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The Concentration Variations of Chemical Constituents Present In Balanites Aegyptiaca Fruit Collected From Different Parts of India: A Study

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ABSTRACT

The objective of the study was to investigate the effect of fruit shape and location on the chemical constituents (Sugars, saponins, protein, oil and moisture content) of *Balanites aegyptiaca* (L.) Del. fruit. *Balanites aegyptiaca* mature healthy fruits, were collected from Jhunjhanu Province in Rajasthan, and Ambala Province in Punjab, in the period (2003-2005). The percentages of the fruit different parts were determined and chemical and statistical analysis (SAS) methods were carried out to obtain the percentages of sugar, saponin, protein, oil and moisture contents in the Balanites' fruit as well as to detect the significant variation between shapes and locations. The study found that, there were significant differences ($P > 0.05$), between the four fruit shapes (oblong, elongate, spherical and oval fruits) in the studied chemical constituents across and within sites and between locations. The highest saponin content (4%) was obtained by oblong fruits while the highest invert sugar (40.9%) and oil content (44.9%) were obtained by oval fruits. Saponin average percentage obtained in Ambala location was double that obtained in Jhunjhanu; the higher sugar percentage was obtained in Jhunjhanu location. Protein content was found to be of higher content than that reported in the literature; it ranged between 49.6 (oval fruit) and 53.8% (oblong fruit). Moisture content was not significantly different between shapes and locations of a mean 9.3%. The study concluded with that, the amounts of sugar, saponin, oil and protein, in Balanites fruit; were found in considerable percentages calling to introduce Balanites different types to the food, pharmaceutical and medicinal industries.

Keywords: *Balanites aegyptiaca*, chemical constituents, saponin

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INTRODUCTION

Balanites aegyptiaca (L.) Del.¹ of the Family Balanitaceae is a small to medium size tree that varies in height from 7m² to 15 m and in diameter from 30 to 50 cm³. It is an ever green tree⁴. Flowering is in November and April^{3, 5}. Fruiting is in December and January and from March to July.

The tree produces about 125 kg of ripe fruit, which resembles date fruit in size and appearance⁶. The fruit is drupe, oblong, elliptic, olive or egg-shaped, about 2.5-4.0 cm long and 1.5-2.5 cm in diameter^{3, 5, 7}. It is green and shortly velvety when young, turning yellow and glabrous when ripe and of stringent and bittersweet mesocarp.

Balanites aegyptiaca is widely spread throughout India, absent only where rainfall is over about 1100 mm. It usually grows in sand, clay, cracking clay and gravel soils. It occurs in pure stands or associated with *Acacia seyal*.⁴ The deep-rooted trees of this species can live for more than 100 years⁶.

Balanites aegyptiaca wood is used for furniture and firewood. Seeds are used for rosary beads, necklaces and in the game of 'seiga' played in India. Flowers and leaves are used as food in Africa. The fruit is edible and the maceration of the fruit is used against constipation and as anti-diabetic². The sapogenin, yamogenin and diosogenin, which are extracted from all parts of the plant, are used in partial synthesis of steroidal drugs⁸. A soap substitute is locally made of saponin extracted from the root, fruit and barks and it is used in washing clothes⁹. This tree is of significance value for its non timber forest products (NTFP). Taking into account the great potentiality of this endangered tree and the great role it could be played in accordance to the climate change mitigation option, as it can be one of the proposed forest trees for carbon sequestration within the REDD+ mechanism, as well as enhancing communities livelihood, by providing some valuable non timber forest products (NTFP) from the fruit.

MATERIALS AND METHOD

Location of study area & Materials used:

Balanites aegyptiaca mature healthy fruits were collected from two different locations, namely, Jhunjhanu and Ambala Provinces in India. The identification and authentication of plant was done at Institute of Pharmacy, SAGE University Indore and the herbarium file no HB/012/022 submitted in the Department of Pharmacognosy, Institute of Pharmacy, SAGE University Indore M.P. for the reference.

Instruments used were:

- Vernier scale (digital)

- Metler P1200 balance
- House field Tensometer machine, type W 2 tons capacity. Maximum rate of loading 500 kgm
- Spectrometer model 6405 uv1vis (JENWAY) nm (544) (wave length)

Chemicals used for the study, were the following solvents:

- Fehling solution (A) consists of hydrous copper sulphate (blue color).
- Fehling solution (B) consists of sodium-potassium tartrate, 346 gm in 100 gm of caustic soda, in one liter of distilled water (white color).
- Saturated sodium hydroxide for neutralization; two indicators methyl blue as redox indicator, for assessing the end point; phenolphthalein white and concentrated hydrochloric acid.
- Catalyst mixture 8g as a tablet (96% anhydrous sodium sulfate, 3.5% copper sulfate and 0.5% selenium dioxide), concentrated sulfuric acid, boric acid, hydrochloric acid, sodium hydroxide and methyl red indicator.
- Petroleum ether 40-60 °C boiling point as a solvent.
- N-butanol, methanol, diethyl ether, anhydrous sodium sulfate, 8% (w/v) vanillin solution: 800gm of vanillin (reagent grade) dissolved in 10ml of ethanol (99.5% v/v, reagent grade), prepared freshly, 72 % (v/v) sulfuric acid: to 28 ml of deionized water, 72 ml of concentrated sulfuric acid (reagent grade, 95%w/w).

Determination of fruit composition:

The fruit consists of four main parts: The outer shell (epicarp), the fleshy pulp (mesocarp), the endocarp (the hard stone) and the kernel embedded in the hard stone. Ten mature healthy fruits, were taken from each fruit shape (oblong, spherical, elongate and oval shape as in figure 1) per location and the weight, diameter, and length of the fruit with epicarp (whole fruit) were found, then the epicarp was removed manually to find out diameter and weight of the mesocarp (fruit without epicarp), then the mesocarp was removed by dissolving in water, air dried for two days to find out diameter, length and weight for endocarp (fruit without epicarp and mesocarp) which had been broken by machine, to release the kernel, and to determine the breaking force. Lastly kernel was weighed out, and the following percentages were determined:

Percent of epicarp = $(TW - MW) \times 100 / TW$

Percent of mesocarp = $(MW - EW) \times 100 / TW$

Percent of endocarp = $(EW - \text{Kernel weight}) \times 100 / TW$ 4-Percent of kernel = $(\text{Kernel weight} / TW) \times 100$

Where:

TW: Total weight of the fruit.

MW: weight of fruit without epicarp.

EW: Weight of fruit without epicarp and mesocarp.



Figure 1: Shows the different shapes of *Balanites aegyptiaca* fruit (Spherical, oval, elongate & oblong shape)

Chemical analysis

The objective was to identify the percentage of the major chemical constituents, namely sugars, oil & protein and moisture content of *Balanites aegyptiaca* (L.) Del fruit of the different shapes in Jhunjhanu and Ambala Provinces.

Sugars determination:

Sugars were determined according to the volumetric method of Lane and Eynon¹⁰. Three types of sugar were determined: Invert sugars fructose and glucose namely reducing sugars. Total sugars (invert sugars plus sucrose). Sucrose percentage was determined as the difference between total sugars and invert sugars.

Protein determination:

Semi-micro Kjeldal method¹⁰ was used for protein determination. It included two steps; firstly, digestion of the powdered matter (0.2 g of the defatted powdered kernel for each sample) then distillation using sodium hydroxide and boric acid. Then titration with with 0.1 normality hydrochloric acid.

Oil extraction and determination:

Oil was determined in the samples (The powdered kernel, of about 20 fruits of each fruit shape) by extraction of the dried material using petroleum spirit in a continuous extraction apparatus soxhlet

type (Pearson 1970)¹⁰. Then distillation and calculation of the oil percentage was done.

Moisture content determination:

The moisture content (MC) was calculated as the loss in weight after drying.

Saponin extraction and determination:

In this study saponin was extracted and purified according to the adapted method¹¹. Saponin (diosogenin) was extracted from the mesocarp and purified to plot a calibration. Absorbance of the solutions of the different fruit shapes was determined by a colorimetric method¹².

Determination of diosogenin content in the mesocarp of different fruit shapes:

Three fruits per each sample were weighed out; the epicarp was removed, and then dissolved in distilled water till the mesocarp completely dissolved. The volume was completed to 500 ml. Then it was filtered using a Whatmann filter paper no. 41. Two ml of each sample were diluted with two ml distilled water.

Then to 0.5 ml of each sample 0.5 ml of vanillin 8% solution in ethanol, then 5ml of 72% sulfuric acid were added, and the method continued as described previously. After recording the samples, absorbance from the spectrometer at (544) nm (wave length), the concentration for each sample was calculated from the standard curve, according to the equation: $Y = 0.0295X - 0.0094$, where Y is the sample concentration and X is the absorbance of the fruit samples. Then saponin (diosogenin) content in Balanites different fruit shapes was determined as follows:

Saponin (diosogenin) % = $\text{Concentration} \times (500 \times 2) \times 100 / \text{sample weight}$ Where:

(500 x 2) = sample volume x dilution factor. All data was analyzed by SAS statistical method, using ANOVA to determine the means of significant differences.

RESULTS AND DISCUSSION

Fruit composition

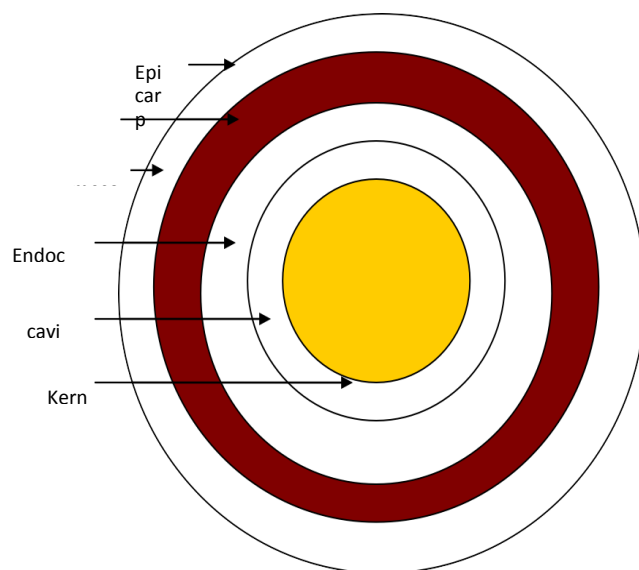


Figure 2: Shows A cross section diagram explaining Balanites fruit's layers

The study found that, the fruit consists of four main parts: The outer shell (epicarp), the fleshy pulp (mesocarp), the endocarp (the hard stone) and the kernel embedded in the hard stone, as shown in the below figure 2:

Fruit chemical constituents

Chemical constituents determined in this study are found in mesocarp and kernel parts of *Balanites aegyptiaca* fruit.

Effect of Balanites aegyptiaca fruit shape on fruit mesocarp chemical constituents:

Oval fruits had significantly the highest value in mesocarp invert sugars (Table 1); followed by spherical and oblong fruits, which were not significantly different from each other. A significantly lowest average percentage of invert sugar was obtained, by elongate fruit (32.5%). The overall mean was 38.88 % and it is in the range of the values given by ^{13,14}: but it is lowest in comparison with the value of 56% given by ¹⁵, and it is relatively higher than the value reported by, ¹⁶ who found that the total value for invert sugars was about 30.7% (20.6% D- glucose and 10.1% D-fructose on dry-base).

Total sugar of the mesocarp was significantly different between shapes and locations with a mean of 42.83% and the higher average percentage of total sugar in mesocarp was obtained by elongate fruit (44.6) and it was greater than the value of 36.5% reported by ¹⁶ and at the same time it was considerably lower than the value of 61% given by ¹⁷ Mesocarp sucrose percentage was significantly different between shapes and locations and the highest value of mesocarp sucrose was obtained from elongate fruit (Table 1), which was significantly different from the other fruit shapes, while oval fruits was significantly different and obtained the least amount of sucrose (3.25%). The average of sucrose (5.83%) is quite agreeable with the percentage of 5.8% that had been reported by ¹⁶. However, *Balanites aegyptiaca* fruits can reasonably be introduced to the beverage and other sugar syrup using industries. The low sucrose content in any sugar syrups is demanded for diet.

Oblong fruit had the highest average percentage in mesocarp saponin and it was significantly greater than the other three shapes, almost double the percentage of spherical ones. There were no significant differences between spherical, elongate and oval fruits in saponin average percentage (Table 1). Mesocarp saponin was significantly different between shapes and location with a mean of 2.95 %, which agreed with that (2.5%w/w) reported by ⁺. Others reported that total saponin content was found to be 7.2% in the mesocarp and 6.7 in the kernel ¹⁸. This result and the obtained results may be regarded as considerable percentages for such valuable compound to contribute in the steroidal industries as intermediate products in sex hormones synthesis, curing cancer,

cholesterols and other pharmaceutical industries. ¹⁹ reported that the steroidal diosogenin from Indiaese Hejlj, showed a relatively high value of nearly 1.26% dry weight in comparison with reports of 0.3% for an Egyptian source. This higher content of *Balanites aegyptiaca* diosogenin grown in India should contribute to India national income; as²⁰ reported that India can supply half of the world consumption of diosgenin and selling this product at 33% of the prevailing world price, it can generate about 36 million USD per year. ¹¹ reported that about 98.95 of high purity saponin content in mesocarp of *Balanites aegyptiaca* can be extracted by foam method and suitable to be used in pharmaceutical industry. However the overall earning from the chemical products (saponin which is shown in figure 3, protein, sugars and oil, shown in figure 4) makes a total of 80 million USD per year as was estimated by ²¹ which is a considerable amount to enhance the contribution of *Balanites aegyptiaca* different types to the national income through establishment of multipurpose factories concerning with *Balanites aegyptiaca* based industries.

Table 1: Effect of *Balanites aegyptiaca* (L.) Del fruit shape on fruit mesocarp chemical constituents

Shape	Invert sugar/ Mesocarp	Total sugar/ mesocarp	Sucrose/ Mesocarp	Saponin/ mesocarp
Oblong	35.5 AB	39.7 A	4.2 B	4.1 A
Elongate	32.5 B	44.6 A	12.08 A	2.5 B
Spherical	37.2 AB	43.7 A	5.9 B	2.2 B
Oval	40.9 A	44.2 A	3.25 B	2.6 B

Means with similar letters, in the same column are not significantly different (P =0.05).

Effect of location on *Balanites aegyptiaca* fruit mesocarp chemical constituents: Significant differences were found between locations, where P=0.05.

Jhunjhanu had the highest sugar content while Ambala had the highest saponin content (double the percentage that obtained in Jhunjhanu location (Table 2).

Table 2: Effect of location on *Balanites aegyptiaca*, fruit mesocarp chemical constituents

Location	Invert sugar	Total sugar	Sucrose	Saponin
Jhunjhanu	38.4 A	45.3 A	6.7 A	2.1 B
Ambala	34.5 B	39.1 B	4.5 B	4.2 A

Means with similar letters, in the same column are not significantly different (P =0.05).

Effect of *Balanites aegyptiaca* fruit shape on its kernel chemical constituents: Moisture content was not significantly different between shapes with a mean of 9.3%, as shown in table 3, and it is in agreement with 10.5% reported by ¹⁶.

Kernel oil was not significantly different between locations and significantly different between shapes with a mean of 41.7% and varied from 38.55% obtained by oblong fruits to 45.3% obtained

by Spherical ones, which is in the range of the values of 46.8% reported by ⁷, 45% ²² and 44-51% reported by ²³ As balanites oil is edible, highly prized by local ¹⁵, it can be used in food and medicine industries, at larger scales.

Protein was determined in the defatted kernel after oil extraction. It ranges between 49.65% (oval fruit) and 53.8% (oblong fruit), with a mean of 52.11% and was significantly different between shape (Table 3). The obtained protein percentages were higher in comparison with 27.5%, 26 to 30%, 32.4% and 38.7 to 41.2% values given by ^{15,20, 24, 25,20} respectively. But still, these protein values obtained in the current study were not considerably different from that range of 49-51% (after oil extraction, before extraction was 27%) given by ²² and 50% reported by ¹⁴. The high protein percentage is an advantage of Balanites kernel cake, which is used for human and animal nutrition.



Figure 3: Shows oil extracted from *Balanites aegyptiaca* Kernel

Table 3: Effect of *Balanites aegyptiaca* fruit shape on its kernel chemical constituents:

Shape	MC	Oil	Protein
Oblong	8.9 A	38.5 B	53.8 A
Elongate	9.8 A	43 AB	52.97 AB
Spherical	9.4 A	45.3 A	52.5 AB
Oval	9.3 A	44.9 A	49.6 B

Means with similar letters, in the same column are not significantly different (P = 0.05).

Effect of location on *Balanites aegyptiaca* fruit kernel chemical constituents:

There were no significant differences between locations in moisture, oil and protein content of fruit kernel (Table 4), where P=0.05.

Table 4: Effect of location on *Balanites aegyptiaca* fruit's kernel chemical constituents:

Location	MC	Protein	Oil
Abu-Jibeiha	9.6 A	52.5 A	40.4 A
Ambala	8.8 A	51.4 A	43.7 A

Means with similar letters, in the same column are not significantly different (P =0.05).

CONCLUSION

The following conclusions are drawn from the study: There was significant difference in fruit mesocarp and kernel chemical constituents between shapes and locations. The highest saponin (4%) was obtained by oblong fruits while the highest invert sugar (40.9%) and oil (44.9%) were obtained by oval fruits. Saponin average percentage obtained in Ambala location represents double that percentage obtained in Jhunjhanu, while the higher sugar percent is obtained in Jhunjhanu. Protein is found to be of higher content than that reported in the literature where it ranged between 49.6(oval fruit) and 53.8% (oblong fruit), with a mean of 52.11%. MC was not significantly different between shapes and locations averaged a mean of 9.3%. The above results of (sugar, saponin, oil and protein), are considerable amounts to introduce our *Balanites* different types to the food and medicinal industries. However the study recommended that:

1. Himachal based industries can be established with regard to oval fruits in food processing (*Balanites* sugar syrups; jam) and oblong ones in saponin extraction for export, as raw material or to be used as intermediate product in sex hormones synthesis and other pharmaceutical industries.
2. Due to the high protein content (52.11%) Hejljij kernel cake can have potential use for human and animal feed.
3. More studies on the relationship between fruit shape, tree location and Hejljij fruits chemical constituents are needed.
4. This multipurpose tree *Balanites aegyptiaca* (L.) Del.is very precious, must be given great attention and care. It deserves intensive protection as natural stand and new more plantations, on purpose of fruit chemical content utilization, to enhance their contribution in the environment conservation and climate change.

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