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Variation in the Levels of Steroidal Sapogenins within the Mature Fruit of *Balanites aegyptiaca* and among Kernels of Balanites Fruit Accessions Collected from Different Geographical Localities in Sudan.

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ABSTRACT

Steroidal sapogenins, diosgenin in particular, are of current research interest as precursors employed by the pharmaceutical industry for the partial synthesis of important steroid drugs as well as for their revealed biological activities of potential medical uses. We report on the content of steroidal sapogenins in mature fruits of *Balanites aegyptiaca*, a wild tree of wide-spread occurrence in Sudan, where large quantities of fruit are collected from the wild and sold in local markets for the edible fruit mesocarp. A simple, selective, infra-reresided spectrophotometric method was used for the determination of both 25α- and 25β- epimeric steroidal sapogenins in crude extracts. Most of the total steroidal sapogenin of the balanites fruit resided in the mesocarp (64%), followed by the kernel (*ca.*25%).The fragile epicarp contained about10%. No sapogenin was detected in the woody endocarp. The ratio of 25α- to 25β-sapogenin considerably varied among the fruit morphological parts. The content of total sapogenin was determined in the kernels of 15 fruit accessions of balanites collected from different parts of Sudan.Values found varied from 2.5% to 5.0%, based on kernel dry-weight. This range compares very well with the sapogenin content reported for *Dioscorea*, so far the major world commercial source of diosgenin. Kernels of the 15 Sudanese balanites fruit accessions were enriched in 25α- (diosgenin-like) sapogenins. It was concuded that whole balanites kernels or de-fatted kernel powder could be commercialized on a world-wide scale as a source of diosgenin, complementing the role of *Dioscorea*. Although the kernel constitutes only one-tenth of the balanites fruit weight,as shown in this study, we claim that Sudan alone could produce fruit material enough to sustain such world-wide commercial exploitatin.
Keywords: *Balanites aegyptiaca*, Sudanese mature-fruit accessions, balanites kernel sapogenins, 25α- and 25β-sapogeninns, within-fruit sapogenin distribution.

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INTRODUCTION

Balanites aegyptiaca (L.) Del. (desert date), family *Balanitaceae*, is a wild, tropical, evergreen, tree native to the Sudano-Sahielian regions of Africa and south Asia. The genus *Balanites* encompasses about ten species, most of which are encountered in Africa, especially *B. aegyptiaca*[1]).All parts of the tree are used in the folk medicine of several nations of the world, mainly in Africa and Asia. In addition, many biological activities have been reported for the plant (see reviews by [2, 3]).

Balanites tree, known locally in Sudan as *hijleej,*bears fruits (*laloab*) with an edible mesocarp, a reason for whichlarge amounts ofwhole fruits are collected from the wild,for trading in local Sudanese markets. Balanites tree isvalued by local ethnic groups in Sudan for its multiple uses including those in foods, feeds, furniture and tool-making as well as in traditional medicine(mainly to treat diabetes, juandice, bilharzia andas a laxative and a contraceptive).

The most important chemical constituents of balanites fruits are saponins, which are composed of sugars (glycones) chemically combied with triterpenes or steoid aglycones. Saponins aresurface-active (soap-like) water-soluble natural compounds which possess many biological effects, e.g., their action on cell membranes including their ability to lyse erythrocytesin addition to a number of potentially useful pharmacological activities[4, 5, 6, 7]. In the case of *B. aegyptiaca*, steroid aglycones (sapogenins) such as yamogenin and diosgenin are the main non-sugar components [8, 9] that give rise to a number of balanites saponins(eg balanitins 1,2,3, etc.) which differ in the type and number of sugars attached to the aglycone and the sugar(s)' position of attachment [2, 7].

We have been interested in Sudanese potential commercial sources of steroidal sapogenins (obtained from saponins after their acid hydrolysis to remove the glycones). These natural chemicals are of considerable research interest as precursors employed by the pharmaceutical industry for the manufacure of steroid drugs [10, 11, 12]. Steroidal sapogenins, particularly diosgenin, are also gaining increasing importance in view of their interesting biological activitiesincluding anticancer [6, 13, 14] and several other activitiesof potential application in medicine [5, 15].

A number of methods were used to assay sapogenins. Theyincludedhigh performance liquid chromatography, HPLC [16, 17], ultra performance liquid chromatography coupled with mass spectrometry, UPLC-MS[18]),capillary gas chromatography[19] and spectrophotometry based on infra-red or visible region spectra.For example, Ghosh et al, 2012 [20])used the simple method ofseparatingcrude sapogenin extracts of *Tribulusterrestris* L. using thin-layer chromatography (TLC), sprayed the TLC plates with anisaldehyde/H_2SO_4 reagentand employed densitometry at 430 nm for quantification. The anisaldehyde-based color reaction, followed by spectrophotometry in the visible region of the spectrum, was also employed for sapogenin quantification byothers [21, 22].

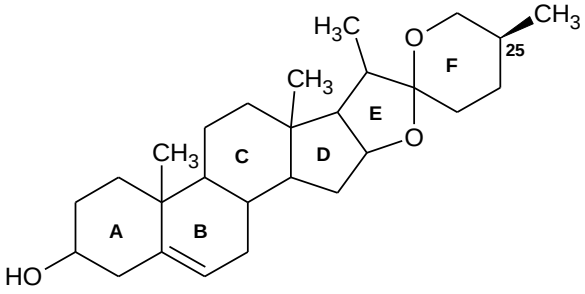

Fig 1: Structure of diosgenin.
Yamogenin is different only in stereoisomerism at Carbon 25 (numbered), the 25-CH_3 group having a β- orientation (α orientation shown for diosgenin). The rest of numbering, ring conformations and other chemical group orientations (including hydrogens), similar for both epimers, are not shown.

In this report, sapogenins (25α-, 25β- epimers and total sapogenin) were determined using anearlier infra-red (i.r.) spectrophotometric method as modified by the Hardman group at Bath University, U.K. [8, 23,

24, 25, 26]. The method is simple, rapid and specific for 25α- and 25β- stereoisomers (epimers). Thus the closely structurally related epimers, diosgenin and yamogenin **(Fig 1)**, the major sapogenins of balanites fruitcould each be quantified when present together in crude extracts, a clear advantage of the method.The method is based on the fact that steroidal sapogenins absorb infra-red radiation of frequencies 900, 915 and 980 cm^{-1}, with 25α- sapogenins (diosgenin) absorbing more intensely at 900 cm^{-1} and their 25β- epimers (yamogenin), at 915 cm^{-1} [23]).In quantitative studies, when 25α- and 25β-epimeric sapogenins were present together, mutual absorption of either epimer at 900 or 915 $cm^{-1}$was corrected for using a ratio graphas explained by Brain et al, 1968 [23].

Using this i.r. method, wepreviously reported on steroidal sapogenins of 33 Sudanese fenugreek seed accessions [27]. We here report on sapogenins of balanites fruit, the second potentialSudanesesapogenin source, mainly on their variability within the different morphological parts of the fruit and variability in sapogeninsof kernels isolated from fruit accessions obtained from different geographical regions of Sudan.

MATERIALS AND METHODS

Balanites fruit material

Mature fruits of balanites, with their epicarps intact, were collected from trees growing wild in different geographical regions of sudan, in the same fruiting season.**Fig 2**is a photograph of two fruits (ripe, and mature but still green) cut in halves to show fruit morphological parts. Epicarp and mesocarp tissues were carefully separated manually using a thin sharp knife. The kernels were separated from the enclosing woody part (the endocarp) by gentle hammering. For larger scale operations, a laboratory- scale Hammer Crusher (Ogawa Seiki Co., Ltd, Japan) was used to separate mature balanites fruits into 'epicarp + mesocarp', 'endocarp' and 'kernel' fractions.

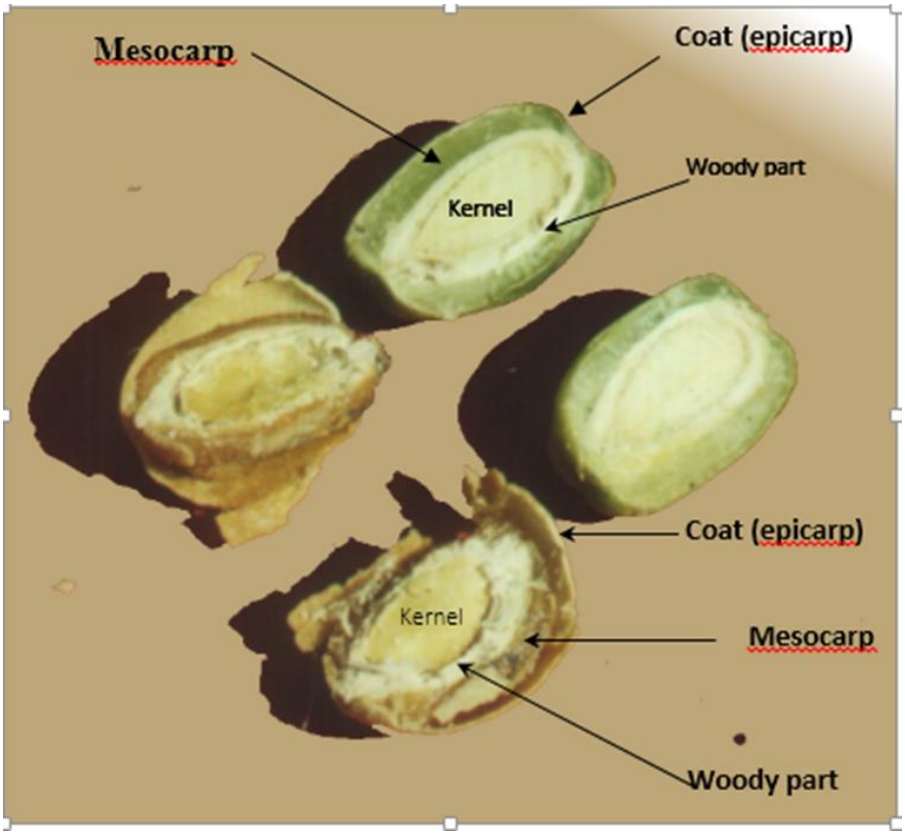

Fig 2: Balanites fruit morphological parts.The photograph shows cutcross sections of ripe (brownish coat and mesocarp) and unripe fruits (greenish coat and mesocarp). The fruits were about 3 cm in length.

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Solvents, chemicals and equipment

Analytical grade solvents and chemicals were used for analysis. Spectrosol grade solvents were used for i.r. spectroscopy.Silica gel G 60 was used as adsorbent for TLC. Precoated TLC plates were obtained from Merck Co. (Germany). When required, particularly for preparative-scale separations, glass plates (20 x 20 cm) were coated manually with the above adsorbent using a TLC spreader (Camag, Switzerland).Antimony trichloride spray reagent was used to detect steroids on TLC plates [28].

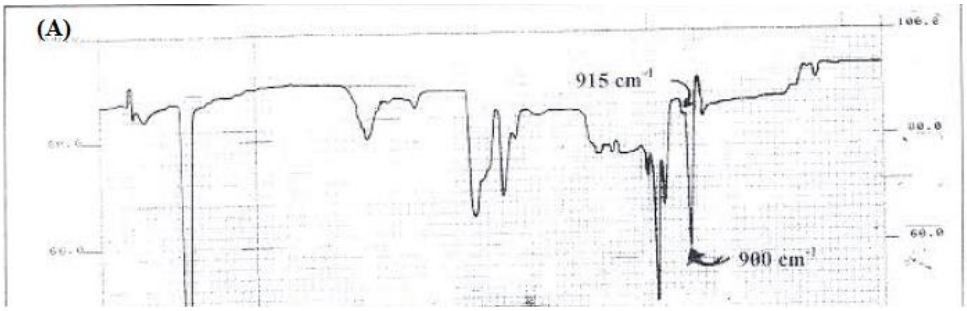
A Shimadzu Infrared Spectrophotometer Model IR 435 was used was for sapogenin analysis by measuring full IR spectra or, for routine quantitative analyses of crude extracts, the spectrograph was set to measure IR absorptions in the wavenumber region 800-1000 cm^{-1}.

Preparation of balanites total sapogenin extract

A weighed amount of dried and powdered plant material (fruit epicarp, mesocarp and endocarp) was hydrolyzed under reflux with 2N HCl (30 ml per gram of plant material) for 3 hours. After cooling, the residue was filtered, briefly washed with water before it was neutralized with NH_4OH (10%). The neutralized residue was washed again and dried over-night at 50 ^oC. The steroidal sapogenins were then extracted with petroleum ether (b.r. 40^0-60^0) in a Soxhlet extractor for 24 hrs. The extracts were concentrated in a rotatory evaporator. The light yellow semi-solid product obtained was dissolved in 10ml of Spectrosol grade $CHCl_3$ and the sapogenins were taken for further analyses (TLC separations or sapogenin quantification by infra-red spectroscopy). The same procedure was followed with kernel powder, only that it was found necessary to de-fat the oil-rich tissue bysoxhlet extraction using hexane prior to hydrolysis and extraction of sapogenins.

Preparation of diosgenin and yamogenin standards

The two epimers diosgenin and yamogenin, which co-chromatographed under ordinary TLC conditions were completely separated from each otheron a preparative scale according to the continuous-flowTLC development method briefly mentionedbyHarborne [29].For this purpose, diosgenin and yamogenin obtained, on a preparative scale, as one TLC band usingthe solvent: n-hexane: acetone (4:1) were eluted and the concentrated eluate used for further continuous-flow TLC.In this method, with the 'spotted' plates inside the tank, the tank-lid was firmly placedso that just the top of the plate was uncovered allowing continuous solvent evaporation off the top edge of the plate while chromatographic development continued for 8 hours. The developing solvent was dichloromethane: diethyl ether (200:3, v/v). Diosgenin and yamogenin, completely separated,were eluted and tested for purity using i.r. spectroscopy. Specific i.r. absorptions at 900 and 915 cm^{-1} characterized the separated diosgenin and yamogenin,rsespectively (**Fig 3, A and B**).



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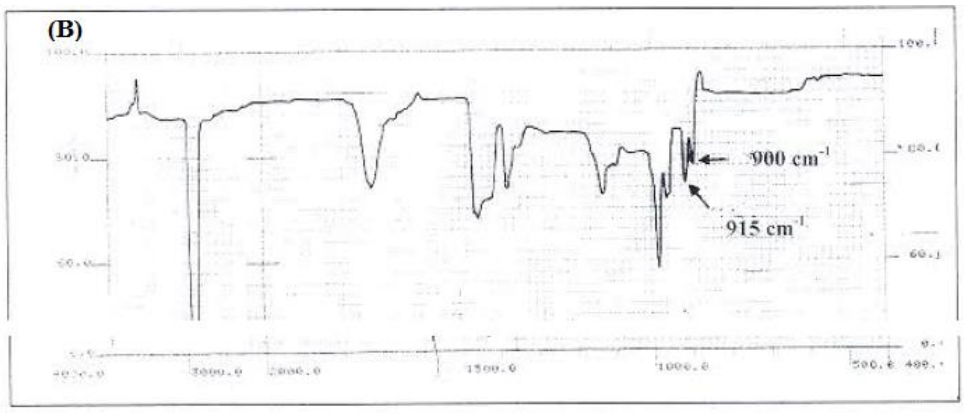

Fig 3: Full infra-red spectra (800 – 4000 cm^{-1}) of diosgenin (A) and Yamogenin (B) separated from each other by continuous-flow TLC.

Calibration graphs

Different concentraions of standard diosgenin or yamogenin solutions were prepared using chloroform and their i.r. absorptions, measured at 980 cm^{-1} in addition to 900 cm^{-1} (diosgenin) or 915 cm^{-1} (yamogenin), were plotted against concentrations. **Figs4 and 5**show typical graphs. The influence of the presence of one epimer on the absorption of the other epimer was routinely corrected for by using a graph prepared according to the "ratio method" [27], For this purpose, chloroform solutions containing both purified diosgenin and yamogenin standards were prepared to give concentration ratios of diosgenin **:** yamogenin from 0.16:1 to 1.60:1. The i.r. absorptionsof these solutions were measured at 800-1000 cm^{-1}. The ratios of i.r. absorbances obtained at 900cm^{-1}**:** 915cm^{-1} were plotted against their concentration ratios. **Fig 6** represents such a graph.

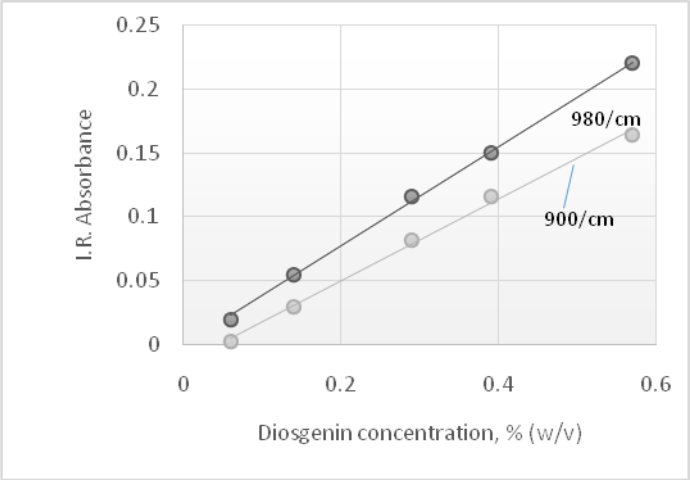

Fig 4: Diosgenin calibration graph.

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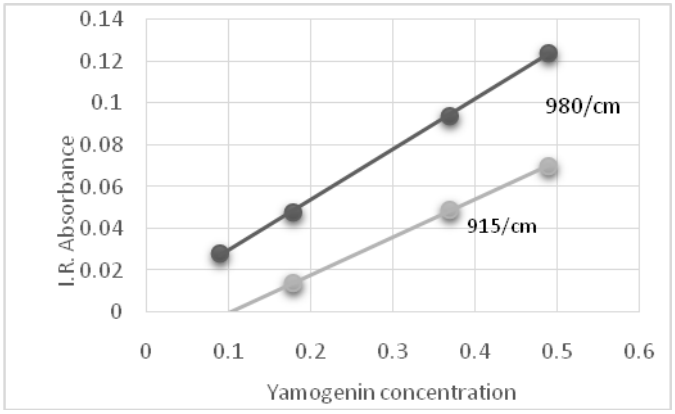

Fig 5: Yamogenin calibration graph.

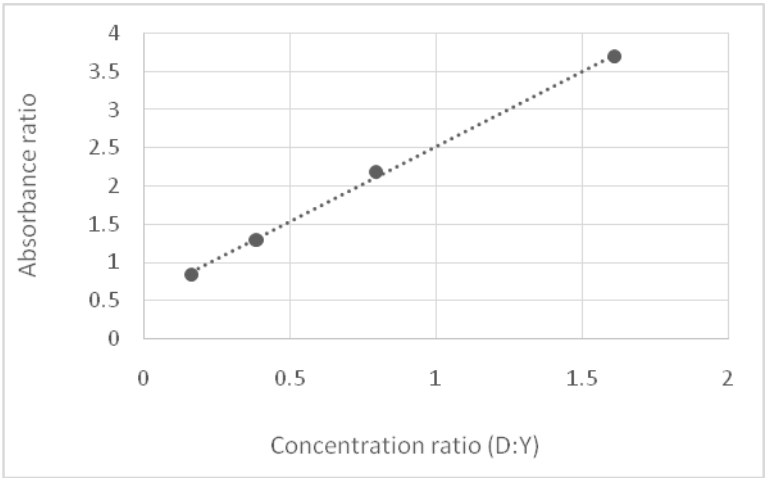

Fig 6: Diosgenin (D) and Yamogenin (Y) 'ratio graph'. Solutions containingdiosgenin and yamogenin in different ratios (by weight) were prepared and the ratio of measured i.r. absorption at 900 cm^{-1} and 915 cm^{-1} was plotted against the concentration ratio.

Infra red spectroscopic assay of total and 25α- and 25β- sapogenins

The final chloroform solutions of crude sapogenins prepared were routinely assayed by scanning in the i.r. region 800 - 1000cm^{-1}. Calibration graphs (Fig 4 or 5) were initially used to determine the concentration of either α or β sapogenin. The amount of the co-absorbing epimer was calculated using the ratio graph (Fig6) and the value subtracted. Total sapogenin values were obtained by summing up values for total25α- and 25β-sapogenins**.**

RESULTS AND DISCUSSION

Contribution of fruit morphological parts to total fruit-weight

Table 1shows the contribution, by weight, of each of balanites fruit parts to the weight of thewhole fruit, determined for 14 fruit accessions collected from different parts of Sudan. Individual fruit weight varied from close to 10g, in the accession from south eastern Sudan, to about 4g, in the western Sudan accession; fruit weight averaging 6.6 g. The major contributors to fruit weight were the sapogenin-free endocarp (woody part) and the sapogenin-rich mesocarp, with the oil-rich kernel constituting about one-tenth of fruit weight. These weight relations are essentially in agreement with those reported for fruits of *B. aegyptiaca* from Sudan [30, 31] and other countries from Africa or Asia [21]. The average values for the relative weights of fruit parts for the fourteen accessions are shown in **Fig 7**.

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Table 1: Weight relations ofbalanites fruit morphological parts(for 14 accession).

Accession (Ser. No., geog. locality)	Weight of fruit part (g), total weight/ fruit (g) and per cent. contribution to whole-fruit weight (in brackets)				
	Kernel	*epicarp*	*mesocarp*	*Woody part*	*Whole fruit*
1 (W)	0.58 (9%)	1.45 (21%)	2.60 (39%)	2.10 (31%)	6.73 (100%)
2 (NE)	0.66 (11%)	1.48 (24%)	2.00 (33%)	1.92 (32%)	6.06 (100%)
3 (W)	0.26 (6%)	0.76 (19%)	1.50 (37%)	1.54 (38%)	4.06 (100%)
4 (SE)	0.92 (12%)	1.16 (15%)	3.03 (39%)	2.71 (34%)	7.82 (100%)
5 (W)	0.59 (13%)	0.83 (19%)	1.41 (32%)	1.58 (36%)	4.42 (100%)
6 (C)	0.60 (10%)	0.97 (16%)	2.25 (38%)	2.10 (35%)	5.92 (100%)
7 (SE)	1.07 (11%)	1.49 (15%)	3.14 (33%)	3.98 (41%)	9.68 (100%)
8 (C)	0.54 (8%)	1.24 (18%)	2.98 (43%)	2.20 (32%)	6.96(100%)
9 (C)	0.56 (8%)	1.30 (18%)	2.97 (42%)	2.24 (32%)	7.07 (100%)
10 (C)	0.62 (9%)	1.28 (18%)	2.89 (41%)	2.30 (32%)	7.09 (100%)
11 (W)	0.40 (8%)	0.83 (17%)	2.47 (49%)	1.30 (26%)	5.00 (100%)
12 (SE)	0.97 (10%)	1.75 (18%)	3.48 (36%)	3.45 (36%)	9.65 (100%)
13 (SE)	0.68 (10%)	1.01 (15%)	2.71 (40%)	2.43 (35%)	6.83 (100%)
14 (SE)	0.53 (10%)	0.90 (16%)	2.18 (39%)	1.94 (35%)	5.55(100%)
Mean (± SE)	0.64 g ± 0.06	1.18 g ± 0.08	2.54 g ± 0.16	2.27 g ± 0.19	6.63 g ± 0.45

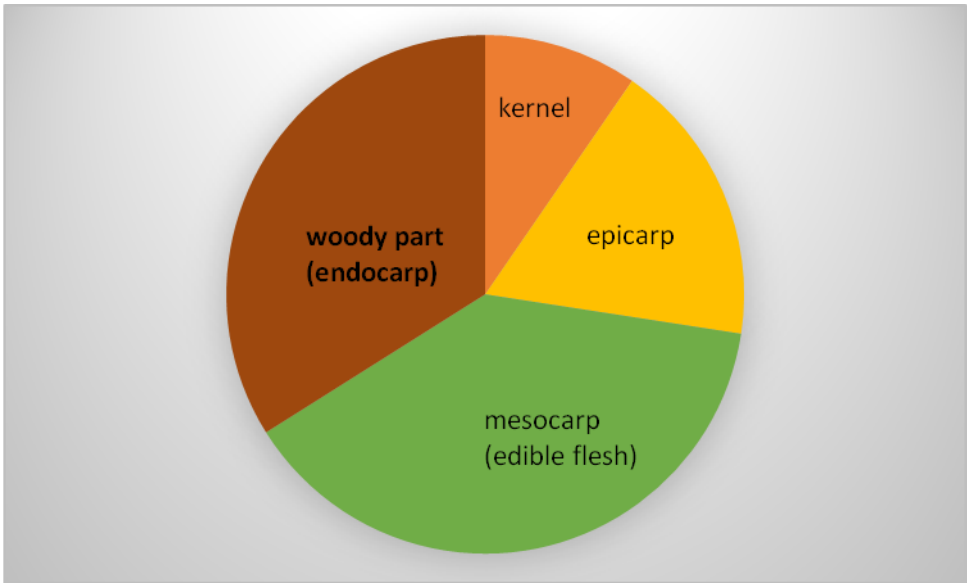

Fig 7: Morphological parts' contribution to whole fruit weight, %. Datarepresent average values for all 14 accessions of Table 1.

Diosgenin and yamogenin calibration graphs

Figs 4 and 5 show the linear relationship between concentrations of diosgenin or yamogenin and i.r. absorptions messured at frequencies of 980 cm-1 (both epimers) and at 900 cm-1 (diosgenin) or 915 cm-1 (yamogenin). Mutual absorption occurs. Yamogenin, for example, also absorps at 900 cm^{-1} frequency (Fig 3, B).Fig 6 shows that, when present together in the same assay sample, the concentration ratio of diosgenin:yamogenin could be predicted (calculated) from the observed i.r. absorbance ratio at 900 and 915 cm^{-1}, thus correcting for mutual absorption.

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Individual sapogenins of balanites kernels

Crude sapogenin extracts,prepared as explained in Materials and Methods, frommature and immature kernels of balanites were subjected to TLC analysis using n-hexane: acetone (4:1) as solvent and antimony trichloride/HCl as detection reagent for steroids. Provision was made to recover (and elute) separated spots free of detection reagent. The sameeightcompounds were separated from both mature and immature kernels of balanites. **Table 2**shows their R_f values and their i.r. absorptions at the frequencies characteristic of steroidal sapogenins. Five TLC-separated spots showed spirostan absorptions typical of sapogenins (at 900 or 915 and at 980 cm^{-1} frequencies). Subsequent studies showed that TLC spot number 6, the major one, was a mixture of co-chromatographing diosgenin and yamogenin. All other four sapogenin spots had a major absorption at 900 cm^{-1}, i.e., of the 25-α epimeric type. Thus, kernel tissue seems to be relatively rich in 25α- sapogenins. No further attempts were made to characterize these four sapogenins. TLC spots 3 and 7 (Table 2) are likely to be phytosterols.Previous literature reports on sapogenins of *B. aegyptiaca*seem to be concerned only with diosgenin and yamogenin as the most important, commercially-sought steroids. Work carried out on the isolation and characterization of *B. aegyptiaca* aglycones [8, 23]or saponins [7, 32]did not report the occurrence of sapogenins other than diosgenin and/or yamogenin. The same was true for reports on two other species of the genus, namely, the ripe fruits of *B. orbicularis*[33] and differentmorphological parts of *B. wilsoniana* [34]where, again, the presence of onlythese two sapogenins was reported.

Table 2: Infra-red spectroscopic absorption of TLC- separated components of the total sapogenin fraction prepared from kernels of mature fruits of balanites. TLC solvent: n-hexane: acetone (4:1); detection: antimony trichloride reagent.

TLC SPOT NO.	R_F (%)	PRESENCE (+) OR ABSENCE (-) OF ABSORPTION AT I.R. FREQUENCY INDICATED (CM^{-1})		
		900 cm^{-1}	915 cm^{-1}	980 cm^{-1}
Origin	**0.0**	**-**	-	-
1+2	**7.0-9.0**	**+**	-	+
3	**16.6**	**-**	-	-
4	**28.7**	**+**	-	+
5	**44.4**	**+**	tr*	+
6	**61.1**	**+**	+	+
7	**77.7**	**-**	-	-
8	**86.1**	**+**	tr*	+

tr* = trace peak of absorption

Sapogenin distribution in different morphological parts of the mature fruit of balanites

Table 3shows values for sapogenin content in the different morphological parts of two fruit accessions procured from western (W) andeastern (E) Sudan. The total sapogenin content was 72.6 and 77.5 mg per whole fruit for the two accessions, respectively. Most of this, about two-thirds of the fruit total content, was contained in the mesocarp of both accessions; the rest resided in kernel tissue (24.6 and 27.3 %, for W and E accessions, respectively) and in the epicarp (about 10%, for both accessions). This thin fragile epicarpis mostly lost during postharvest handling, despite its considerable sapogenin content. **Fig 8**, constructed from data of Table 3,alternatively shows the contribution of each morphological part (%) to whole fruit sapogenin.

Table 3: Sapogenin distribution in different morphological parts of the mature fruit of two accessions of balanites from western (W) and eastern (E) Sudan.

Sapogenin	*Fruit part* (or whole fruit)									
	Kernel		*Epicarp*		*Mesocarp*		*Woody part*		Whole fruit	
	W	E	W	E	W	E	W	E	W	E

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Total sapogenin/ fruit part, mg	**18.0**	**21.0**	**8.2**	**7.1**	**46.4**	**49.4**	**0.0**	**0.0**	**72.6**	**77.5**
Total sapogenin content, % of tissue dry weight	**2.71**	**2.70**	**0.57**	**0.62**	**1.80**	**1.90**	**0.0**	**0.0**	**-**	**-**
Percentage contribution to whole fruit total sapogenin	**24.6**	**27.3**	**11.2**	**9.3**	**64.2**	**64.3**	**0.0**	**0.0**	**100%**	**100%**
25α- : 25β- sapogenin ratio	**2:1**	**4:1**	**1:1**	**1.5:1**	**0.4:1**	**1.4:1**	**-**	**-**	**-**	**-**

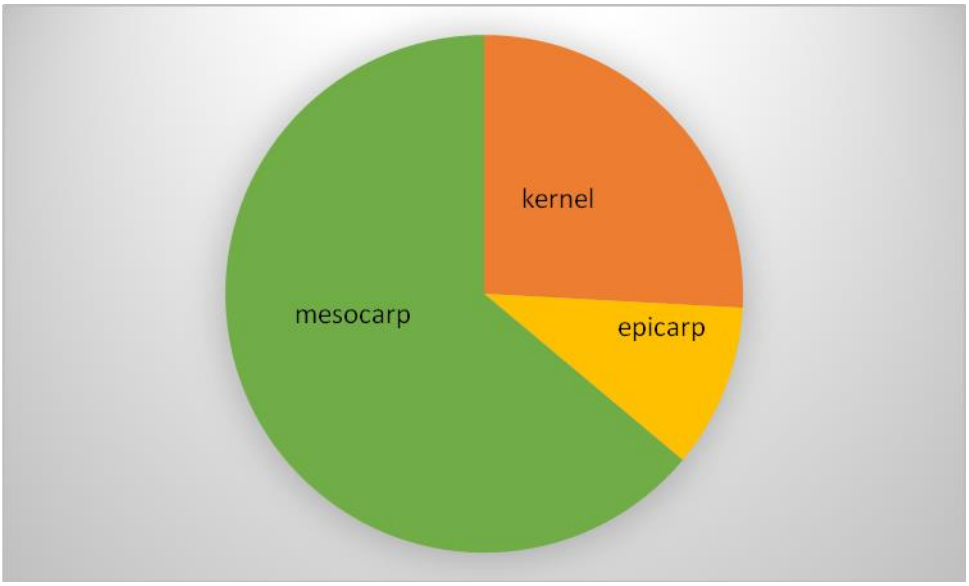

Fig 8: Fruit part contribution (%) to total sapogenin of whole fruit. Data used were averages for W and E fruit accessions of Table 3.

When the total sapogenin values were expressed as a percentage of tissue weight, the kernel turned out to be the richest (well over 2%), followed by the mesocarp (slightly less than 2%; Table 3). This is in close agreement to the value of 2.0 % reported by Hardman and Sofowora, [24]) for kernels of a fruit sample of *B. aegyptiaca* from West Africa, and the value of 2.2 % for kernels from Israeli balanites fruits [21]). The latter authors[21] reported lower values (around 1.0 %) for balanites kernels from West Africa and India.

Table 3 also shows that the woody epicarp was void of sapogenin. Parts of the balanites tree other than the fruit were reported to contain little amounts of steroidal sapogenins.The roots and stem barkof *B. aegyptiaca* contained 1.0% and 0.7%, respectively [8] and even less values were reported for the root (wood and bark), stem bark and leaf of the other species, *B. wilsoniana*.

Table 3 also shows that kernel tissue is enriched in diosgenin-like sapogenins while the mesocarp contained more of the 25β- (yamogenin-like) sapogenins.

Sapogenin content of kernels of 15 balanites fruit accessions

Kernels were found easy to isolate in intact form from balanites fruits either manually or using machines such as that referred to in Materials and Methods.Kernel tissue is more available than any other part of the balanites fruit. When the edible fruit fleshis consumed by humans or animals, the kernels, still enclosed within the woody endocarp, could be wholly recovered.

Table 4shows the sapogenin content of kernels isolated from 15 fruit accessions obtained from different parts of Sudan. The results show remarkable variability in content. Total sapogenin values varied from as low as 9.0 to as high as 42.0 mg per whole kernel in accessions from western and south-eastern Sudan, respectively. These results (of total sapogenin in mg/kernel) for all 15 accessions are also shown in **Fig 9**, for easier comparison.

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Table 4: Sapogenin content and ratio of 25α to 25β- sapogeninof balanites kernels of 15 fruit accessions collected from different parts of Sudan.

Accession (Ser. No., geographical locality)	Sapogenin content		25α:25β sapogenin ratio*[8]
	in mg per kernel	as a % of kernel tissue dry weight	
1 (W)*	18.0	2.91	2:1
2 (E)	39.6	3.40	4:1
3 (W)	26.2	3.00	4:1
4 (SE)	24.4	2.90	4:1
5 (S)	33.6	4.00	3:1
6 (W)	18.2	3.20	3:1
7 (C)	23.0	3.50	3:1
8 (SE)	42.0	5.00	2:1
9 (C)	31.6	4.00	2:1
10 (C)	26.5	2.70	4:1
11 (W)	9.0	2.50	4:1
12 (C)	16.0	2.80	4:1
13 (NE)	24.0	4.30	4:1
14 (SE)	20.4	2.60	4:1
15 (C)	18.0	4.40	3:1

- **(W), (N), (C), etc. = western, northern, central, etc., parts of Sudan.**

****numerator approximated to nearest whole number**

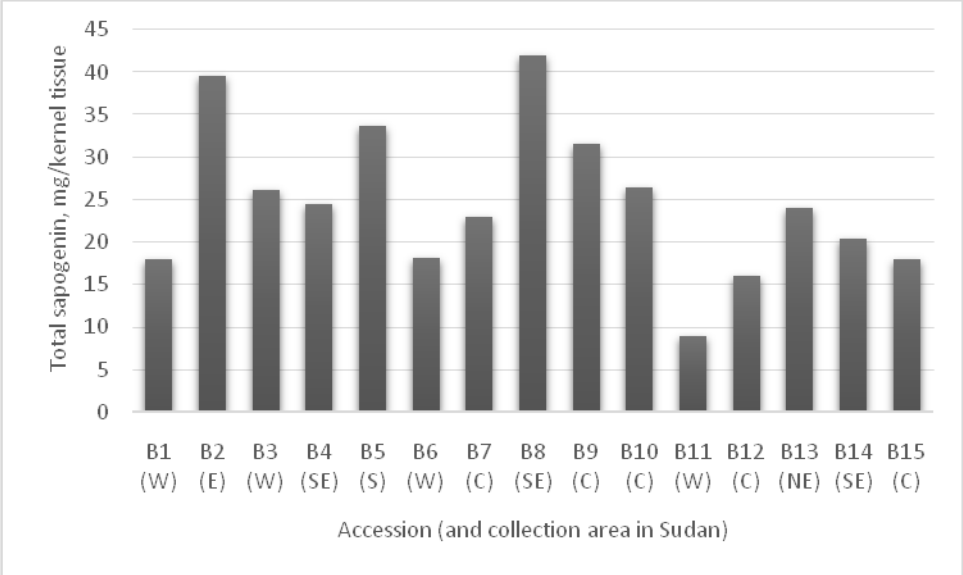

Fig 9: Total sapogenin content of balanites kernels (mg/ kernel) isolated from different Sudanese fruit accessions.B_1, B_2, etc. refer to accession serial numbers of Table 4 ; (W), (E), etc. Indicate locality of collection in Sudan

As a percentage of kernel tissue dry weight the total sapogenin value was close to 3 % in most accessions, with five accessions reaching 4.0 % or more.

Table 4 again shows that 25α- sapogenins were predominant over the 25β- epimers in kernels of all fifteen accessions analyzed, the ratio pointing to a four-fold predominance of sapogenins of the diosgenin-type.

Diosgenin is an important precursor stillin demand by the pharmaceutical industry [12, 35]for the commercial partial-synthesis of steroid drugs such as sex hormones and corticosteroids widely used as anti-inflammatory, androgenic and contraceptive drugs. Although other raw-materials are in commercial use such as hecogenin, the sapogenin obtainable from *Agave* species [10] or phytosterols[35]it was estimatedby Dangi et al, 2014 [11] that two-thirds of annual total world consumption of steroid precursors (*ca.* 3000 tons)came from diosgenin. This dependence on diosgenin as a raw material, in the absence of a feasible total-synthesis method, continued despite some decline in the production of its major source, i.e., tubers of the yam species of *Dioscorea*[28]. The sapogenin (diosgenin) content of species of *Dioscorea* reported in the literature was variable [35]. Ageneral, wide range of diosgenin (plus yamogenin) content of 3-7 % of the weight of mature tubers of *Dioscorea*species was often quoted [11,21]. Other sapogenin (diosgenin)-content ranges, with lower maximum values, were reported. Chen and Wu, 1994 [10] reported a range of1.5 to 3.6 % of the fresh weight of tubers of the major five *Dioscorea* species growing in China, while Yang et al, 2003 [36]quoted diosgenin values of 0.62, 1.42 and 3.48% of the dry weight of three different Indian *Dioscorea* species.

Thus, our results with *Balanitesaegyptiaca*qualifythe fruit kernels of this plant as a commercial source of diosgenin for use as a raw material by the pharmaceutical industry, complementing the role of *Dioscorea* tubers. Balanites kernels had a sapogenin content, mostly diosgenin, of 2.5 to 5.0 % (Table 4), in line with the ranges reported for Dioscorea. The kernels are also rich (~ 40 % of dry weight) in an edible oil that could be recovered prior to sapogenin extraction. We have shown that soxhlet extraction of balanites kernel powder with hexane results in a sapogenin-free oil fraction. The cake powder left is therefore as good a source of sapogenins as the whole kernel.

Sudan could provide significant quantities of balanites fruits, enough to sustain a diversified industrial exploitation. Although kernel weight is only one-tenth of that of the whole fruit (Table 1),it had been estimated that 400 000 tons of balanites fruits were annually collected in Sudan and locally marketed for their edible mesocarp[37].An even higher estimate of fruit tonnage possible to produce in Sudan could be arrived at given the report that > 90 million balanites trees grow wild in Sudan [38] and that a fruit yield of 100-150 Kg/ tree/ annumcould be expected[39].

Of course, muchcould be done to future improve yields of fruits and kernels of wild balanites trees, includingnew biotechnological approaches, cultivation and selection breeding, for which an encouragingly wide basis of variability was shown.

CONCLUSIONS

The present study was concerned mainly with steroidal sapogenin distribution in the different morphological parts of mature, ripe fruits of *Balanitesaegyptiaca*. Weight relations of these fruit parts were also reported. Total sapogenin per fruit (~75 mg) was mainly contained in the mesocarp. The fruit kernels, which represent about 10% of fruit weight, contain about one-fourth of the whole fruit's total sapogenin. However, when results of total sapogenin content were exprssedas percentages of tissue weight, kernel tissue was found to be very rich in sapogenins, particularly of the 25α- (diosgenin) type. It was suggested that balanites whole kernels or defatted kernel powder could be commercially exploited as a sourceof diosgenin, augmenting the current commercial source (*Dioscorea* tubers). Sudan is capable of producing large amounts of balanites fruit material, enough to sustain an agro-industry based on balanites kernels and other fruit parts.

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