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Full Length Research Paper

Variation in diosgenin level in seed kernels among different provenances of *Balanites aegyptiaca* Del (Zygophyllaceae) and its correlation with oil content

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Balanites aegyptiaca **(Zygopyllaceae) is a widely grown desert plant with multi-use potential. It is found in most of the African continent, the Middle East, and South Asia; however, this plant remains one of the most neglected plant species. Its seed kernel is used for oil extraction and the oil is used for human consumption and cosmetics. However, the oil cake is regarded as unsuitable for feeding because of the presence of many toxic substances. In this study, a spectrophotometric determination of diosgenin level and subsequent oil percentage analyses were carried out using the seed kernels of *B. aegyptiaca* collected from five Israeli provenances (Bet -Shean, Ein -gedi, Sapir, Samar, and Eilat) and five international locations (Burkina Faso, Senegal, Mali, Niger, and India). The results suggested that the sample from the Bet Shean Valley, which is considered the northern- most latitude where *B. aegyptiaca* naturally grows, contained the highest level of diosgenin as well as oil percentage; the Indian sample contained the lowest levels of both diosgenin and oil. The result also showed that there is a strong positive correlation (R^2 = 0.849) between diosgenin level and oil percentage in the *B. aegyptiaca* seed kernel.**

Key words: *Balanites aegyptiaca*, provenances, kernel cake, diosgenin, oil content.

INTRODUCTION

Diosgenin is a steroidal sapogenin compound (Figure 1) that is very useful in pharmaceutical industries as a natural source of steroidal hormones. Diosgenin is found in a few higher plant species and interest in its medicinal properties has increased recently (Liu et al., 2005). Recent studies have found that diosgenin can be absorbed through the gut and plays an important role in the control of cholesterol metabolism (Roman et al., 1995). Other authors have reported that it has estrogenic effects (Aradhana et al., 1992) and antitumor activity (Moalic et al., 2001; Corbiere et al., 2003). Studies have also revealed that diosgenin produces changes in the lipoxygenase activity of human erythroleukemia cells and is responsible for morphological and biochemical changes in megakaryocyte cells (Beneytont et al., 1995; Nappez et al., 1995). Furthermore, diosgenin was found to be the most effective cell death inducer compared to the other two plant steroids (hecogenin and tigogenin) in the human osteosarcoma 1547 cell line (Corbiere et al., 2003). Diosgenin is generally used as starting material for partial synthesis of oral contraceptives, sex hormones, and other steroids (Zenk, 1978). The partial synthesis of steroids from plant-based precursors has been a boon because of the increasing demand for corticosteroids, contraceptives, sex hormones, and anabolic steroids since about 1960 (Hall and Walker, 1991). To date, diosgenin and related steroidal saponins were commercially obtained from the tubers of various *Dioscorea* species; however, it is crucial to discover new

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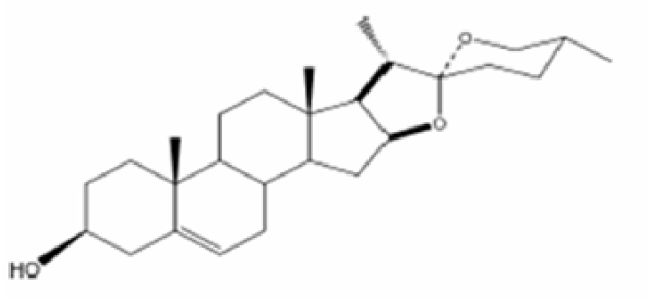

Figure 1. Chemical structure of diosgenin.

and alternative sources of these compounds due to decreasing plant resources as well as increasing demand (Savikin-Foduloic et al., 1998).

Balanites aegyptiaca Del (Zygophyllaceae), popularly called the 'desert date,' is a highly drought-tolerant evergreen desert plant species. It is widely grown in the Sudano-Sahielian region of Africa, the Middle East, and South Asia (Hall and Walker, 1991). *B*. *aegyptiaca* plant tissues have been used as various folk medicines in Africa and Asia. Although this plant has various uses from ethnobotanical to fire wood, it is considered one the most neglected tree species in arid regions (Hall and Walker, 1991). The fruit consists of epicarp (5–9%), mesocarp (28–33%), endocarp (49–54%), and kernel (8–12%) . The kernel of *B. aegyptiaca* fruit is rich in edible oil used by local people (Newinger, 1996), however, the oil cake is regarded as unsuitable for feeding because of the presence of many toxic substances. Studies have shown that *B. aegyptiaca* plant tissues contain steroidal saponins primarily with diosgenin or its isomer yamogenin as a sapogenin (Neuwinger, 1996). In a detailed examination, Hardman and Sofwora(1972) have reported 2% diosgenin in seed kernels from the West African sample. Ognyanov et al. (1977) have reported 3% sapogenin in the fruit mesocarp and 2% in seed kernels from the East African sample. Desai et al. (1978) have reported 0.994% diosgenin from fruit pericarp, 0.94% from seed kernels, and 0.45% from the oil in the Indian sample.

Literature survey revealed few studies about the diosgenin analyses in various *B*. *aegyptiaca* tissues with samples mainly from African and Indian provenances, but no study so far has been reported from samples of Israeli provenances. Israel is one of the native homelands of *B. aegyptiaca;* the Bet-Shean Valley (35° 25′ N) area in particular, is considered to be the northern-most latitude where *B. aegyptiaca* grows naturally (Zohary, 1973).

Considering the high market potential of diosgenin and vivid reports of diosgenin level in *B. aegyptiaca* kernels, and lack of data for samples with Israeli provenances, this study was conducted to seek the provenances with higher diosgenin content in seed kernels. We believe that identifying provenances of *B. aegyptiaca* with high levels of diosgenin might help to make this neglected plant species economically competitive with tubers of Dioscorea, the traditional source of diosgenin used for the synthesis of steroid drugs, and ultimately help the domestication process of *B*. *aegyptiaca*.

MATERIALS AND METHODS

Sample collection

Ripe fruits were collected from *B*. *aegyptiaca* the trees grown in five areas of Israel namely: Bet-Shean Valley (close to the Sea of Galilee), Ein-Gedi (close to the Dead Sea), Sapir (Central Arava Valley), Samar (South Arava Valley), and Eilat (close to the Red sea), and authenticated by Prof. Uzi Plitman from the herbarium in the Hebrew University of Jerusalem. Voucher specimen (76816) was deposited in the herbarium of the Hebrew University of Jerusalem. Five other samples were obtained from India (Jodhpur), Mali (Bamako), Niger (Zinder), Senegal (Dakkar), and Burkina-Faso (Ouaga). All non-Israeli samples were collected from street vendors.

The epicarp (outer cover) and mesocarp (pulp) of the fruits were removed by hand and the nuts were washed with tap water. After washing, the nuts were oven dried at 70ºC for 72 h. Decortications of the nuts were carried out by hand and released the kernel (approximately 10% of the total fresh weight). The kernel to stone ratio was 30:70 by weight. The kernels were ground in a mortar and pestle using liquid nitrogen.

Oil extraction

Oil percentage was determined gravimetrically by dividing extracted oil weight by kernel weight after solvent evaporation (IUPAC, 1979; AOAC, 1996). In brief, 3 g of pulverized kernel powder were placed in a plastic tube (50 ml capacity, centrifugable grade) and 30 ml n-hexane was added. The tubes were left over-night in an electric shaker (Z. Tuttnauer Ltd., Jerusalem) at high speed, followed by centrifuging at 3500 rpm, 18 min., 20^{o}C (Hermble, Germany), and collecting the supernatant. Two subsequent extractions were carried out on the residue using only vortexes and centrifuging. After three successive extractions, the supernatant was clear; all the supernatants were collected, the n-hexane was evaporated using a rotary evaporator (Heta-Holten A/S, Allerod, Denmark), and the oil collected. Each sample was repeated thrice.

Saponin extraction

The defatted cake (residue after oil extraction) was kept under the hood overnight. The next day, 30 ml methanol was added to the tubes and left on the shaker overnight, followed by centrifugation. The second and third extractions by methanol were also carried out as with n-hexane. At the end, all supernatants of methanol extracts were pooled and the methanol was evaporated using a rotary evaporator. Finally, a yellowish crystal powder of crude saponins was obtained (approximately 12.2% kernel by weight).

Diosgenin determination

Diosgenin was determined as described by Baccou et al. (1977) and Uematsu et al. (2000), with some modification. Standard

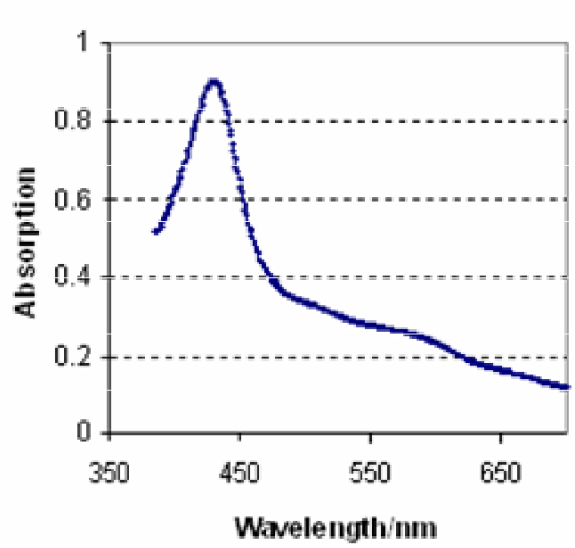

Figure 2. Absorption spectrum of diosgenin.

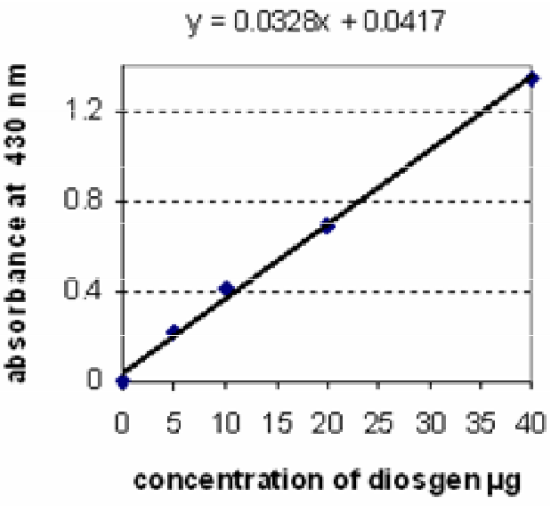

Figure 3. Calibration curve for the determination of diosgenin.

sapogenin (diosgenin) and *p*-anisaldehyde (4-methoxybenzaldehyde) were purchased from Sigma. Sulfuric acid and ethyl acetate were both analytical grade and obtained from Frutarom. The diosgenin level was determined by measuring absorbance at 430 nm (Figure 2), based on the color reaction with anisaldehyde, sulfuric acid and ethyl acetate. In brief, two color developing reagent solutions were prepared: (A) 0.5 ml *p*-anisaldehyde and 99. 5 ml ethyl acetate, and (B) 50 ml concentrated sulfuric acid and 50 ml ethyl acetate. 200 µg of the methanol extract of defatted kernel was placed in a glass tube. To this, 1 mg of defatted methanol extract of kernel powder was first dissolved in 1 ml methanol, and 200 µl of this solution was placed in another tube; the methanol was evaporated under reduced pressure. This residue was dissolved in 2 ml of ethyl acetate; 1 ml each of reagents A and B were added to the tube and stirred. The test tube was placed in a water bath maintained at 60ºC for 10 min to develop color, then allowed to cool for 10 min in 25ºC water bath. The absorbance of the color developed solution was measured in a spectrophotometer (V- 530- UV/VIS, JASCO Corp., Japan). Ethyl acetate was used as a control for the measurement of absorbance. As a reagent blank, 2 ml ethyl acetate was placed in a tube and assayed in similar manner. For the calibration curve, 2–40 µg standard diosgenin in 2 ml ethyl acetate was used (Figure 3). Each sample was repeated thrice and the average was taken.

Statistics

Data were statistically analyzed with JMP software (SAS, 2000), using the Tukey- Kramer HSD test for determining significant differences among treatments at *p* 0.05.

RESULTS AND DISCUSSION

Since first isolated from *Dioscorea tokoro* in the 1930s (Yang, 1981), diosgenin, a plant steroid (5 -spirostan-3 -ol) has been used for various steroidal drugs. Steroidal drugs are considered to be some of the costliest and most important medicines used throughout the world today. With recent reports of diosgenin's function in inducing differentiation of erythroleukemia through changing lipoxygenase activities (Beneytout et al., 1995) and inducing apotosis and cell cycle arrest in the human oesterosascoma 1547 cell line (Moalic et al., 2001), the value of diosgenin has further increased. Until now, *Dioscorea* has been the main source of diosgenin, although there are many plant species that contain diosgenin. However, it is now crucial to find alternate source for diosgenin production.

There have been reports that most of the *B. aegyptiaca* plant tissue, including the seed kernel, the fruit, and oil contain steroidal saponins. The highest level of diosgenin is found in the fruit mesocarp; however, the fruit is edible and used for various purposes such as juice preparation and even as a preparation for alcoholic beverages. There is a little trade in kernel oil however, there almost no use is made of its cake. This study was basically focused on the diosgenin in *B. aegyptiaca* seed kernels. The main objective of this study was to check the diosgenin level in *B. aegyptiaca* grown in various provenances in Israel and abroad.

Figure 4 clearly shows that there is a large variation in diosgenin percent among the 10 provenances that were analyzed in this study. The highest level of diosgenin was obtained in the sample from the Bet-Shean Valley (2.22%), the lowest was in the sample from India (1.09%). The comparatively small error bars (± standard deviation) in the figure show that there was less variation within the samples. When we see the percent oil recovery from the seed kernel (Figure 4 bottom), a similar pattern was found i.e., as with diosgenin, the highest oil recovery was obtained from the Bet- Shean sample (50.22%) and the lowest was again in the Indian sample (39.20%).

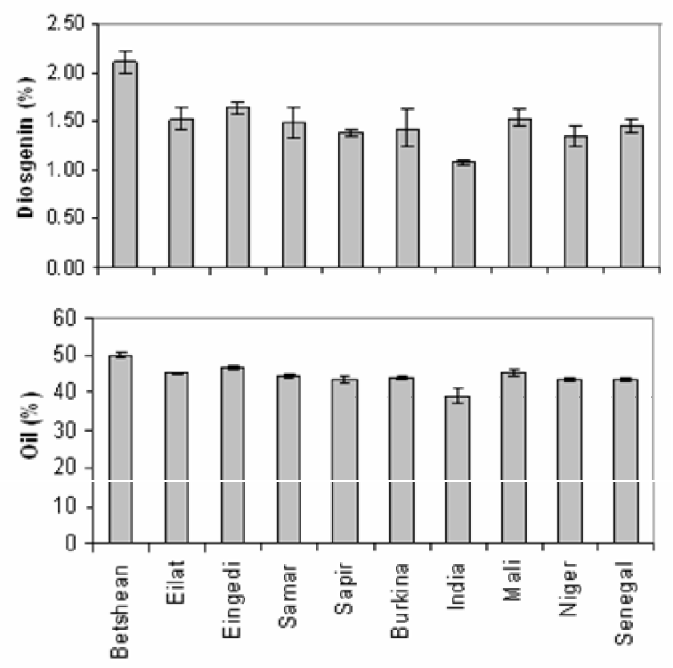

Figure 4. Percent diosgenin and oil content found in *B. aegyptiaca* kernel from the different provenances (on dry weight basis).
Top: % diosgenin; Bottom: % oil. The first five provenances were from Israel. Error bars illustrate standard deviation from triplicate analysis of each sample.

Table 1. Total sapogenin (diosgenin and yamogenin) and oil recovery of the *B. aegyptiaca* seed kernel from different regions. The total sapogenin value was calculated as the spectrometric value of the diosgenin.

Region	Sapogenin (%)	Oil recovery (%)
Israel (combined)	2.12 b	46.12 b
Israel (Bet-Shean only)	2.74 a	50.22 a
Africa (combined)	1.87 b	44.17 b
India	1.41 c	39.20 c

Combined value is the mean value of all samples in that region. Different letters in each column after the mean value are significantly different at 5% level of significance according to Tukey-Cramer HSD.

Actually, the total sapogenin in the *B. aegyptiaca* seed kernel in the analyzed samples would be higher because *B. aegyptiaca* also contains yamogenin, an epimer of diosgenin, and of equal utility (Fazil and Hardman, 1971). In *B. aegyptiaca* seed kernels, an approximate ratio of diosgenin to yamogenin of 77:23 has been reported (Abu-Al-Futuh, 1983). Since a pure sample of yamogenin is unavailable, it will be reasonable to calculate the amount of total sapogenin using the ratio (Taylor et al., 2002). So total sapogenin comprised of both diosgenin and yamogenin, can be 30% higher (Table 1). In Table 1,

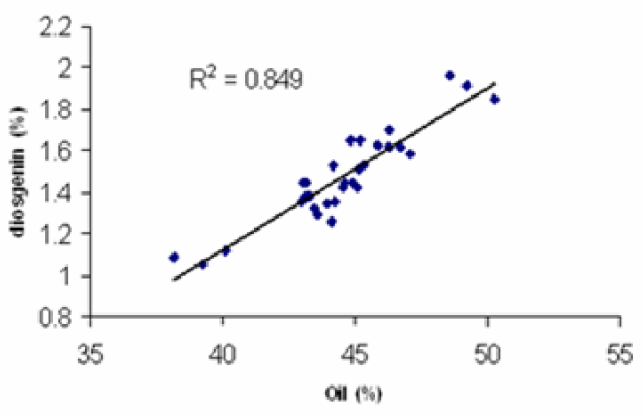

Figure 5. Relationship between oil and diosgenin content in the Balanites seed kernel (n=30). Values are taken from all 10 provenances.

the total sapogenin as well as oil recovery values are presented on a regional basis. From this table we can clearly see that the Bet-Shean sample has a significantly higher level of both sapogenin and oil content compared to Israel (all Israeli samples combined), Africa (all African samples combined), and the Indian sample. Similarly, the Indian sample had significantly lower levels of both sapogenin and oil content, among all regions. However there was no significant difference between combined Israeli and African samples in their sapogenin and oil content. Earlier studies have reported 2% sapogenin in *B. aegyptiaca* seed kernel (Hardman and Sofowora, 1972; Ognyanov et al., 1977) in African *B. aegyptiaca* samples, which is very close to the results of this study when we calculated combined results for Africa and Israel. In this study, we found a slightly higher level of total sapogenin in Indian samples than the earlier report of 0.99% (Desai et al., 1978). However, the Bet-Shean provenance itself shows the highest level of sapogenin (2.74%) so far reported. The differences in the sapogenin content in the African and Indian seed kernels might be from the different determination procedures used.

Interestingly, we found a positive correlation (R^2 = 0.849) between the diosgenin content in the seed kernel and oil content (Figure 5). Since both these parameters are desirable characteristics, these findings may play a vital role during germplasm selection for the domestication of *B. aegyptiaca*. However, this rule didn not follow for diosgenin content in fruit mesocarp and kernel seed oil content. In fact, there was a very weak negative correlation (R^2=0.3078) between the diosgenin content in the fruit mesocap and kernel seed oil content (results not shown).

Although *Dioscorea* root contains a higher level of sapogenin (3–7% combined diosgenin and yamogenin) especially in its mature stage, it may take up to 3 years to mature (Savikin-Fodulovic et al., 1998). In this

circumstance, 1.08–2.2% diosgenin (equivalent to 1.41–2.8% sapogenin) content in *B. aegyptiaca* seed kernels is a considerable amount, especially from the neglected by-product of the seed kernel. One estimate shows that more than 400,000 tons of *B. aegyptiaca* fruits are produced in the Sudan annually (Mohamed et al., 2002) and a significant quantity of this is used for oil extraction; so thousands kilograms of diosgenin can be extracted from this production alone. The seed kernel of *B. aegyptiaca* would be an alternate for diosgenin extraction in the global market. Furthermore, *B. aegyptiaca* selected from provenances like Bet-Shean, which contain high percentages of both oil and diosgenin, could be a resource for germplasm for the future. Furthermore,
a nine-year-long trial of *B. aegyptiaca* in the Kibbutz Samar in Israel, clearly shows that *B. aegyptiaca* can easily be grown, even in hyper-arid desert conditions with minimum irrigation (data not yet published).

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