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STUDIES ON THE STEROID HORMONE PRECURSORS OF TWO TROPICAL WILD YAMS
(Dioscorea bulbifera and Dioscorea manganotiana)

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ABSTRACT

Dioscorea bulbifera and Dioscorea manganotiana were evaluated for their potential as a source of saponin and sapogenin. The levels of these steroid hormone precursors were determined by solvent extraction and characterized by froth test, haemolytic test, colour, taste and TLC analysis. The saponin contents of both yams were 1.04±0.08% (*Dioscorea bulbifera)* and 1.58±0.26% (*Dioscorea manganotiana*). The sapogenin content of *Dioscorea manganotiana* was 6.04±0.06mg/g, while that of *dioscorea bulbifera* was 3.36±0.37mg/g. The saponin had a dark-brown colour, bitter taste, frothing ability and haemolysed blood. TLC analysis gave a purple spot with R_f ranging from 0.55 to 0.56. Since the wild yams used for the present study are neither consumed by man nor used for livestock feeding, coupled with their relative abundance and low cost, they hold a good promise with respect to sourcing precursors for commercial production of steroid hormones.

INTRODUCTION

Diosgenin, which is a type of sapogenin, occurs in yams of the genus *Dioscorea;* it also occurs in both root and tubers of *Testudinaria sylvatica* an African plant known as elephant foot [1]. Species of *Dioscorea* have given up to 8.5 – 10.0% diosgenin while *Testudinaria sylvatica* gives 11.5%. However, two Central American species of *Dioscorea floribunda* and *Dioscorea composita* have been found to be the most productive sources. Both of these species yield diosgenin, a good starting point for chemical synthesis of steroid hormones. The sapogenin is extracted from the tubers, which are washed, chopped and dried, for later extraction. Infact diosgenin isolated from *Dioscorea spp* is at present the principal starting materials for the synthesis of steroidal hormones such as testosterone, estradiol and progesterone [1].

The majority of the hormones synthesized from diosgenin is used in birth control pills, for the production of hormone to regulate menstrual cycle, or as a component fertility drug. Cortisone and dihydrocortisone, are two other important hormones that are synthesized from diosgenin. They are used for the treatment of severe allergic reactions for arthritis and for Addison disease caused by malfunction of adrenal gland [1].

A certain amount of steroids come from animal sources. When such steroids are given, there is an adverse reaction from administration of the synthetic variety. Placentae are a rich source of steroids

and have also been found to contain hormones, minerals and enzymes [2]. Companies in Germany, Switzerland and Sweden are buying placentae from hospitals, paying 20p for each placenta. They are taken to an industrial plant in Northern France where the hormones and minerals are extracted and made into pills [2]. Although it is possible to make the contraceptive pill from placental extract, it is not commercially viable, over 600 varieties of yams exist and at the moment there is no danger of the supply running out. Wild yam species has being used extensively for the preparation of the steroidal sapogenins in the developed countries. The sapogenin are in turn converted into cortisone, testosterone (male hormone), oestrogen and progesterones (female hormones) [3]. Wild yams species vary from one location to the other, however not much data on this source is available in Nigeria with respect to providing raw materials for the production of pharmacologically important compounds. This study therefore sought to address the potentials of two tropical wild yam *Dioscorea bulbifera* and *Dioscorea manganotiana* as sources of steroid hormone precursors.

MATERIALS AND METHOD

Materials

Dioscorea bulbifera and *Dioscorea manganotiana* (wild yams) were collected from Igbara-odo in Ekiti South West Local Government Area of Ekiti State, as well as Ibafon, A farm settlement about 2km from Akure in Ondo State, Nigeria. The authentication of the yams was done at Crop Production Department of the Federal University of Technology, Akure, Nigeria. Voucher specimen is available from the authors. The entire chemical used was of analytical grade, while the water used was glass distilled.

Methods

Preliminary screening for saponins and sapogenins

Phytochemical analysis of the samples for saponins and sapogenins was carried out using Gunatilaka *et.al.* [4] methods.

Froth test for saponins

The tuber (100mg) was chopped and shaken with 5ml water in a test-tube, the length of the froth was measured, and taken for the froth to disappear was equally determined.

Extraction of saponins

The tubers (0.5kg) which showed positive froth test were chopped and immediately extracted in a soxhlet, with rectified spirit (1L) for 24hrs. The residue obtained after evaporation of the solvent was extracted in a soxhlet with light petroleum (40 – 60°) (250ml) and the defatted residue was partitioned between butanol and water. The n-butanol layer was dried (Na_2SO_4) and then evaporated to dryness. The percentage weight of the butanol - soluble residue (saponin) was calculated [4].

Isolation of sapogenins

Each sample (25g) of *Dioscorea bulbifera* and *Dioscorea manganotiana* was cut into small pieces and heated with 50ml of 10% HCl (v/v) for 4hrs. After cooling, the hydrolysis product was extracted with chloroform (2 x 25ml). The organic layer was dried over Na_2SO_4 and solvent evaporated, the residue was extracted with light petroleum (60° – 80°) (10ml) and the weight determined [4].

Treatment of erythrocytes

Fresh blood was collected from the University Health Centre. It was centrifuged (x 2000g) for 10 minutes. The supernatant was discarded and the pellets (packed cells) were washed with isotonic phosphate buffer (pH 7.4) several times until there was a clear supernatant [5].

Haemolysis test

A medium double-layered plate of human red blood cell suspension in isotonic phosphate buffer (pH 7.4) in 1.5% agar-buffer, overlaid in 1.5% agar was used. A disc of filter paper saturated in the appropriate solution of the test substance dissolved in phosphate buffer and diluted serially two-fold in the same buffer was placed on the surface of the dried plate. The plate was left for 24 hrs and haemolytic zone, if any was observed [4].

Chromatographic analyses

Each sample of saponin extracts (100mg) was treated with rectified spirit (10ml) and 10% HCl (10ml). The mixture was treated under reflux for 4hrs. The solvent was evaporated under reduced pressure and the residue was taken up in chloroform, washed with water and organic layer was dried over Na_2SO_4. The organic layer after concentration was subjected to TLC analysis using chloroform – methanol (9 : 1) as developing solvent and vanillin-sulphuric acid solution (I and II) spray as the locating reagent.

The plate was sprayed vigorously with 10ml solution I and followed immediately by 10ml solution II. The plates were then heated at 110°C for 10minutes. Further TLC studies were carried out using n-hexane – ethyl acetate (4 : 1) solvent system and 10% H_2SO_4 as the spray reagent [5].

RESULTS AND DISCUSSION

Wild yams are widely distributed in the tropics of Africa and Asia. They are underutilized in Africa in view of the fact that these yams contain some toxic alkaloids which must be removed by either boiling or prolonged soaking in water [6]. Presently, they are used for hunting as well as traditional medicine, both in Africa and Asia [6]. According to FAO production yearbook [7], 95% of yam were grown in Africa, particularly in Nigeria. Though preliminary studies showed that wild wild yams are very abundant in Ondo – state, little information is known about their chemical potentials. Simpson and Ogorzally [1] reported on the pharmacologically potential of some wild yams. The tubers of some wild yam species contain steroidal sapogenin-related sex hormones (corticosteroids) which provide a source of diosgenin, which can be used in the manufacture of oral contraceptives, sex hormones and cortisone.

Phytochemical screening recorded positive froth test by both wild yams. *Dioscorea bulbifera* had foam length of 3.18cm and foam disappearance time of 36.0±5.2min, *Dioscorea manganotiana* had a foam length of 3.98cm and foam disappearance time was 32.0±1.2min. These values tend to agree with Gunatilaka *et.al.* [4] for presence of saponin, whose foam will persist for about half an hour (Table 3). In accordance with earlier reports [8-10], the ethanolic extract of both wild yams had haemolytic effects on human erythrocyte. This test would tend to support the fact that the ethanolic extract contains saponin. The saponin contents of both wild yams were 1.58±0.26% (*D. Manganotiana*) and 1.04±0.08 (*D. bulbifera)* (Table 3). The higher saponin content of *D. manganotiana* would probably explain its higher foam length. In similar studies carried out on *D. bulbifera* in Sri-Lanka, the yam bubils were found to contain 2.2% which is still higher than the value obtained in the present wild yam species. This indicates that saponin contents of wild yam species vary from one location to another.

The saponins from D.bulbifera and D. manganotiana were further characterized as shown in table 2. The attributes, namely, taste, colour and solubility agreed with Merck Index [11] description of saponins. The melting point of saponin from *D. bulbifera* was above 200°C, while that of *D.*

managanotiana was 185°C. The difference in the melting points would tend to suggest that though both extracts are saponins, they differ in some respects.

The sapogenin extract which were light brown in colour with pungent smell were 3.36±0.37mg/g (*D.bulbifera)* and 6.04±0.06 mg/g (*D. managanotiana*) (Table 4). From the results, it would seem that *D. manganotiana* has more sapogenin than *D. bulbifera*. This trend agrees with their saponins content, which were 3.98±0.14% (*D. manganotiana*) and 3.18±0.14% (*D. bulbifera*) as stated earlier. The sapogenin extract from *D. bulbifera* and *D. manganotiana* has a single spot each of which stained purple with vanillin-sulphuric acid solutions (I and II) with the same R_f (0.86). The results of thin layer chromatographic studies on sapogenin would tend to support the result of froth test and heamolytic test, that both wild yam extracts contain saponins. The purple colour of the stain on the spot would tend to confirm sapogenin from saponin and their R_f values indicate that the saponins and sapogenins from *D. bulbifera* and *D. manganotiana* is very much similar (Table 5). Furthermore TLC studies using n-hexane and ethyl acetate (4 : 1) solvent system revealed that *D. manganotiana* contained four spots while *D. bulbifera* contained two spots, the standard saponin hydrolysate contained three spots.

Spots a (*D. manganotiana*), h (*D. bulbifera*), g (standard saponin) were very much alike in colour (purple) and R_f. The purple colour typifies sapogenin, and the R_f value ranged from 0.55 – 0.56 that is close to the characteristics R_f for diosgenin [5, 12] which is a type of steroidal sapogenin. Although there were other unidentified purple spots from both yams, the purples colour will suggest there are other types of sapogenins in the yams other than diosgenin [5, 12,13]. Moreover, since both yams were found to contain diosgenin, a type of steroidal sapogenin, and the saponin would likely be steroidal, sapogenin being the acid hydrolysis product of saponin [14]. Although only diosgenin was identified, the fact that the saponins extracts completed their haemolysis within three minutes in the time-course haemolysis studies indicated that the unidentified haemolysis would likely be steroidal [5]. Since the wild yams used for the present study are neither consumed by man nor used for livestock feeding, coupled with their relative abundance and low cost, they hold a good promise as sourcing precursors for commercial production of steroid hormones.

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REFERENCES

[1] Simpson, B.B. and Ogorzally, M.C. (1986): Plant in Medicine, Economic Botany Plants in our World 364 – 368.

[2] Almar R. (1985): The day of the Yam , Nursing Times, May1 p47.

[3] Kochar, S.L. (1986): Tropical Crops, Textbook of Economic Botany, Macmillan Publishers Ltd. London, 232-233.

[4] Gunatilaka, A.A.I. Sotheswaran, S. and Balasubramanin S. (1978): Economically useful plants of Sri-Lanka II: Commercially important steroidal sapogenins from Sri Lanka Plants. J. Natn. Sci. Coun. Sri Lanka 6 (2) 121 –128.

[5] Oboh, G., Akindahunsi, A.A. and Ekperigin, M.M (1997): In.vitro studies on steroidal extracts from two wild yams (Dioscorea spp.), Nigerian Journal of Biochemistry and Molecular Biology 12 , 53-60

[6] Purseglove, J.W. (1985): Dioscoreaceae, Tropical Crops Monocotyledon Vol.1 and 2 Combined 67-116.

[7] FAO production year book (1985): Food and Agriculture Organization of the United Nations, Rome, 1986 vol. 39.

[8] Ponder, E. and Cox, R.T. (1952): Haemolysis considered as progressive reaction in a heterogenous system J. Gen. Physiol. 35 (4) 599 – 603.

[9] Teschesche, R. and Wulff, G. (1964): Constitution and properties of saponin. Planta Medica, 12 (3) 272- 292.

[10] Sodipo, O.A. Akanji, M.A. and Odutuga, A.A. (1990): Comparative study on the effect of saline extracts of the pulp and seeds of *Garcinia Kola* (Hecke) on some mammalian erythrocytes. Nig. J. Sci. 24 (182), 95 – 99.

[11] Merck Index (1983): An Encyclopedia of Chemicals Drugs and Biologicals, 10th ed. (Windholz ed.), 1204 Merck and Co. Inc. Rahway, N.J. USA, 8218p.

[12] Oboh, G., Akindahunsi, A.A., Famutimi, R. and Adetuyi, F. C (1998):Antimicrobial studies on saponin extracts from two wild yams(Dioscorea spp.), Nigerian Journal of Biochemistry and

Molecular Biology, 13 , 47 - 50

[13] Oboh, G., Akindahunsi, A.A. and Ekperigin, M.M.(2000): Biotransformation of steroidal saponins from two wild yams (*Dioscorea spp.*) to sapogenins, Nigerian Journal of Biotechnology, 11 (1) 77 - 81

[14] Agrawal, P.K., Jain, D.C., Gipta, P.K. and Thakur, R.S. (1986): Review Article II, Carbon-13 NMR Spectroscopy of steroidal sapogenins and steroidal saponins. Phytochem. 24(11) 2479 – 2496.

Table 1: Physical characteristics of *D.bulbifera* and *D.manganotiana*

Properties	*D. bulbifera*	*D. manganotiana*
Weight (g)	19.30 ±6.06*	ND
Texture of the Back	Rough	Rough
Colour of the Back	Gray	Black
Colour of the endosperm	Yellow	White

*Value represent means of 20 replicates; ND= not determined

Table 2: Physico-chemical properties of saponins from *D. bulbifera* and *D. manganotiana*

Property	*D. bulbifera*	*D. manganotiana*
Colour	Dark brown	Dark brown
Taste	Bitter	Bitter
Odour	Coffee-like	Coffee-like
Texture	Crystalline	Crystalline
Melting point	>200°C	185°C
Solubility in		
-water	Soluble	Soluble
-methanol	Soluble	Soluble
-ethanol	Soluble	Soluble
-butanol	Soluble	Soluble

values represents means of triplicates.

Table 3: Phytochemical tests on steroidal saponins from *D. bulbifera* and *D.manganotiana*

Test	*D.bulbifera*	*D.manganotiana*
Froth test	Positive	Positive
-Foam length (cm)	3.18 ±0.14	3.98 ±0.14
-Time taken for foam to disappear (min)	36.0 ±5.2	32.0 ±1.2
Saponin content (%)	1.04 ±0.08	1.58 ±0.26
Haemolytic test	Positive	Positive

Values represent means of triplicates

Table 4: Physico-chemical properties of sapogenins from *D. bulbifera* and *D. manganotiana*

Property	*D. bulbifera*	*D.manganotiana*
Colour	Light brown	Light brown
Odour	Pungent	Pungent
Content (mg/g)	3.36 ±0.37	6.04 ±0.06

Values represent means of triplicate

Table 5: TLC analysis of saponin from *D. bulbifera* and *D. manganotiana* using chloroform-methanol (9 : 1) solvent system and Vanillin-H_2SO_4 solutions I and II stain as spray

Property	*D. bulbifera*	*D.manganotiana*
Numbers of spot	One	One
Colour with stain	Purple	Purple
R_f	0.86	0.86

Table 6: TLC analysis of saponin from *D. bulbifera* and *D. manganotiana* using n-hexane - ethyl acetate (4 : 1) solvent system and 10% H_2SO_4 solution as spray

Sample	No. of Spot	Spot code	Colour of spot	R_f
D. manganotiana	4	a	Purple	0.55
		b	Purple	0.45
		c	Purple	0.33
		d	Blue	0.21
D. bulbifera	2	h	Purple	0.56
		i	Purple	0.45
Standard	3	e	Pink	0.08
		f	purple	0.18
		g	Purple	0.55