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## Antioxidant and Antibacterial Activities of *Balanites aegyptiaca delil* from Northern Ethiopia

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### ABSTRACT

Leaves of *Balanites aegyptiaca* have been used in Africa as traditional medicine in the form of juice to treat diarrhea, dysentery, cuts and wounds. So this study is intended to discover the antioxidant and antibacterial activity of *Balanites aegyptiaca* leaves. Leaves were macerated using methanol to get a crude extract. Antioxidant activity of methanol extract was found to be 89.6 % at 1 mg/mL with IC<sub>50</sub> value of 182.02 µg/mL ( $r^2 = 0.9883$ ) using DPPH (2, 2-Diphenyl-1-picryl hydrazyl) free radical scavenging method. In addition, the antibacterial activity of the methanolic leaf extracts was assessed on one gram-positive and one gram-negative bacterium using the disc diffusion assay. Antibacterial activity of methanol extract was higher for *Escherichia coli* with  $8.15 \pm 1.22$  mm zone of inhibition and for *Staphylococcus aureus* methanol extract was not effective with the zone of inhibition of  $0.00 \pm 0.00$  mm. The methanolic leaves extract of *B. aegyptiaca* revealed potential antioxidant activity but antibacterial activity was found to be relatively lower to that of the standard antibiotics used.

**Keywords:** *Balanites aegyptiaca*, DPPH, antioxidant activity and antibacterial activity.

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## INTRODUCTION

The plant has up to 10 m high and is a multi branched tree <sup>1,2</sup>. The name *Balanites* (from the Greek for acorn, referring to the fruit) was given in 1813 by Alire Delile and replaced Agialid (derived from the Arabic name for the tree 'heglig') <sup>3</sup>.

*Balanites aegyptiaca* is spiny branches, highly drought tolerant evergreen plant, a dicotyledonous flowering species, belongs to Zygophyllaceae family popularly named as the 'desert date' and Bedena in Amharic. It is the one of the furthestmost neglected common tree, usually found throughout in dried regions of Africa, the Middle East, India and Burma <sup>6,7</sup>. And also *B. aegyptiaca* is one of the main tree species purposely reserved on farmers' land in the Tigray region of Ethiopia <sup>4</sup>.

The plant possesses the botanical name of *Balanites aegyptiaca* and is assigned to the following scientific plant classification <sup>5</sup>.

Kingdom: Plantae

Subkingdom: Tracheobionta

Super division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Sapindales

Family: Zygophyllaceae

Genus: *Balanites Delile*

Species: *Balanites aegyptiaca (L.) Delile*

The tree has bushy, tough leaves, and a double root system, and produces date-like fruits. It is also a thorny species which has 2.5-3.5 cm lengthy thorns, the diameter of its flowers is 5-6 mm and greenish white odorous <sup>6-9</sup>.

*Balanites aegyptiaca* fresh fruits contain 35% a glycoside pulp, 45% nut and 25% kernel <sup>4</sup>. The kernel contains about 45 % oil. *Balanites aegyptiaca* trees frequently live and stay productive for dozen of years. Current studies on the productivity of irrigated *Balanites aegyptiaca* trees, evidently showed that the productivity of low quality incompletely purified waste salted water were significantly increased up to 15,000 kg per hectare <sup>8-10</sup>.

Different part of *Balanites aegyptiaca* contains protein, lipid, carbohydrate, saponin, flavonoid, organic fatty acid, tannins and glycoside alkaloid, steroids and anthraquinone <sup>11-14</sup>.

Almost all the parts of *Balanites aegyptiaca* plant are traditionally used in several folk medicines. In the Sahara region of Africa, the fruits are used as oral hypoglycemic drug <sup>15</sup>. While the stem, root and leaf extracts of *Balanites aegyptiaca* have commonly been used as various traditional folk medicines especially in Africa and southern Asia <sup>16, 17</sup>. The fruits are also commonly used as purgative, antiparasitic and schistosomicide <sup>16</sup>.

A literature study has shown anthelmintic, antivenin, anticancer, antioxidant, mosquito larvicidal, anti-inflammatory, antidiabetic, wound healing, hepatoprotective, antiviral, hypocholesterolemic, diuretic & contraceptive activities in various parts of *Balanites* extracts <sup>13-17</sup>.

## MATERIALS AND METHOD

### Plant Sample Collection and Identification

Fresh leaves of *Balanites aegyptiaca delile* were collected from Sefeho around the town of Axum in the Tigray People Region, 275 km north-western of Mekelle in January 2013. The plant was botanically authenticated in the Addis Abeba University and voucher specimen was deposited at National Herbarium, Department of Biology, Addis Ababa University.

After the washed leaves of *Balanites aegyptiaca* were shade dried at room temperature for 5 days, Dried powdered leaves (300 g) of *Balanites aegyptiaca* were extracted using one liter methanol at room temperature for three days by maceration and then the obtained extract was filtered using Whatman filter paper. The second successive extraction was performed using recycled methanol on the residue left, following formerly mentioned technique to get the methanolic extract. The obtained methanolic extracts were concentrated in rotary evaporator and dried in a vacuum oven so as to get thick, viscous mass.

### Biological Activities of Crude Extract

#### Free Radical Scavenging Activity using Spectrometric Method

Two milliliter of 0.04% DPPH methanolic solution was added to two milliliter of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 50 µg/mL and 25 µg/mL of sample solution. Different concentrations and mixed thoroughly in clean and dried test tubes. Also the same concentration of ascorbic acid was prepared separately as an extracted sample. Both leaves extract and ascorbic acid samples were incubated for 30 min at room temperature (23 °C) in the dark; the absorbance of the mixture in the samples was recorded using a UV-Visible spectrophotometer at 517 nm against methanol as blank <sup>18-20</sup>.

The percentage radical scavenging activity (RSA) was calculated using the following equation <sup>21</sup>.

$$\% \text{ Radical scavenging activity RSA} = [(A_0 - A_1)/A_0] \times 100$$

Where;

$A_0$  is the absorbance of the control, and

$A_1$  is the absorbance of samples after 30 min

The free radical scavenging activities of the crude extract and ascorbic acid were expressed as  $IC_{50}$ .

### Antibacterial Activity Test

#### Disc Diffusion Assay:

The disc diffusion method was implemented to determination the antibacterial activity of extract using *E. coli* ATCC and *S. aureus* ATCC 25923 microorganisms<sup>22</sup>. 6 mm diameter disc of filter paper was boiled for 30 minutes .After it has boiled, the disc was kept in the oven till it became dry and stored in a 21 °C in Petri dish glass.

#### Determination of Minimum Inhibitory Concentration

Two fold serial dilutions of the methanolic leaves extract were prepared to obtain 62.5 - 4000 µg/mL concentration range. After inoculation, the plates were incubated at 37 °C for 24 hours and the plates were observed for inhibition zones. The minimum concentration which caused inhibition was taken to be the MIC<sup>23</sup>.

#### Statistical Analysis

All experimental measurements were carried out in triplicate with each assay and are expressed as average of three analyses ± standard deviation for each assay recorded and the difference between the two groups were considered to be significant if  $p < 0.05$ . The magnitude of correlation between variables was done using a SPSS 20 statistical software package.

## RESULTS AND DISCUSSION

### Biological Activities

#### Antioxidant Activity toward DPPH Radical

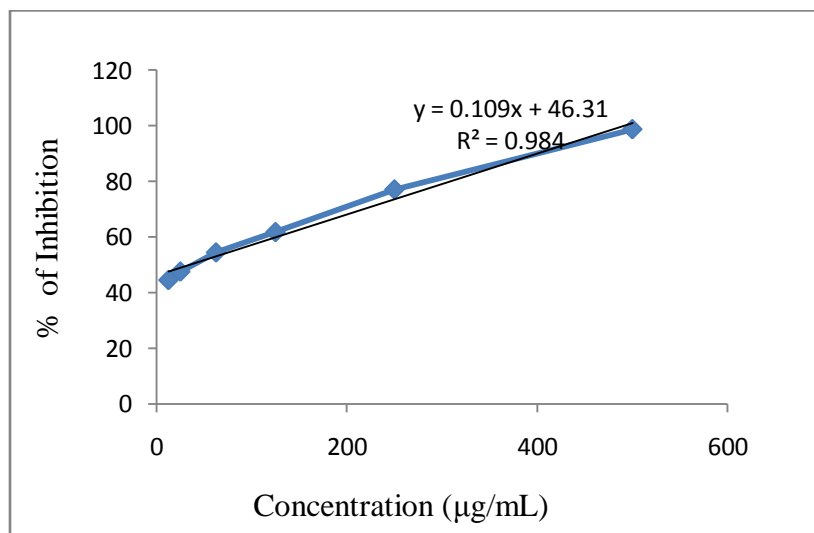
The % inhibition of methanolic leaf extracts & ascorbic acid was calculated as follows (Table 1).

**Table 1. Percentage inhibition of DPPH using extract and ascorbic acid at 517 nm**

S.N.	Concentrations of extract in µg/mL	% inhibition of DPPH	
		Methanol extract	Ascorbic acid
1	25	23.53 ± 0.57	44.62 ± 0.19
2	50	29.88 ± 0.89	47.66 ± 1.42
3	125	36.65 ± 0.64	54.51 ± 0.31
4	250	43.90 ± 1.67	61.81 ± 0.26
5	500	61.18 ± 0.07	77.08 ± 0.50
6	1000	89.6 ± 0.31	98.70 ± 0.19

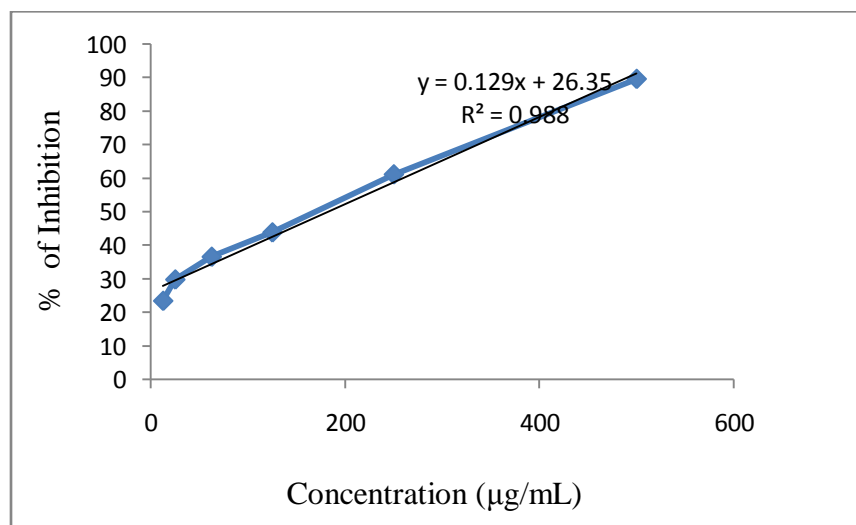
The above, table 1 shows the percentage of free radical scavenged of DPPH by methanolic leaf extracts and ascorbic acid at different concentrations.

In all concentrations of both samples shows DPPH scavenging activity. Ascorbic acid DPPH scavenging activity was found to be greater than methanolic leaf extracts of *Balanites aegyptiaca* at all concentration. From the current results it may be suggested that the extract of *Balanites aegyptiaca* reduces the DPPH radical.



**Figure 1. Calibration curve for ascorbic acid**

The  $IC_{50}$  value of ascorbic acid from the data of linear regression analysis was found to be 33.71  $\mu\text{g/mL}$



**Figure 2. Calibration curve for methanol extract**

The  $IC_{50}$  value of the methanolic leaf extracts from plotted graphs (Figure 2) was 182.02  $\mu\text{g/mL}$ , as opposed to that of ascorbic acid ( $IC_{50}$  33.71  $\mu\text{g/mL}$ ), which has a good antioxidant activity.

#### **Antibacterial Activity of Plant Extract**

Using agar disc diffusion, *in vitro* antibacterial activity of methanolic leaf extracts of *Balanites aegyptiaca* was determined against *E. coli* and *S. aureus* bacterial species. Table 2 shows the inhibition of microbial growth of methanolic leaf extracts of *Balanites aegyptiaca*. The methanol extract of *Balanites aegyptiaca* had visible zone of inhibition on *Escherichia coli* ranging from  $8.15 \pm 1.22$  to  $3.05 \pm 1.1$  mm in diameter. Whereas, test organism *S. aureus*, it had no zone of inhibition.

In addition, Penicillin indicates no zone of inhibition on *S. aureus* organism but had a wide effect on *E.coli*. However, 1mg/mL gentamycin showed wide-ranging zones of inhibition for both *E. coli* and *S. aureus* test organisms,

**Table 2. Result Diameter of zone of inhibition**

Test Organism	Zone of Inhibition in mm of methanolic leaves extract ( $\mu\text{g/mL}$ )							Control	
	4000	2000	1000	500	250	125	62.5	Gentamicin	Penicillin
<i>S. aureus</i>	-	-	-	-	-	-	-	$25 \pm 0.00$	-
<i>E. coli</i>	$8.15 \pm 1.22$	$6.4 \pm 1.39$	$4.9 \pm 1.47$	$4.3 \pm 2.05$	$3.5 \pm 0.74$	$3.05 \pm 1.1$	-	$28 \pm 0.00$	$16 \pm 0.00$

Table 2 shows narrow spectrum antibacterial activity since the antibacterial activity of the tested sample has visible effect against the growth of *E. coli* strains compared to *S. aureus* bacteria strains.

The MIC of *S. aureus* as observed from the above table was zero, not any of the dilutions showed any zone of inhibition. Conversely, MIC value of methanolic leaves extract against *E. coli* was 125  $\mu\text{g/mL}$ .

## CONCLUSION

The methanol leaf extracts of the *B. aegyptiaca* has good antioxidant potential but it has less efficiency than standard ascorbic acid. This indicates that this plant can have great scope of important antioxidant molecules which can be formulated to make antioxidant dosage forms. The antibacterial activity test showed certain antibacterial effectiveness of the extract even if it was very lower as compared to the standard antibiotics. Antibacterial activity of methanolic extract against *E. coli* showed the maximum degree as compared to the *S. aureus*. From this study the *in vitro* antibacterial evaluation confirms that the plant contains biologically active compounds which exhibit significant antibacterial activity against some pathogenic bacteria.

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