PARTIAL CHARACTERISATION OF SAPONINS AND SAPOGENOLS OF CONOPHOR NUT *(TETRACAPIDWM CONOPHORUM.* HUTCH AND DALZ)

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Abstract

The deleterious or beneficial role of saponins made it a necessity to investigate its occurrence in *Tetracarpidiwn conophorum* nut (conophor nut). Triterpenoidal saponins were found present in conophor nut with a yield of 0.32% (299mg/kg). Melting print of the saponins was in the range of 155-158"C. Two (2) different sapon-ns (R_f02 and 0.5) were resolved. Sapogenin portions of acid and alkaline hydrolysates gave single spot each (R_f 0.95) similar to sapogenol standard C. Sugar portion of hydrolysates suggest the presence of glucose. The sapouins evident from the study possibly suggest two saponins with the same sap'Jgenol and sugar, but differed by the number of sugar moieties present or mo* !c of arrangements of the sugars.

Key words: *Tetracapidwn Conophorum* (Q nophor Nut), Saponins, Sapogenol, Sugars.

Introduction

Tetracapidium conophorum belong;, to the family *Euphobiaceae* mainly found in tropical Africa (Hutchinson and Dalziel, 1978). Tree nuts have been used for food since antiquity; however, the -potentials of most tree nuts have not been fully exploited. The vast majority of Nigerians are not only improperly fed but underfed and the widespread use of wild fruits, vegetables and nuts to supplement the traditional diets help alleviate this problun (Adesioye, 1991). Conophor nut is edible and yields a drying oil (Howes, 1974). The nut was chemically analysed and found to contain 53.8% moisture, 23.4% crude fibre, 2.9% ash and 47.4% fat (Adesioye, 1991). The oil contains 60% linoleic acid (Ogunsua and Adebona, 1983). The nuts are eaten locally and the leaves as vegetables with rice (George, 1974). Although conophor nut has been used in Nigeria, no attempt has been made to study the occurrence of saponins in the nut. The aim of this study was to isolate, purify and partially characterise the saponins and sapogenols of conophor nut.

The term saponins generally refers 1 > a group of natural products which have in common the properties of forming foam when shaken with water, haemolysing red blood cells (Milgate and Robert, 1995). They generally have a bitter and astringent taste or are tasteless, although one exception to this is the saponins in liquorice, which is sweet tasting (Irene *et al,* 2001). These compounds comprise monosaccharide moieties linked to triterpencid or steroid aglycones which, depending on the vegetable species and on the chemical structure, may exhibit deleterious or beneficial effects upon human consumption (Cuadrado *et al,* 1995). Saponins have been shown to have hypocholesterolemic, anticoagulant, anticarcinogenic, hepatoprotective, hypoglycemic, immunomodulatary, neuroprotective, anti-inflammatory and antioxidant activity in animals and *in vitro* models (Rao and Gurfinkel, 2000). Saponins are also studied for their insecticidal, antibiotic, fungicidal and mullusicidal properties (Irene *et al;* 2001). The dietary intake for saponins has been estimated at 15 to 240mg daily, depending on the amount and type of substance consumed (Rao and Grunfinkel, 2000).

Materials and Methods

Conophor nut was collected fresh from Omi Adio near Ibadan, Nigeria and subjected to further treatment. Sapogenol standards of Soya sapogenols A, B, C and E were supplied as gift from Professor Isao Kitagawa of the Faculty of Pharmaceutical Sciences, Osaka University, Japan. Octadecylsaline (Ci_8) bonded to silica gel, the matrix used for flash chromatography (reverse phase) was from Dr. Keith R. Price of the Agricultural and Food Research Council (AFRC) Institute of Food Research, Norwich Laboratory, U.K.

Treatment of Conophor Nut/Extraction

The nut was deshelled using a small baton, and heated at 80°C for 10 min, prior to rapid drying at 60°C (Joslyn, 1970). Further drying was earned out under the sun before being pulverised into fine powder to pass

through a 0.25mm sieve (Endecotts Ltd. London). The dried plant part 0.5kg was defatted with chloroform in glass soxhlet followed by methanol (Oleszek, 1988). The extract was then evaporated to dryness using rotor evaporator and the residue weighed.

Isolation of crude saponins was carried out using thejnethod of Kitagawa *et al,* (1976). The aqueous-solution of crude saponins was purified using reverse phase flash chromatography column (9.5xl.0cm Octadecylsalane bonded to silica gel, J.T. Baker G18g). The column was equilibrated with 10ml of methanol followed by 20m3 of distilled water. The crude saponin was applied unto the column in aliquots of 2ml under pressure using N_2 gas. The column was eluted with 2ml volumes of 10, 20... 100% (V/V) methanol in water. Fraction in 2ml elute were collected, concentrated *in vacua* and dissolved in 1ml methanol and chromatographed on TLC plate. Regeneration of column for use was carried out by washing successively with 20ml of acetone followed by 20ml of methanol (Curl *et al.',* 1985, Price *et al,* 1987 and Oleszek, 1988).

Analytical Thin Layer/paper Chromatography (TLC/PC)

Analytical TLC/PC were carried out as described by Price *et al.,* (1987) and Oleszek, (1988) using precoated plate (Kieselgel F254 Merck, 0.25mm thick, 20x20cm) and Whatman No: 3 chromatographic paper, (0.38mm thick, 5.0x20cm, medium flow rate, Whatman Ltd. England.) Saponins were resolved with n-BUOH: EtOH: Cone NH_3 (7:2:5V/V) Wolf and Thomas, (1970);. Detection of saponins was carried out by saturating the plate with Lieberman-Buchard's reagent (10% V/V sulphuric acid, modified) and heating at 120°C for 15min (Gurfinkel and Rao, 2002). Sugars were resolved using n-BuOH: acetic acid:H_2O (4:1:5V/V) and sprayed with resorcinol reagent (visualised at 90°C).

Acid/Alkaline Hydrolysis

A 2mg portion of the purified saponins was dissolved in a 5ml 2N HCl:MeOH (1:IV/V) in 25ml round bottom flask and heated under a condenser on a steam bath for 60 min. The mixture was then neutralised with 0.5% KOH. After evaporation the residue was dissolved in a small quantity of MeOH:H_2O (1:1 V/V) and chromatographed to determine if hydrolysis had occurred or not. The aqueous methanol solution was evaporated to half its volume to remove methanol. Sugars and sapogenins were isolated by extracting the aqueous solution with ethylacetate and examining the ethylacetate fraction for sapogenins and the aqueous fraction for sugars. Alkaline hydrolysis was carried out by dissolving the sample in 0.5% KOH and neutralised with 2NHC1 and treated as in acid hydrolysis (Markham and Chari, 1982).

Melting point was determined using an electro-thermal melting point apparatus (Electro thermal Engineering Ltd. England) Chandell and Rastogi, (1980).

Results

The plant material was defatted using chloroform and extracted with methanol. The yield was 93.53g/kg. Total purified saponins content of conophor nut was 299mg/kg.

Thin-layer chromatographic analysis of the crude extract gave a single spot with R_f value of 0.47, while the purified saponins gave two spots with R_f values of 0.2 and 0.58. Both acid and alkaline hydrolysates of the saponins of conophor nut revealed only the presence of single sapogenol spot each, with the R_f value of 0.95. Soya sapogenols A, B, C and E were also resolved as standards. Soya sapogenol A revealed the presence of 3 spots, Soya sapogenol B 2 spots, while Soya sapogenol C and E had 4 spots each (Table 1).

Paper chromatography of the sugars in the aqueous portions of both acid and alkaline hydrolysates were resolved with n-BuOH: acetic acid: H_2O (4:1:5V/V). The presence of a single spot corresponding to the R_r value of standard glucose 0.29 was observed (Table 2). The melting point of the purified saponins was found to be in the range of 155-158°C.

Discussion

The presence of saponins in conophor nut has further confirmed that saponins occur in a wide variety of plants (Milgate and Robert, 1995) and that some of the saponins containing plants are component of human diet and animal food (Sodipo and Arinze, 1985). Elution of the saponins using various grades of methanol in water (10-100% V/V) revealed that fraction 10,20 and 30% eluted the bound saponins. The yield of 0.32% (299mg/kg) total saponins content of conophor nut was lower than *lupinus mutabilis* CV SCG-9 (390.5mg/kg). Slightly higher than *L. mutabilis* sennca (284mg/kg) and -*L. mutabilis* accha (271mg/kg) Cuadrado *et al,* (1995). The saponins yield of conophor nut was also less than defatted soya flour (0.58%), same with dried

navy beans (0.32%) and higher than dried kidney beans (0.29%), Gurfmkel and Rao (2002). This confirms that the amount of saponins present-in a particular plant material or specie may differ from another (Cuadrado *et al.*, 1995).

Thin-layer chromatography of the purified saponins suggests the existence of a mixtureyof saponins in the plant material (Oleszek, 1988). The R_f value (0.95) of sapogenols from both acid and alkaline hydrolysates, which correspond, with one of the R_f values of Soya sapogenol C (std C) may indicate possible structural similarity. The pinkish colour of the sapogenol spots observed after spraying with Liebermann-Buchard's reagent (modified) was indicative of the fact that the sapogenol of conophor nut were triterpene (Oleszek, 1988). Hence the saponins of conophor nut were triterpenoidal in nature.

The sugar fraction with the R_f value of standard glucose (0.29) was a probable indication that the sugar in the fractions might be glucose. Other studies had also reported the presence of glucose as sugar portion of saponins (Mahato *et al*, 1982; Oleszek, 1988; Irene *et al.*, 2001 and Oleszek, 2001). Rao and Gurfmkel, (2000) reported considerable variations in the number and type of sugars present and the manner in which they are attached to the sapogenins. 'It suggests that the sapogenins in the two saponins of conophor nut might possibly be the same but had variations in the number of glucose (sugar) present or the manner in which they were attached to the sapogenins. The attachment of sugar could be at carbon 3 only or carbon 3 and 22 of the sapogenin moiety (Rao and Gurfmkel, 2000).

The melting point of the purified saponins of conophor nut in the range of 155-158°C could be as a result of the mixture of saponins present (Chandell and Rastogi, 1980).

Conclusion

The study has revealed that conophor nut contains saponins which were triterpenoidal in nature. The saponins might be two (2) types with variations in the number of sugar moieties or the mode of attachment to the sapogenin. Further studies are needed to actually characterise the structure of the saponins of conophor nut and the mode of attachment of the sugar to the sapogenins using ESI-HRMS and NMR spectroscopic analysis. Also studies should be carried out to establish the beneficial or otherwise deleterious role of the saponins of conophor nut.

Table 1: R_f Values of Different Sapogenol of Purified Saponins of Conophor Nut and Soya Sapogenols

Fraction/Standard	Number of Spots	R_r Values
Acid hydrolysate	1	0.95
Alkaline hydrolysate	1	0.95
Std A	3	0.22,0.37,0.46
Std B	2	0.59, 0.67
Std C	4	0.70,0.78,0.85,0.95
Std E	4	0.56,0.65,0.70,0.76

Table 2: R_f Values of Different Sugar Spots

Fraction/Standard	Number of Spots	R_f Values
Acid hydrolysate	1	0.29
Alkaline hydrolysate	1	0.29
Galactose	1	0.26
Glucose	1	0.29
Mannose	1	0.37
Fructose	1	0.38

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