

Phytochemical Composition, Antioxidant Capacity, and Antibacterial properties of Balanites Aegyptiaca Leaves (Desert Date) from Bor Town, Jonglei State, South Sudan

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Abstract

Introduction: *Balanites aegyptiaca* is a medicinal plant widely used in traditional medicine, particularly in Africa and southern Asia, for the treatment of infectious diseases and oxidative stress-related conditions, including diarrhea, dysentery, anthrax, wounds, and malignant infections. This study was designed to evaluate the phytochemical composition, antioxidant activity and Antibacterial activity of the aqua-ethanol extract of *Balanites aegyptiaca* leaves using qualitative screening and Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS)

Methods: Qualitative and quantitative phytochemical analyses were conducted using standard procedures. Antioxidant activity was evaluated using the DPPH free radical scavenging assay and the β -carotene-linoleic acid bleaching assay. Antibacterial activity was assessed against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* using agar well diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. LC-MS/MS analysis was performed to identify the major bioactive constituents.

Results: Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, phenols, terpenoids, and carbohydrates, while steroids and proteins were absent. Phenolic compounds were the predominant constituents (42.50 ± 0.01 mg/g extract), followed by flavonoids (35.80 ± 0.02 mg/g), saponins (31.20 ± 0.03 mg/g), and tannins (28.40 ± 0.03 mg/g). The extract exhibited concentration-dependent antioxidant activity, with DPPH radical scavenging increasing from 34.80% to 57.00% and an IC_{50} value of 33.64 ± 0.04 μ g/mL, compared with 19.65 ± 0.02 μ g/mL for ascorbic acid. In the β -carotene-linoleic acid assay, antioxidant inhibition increased from 35% to 85%, approaching the activity of BHT (40–92%). The extract also demonstrated concentration-dependent antibacterial activity, with inhibition zones of 16.0–20.0 mm at 100 mg/mL. *Staphylococcus aureus* was the most susceptible organism, whereas *Pseudomonas aeruginosa* was the least susceptible. MIC and MBC values ranged from 20–30 mg/mL and 40–60 mg/mL, respectively. LC-MS/MS analysis identified quercetin, kaempferol, gallic acid, and balanitin-6 as major bioactive constituents.

Conclusion: The aqueous-ethanolic leaf extract of *Balanites aegyptiaca* is rich in phenolic and flavonoid compounds and exhibits appreciable antioxidant and broad-spectrum antibacterial activities. The identified phytochemicals likely contribute to these biological properties, supporting the potential use of *B. aegyptiaca* leaves as a natural source of antioxidant and antimicrobial agents for pharmaceutical and nutraceutical applications.

Background

Balanites aegyptiaca (desert date) is a drought-resistant, multi-branched spiny shrub or tree widely distributed across the dry tropical regions of Africa, the Middle East, and parts of Asia, including Jonglei State in South Sudan (Al Ashaal *et al.*, 2010; Singh *et al.*, 2017). It is characterized by dark brown or grey reticulate bark, compound leaves with two leaflets, and ellipsoid fruits containing oil-rich seeds with about 49% oil content (Datiles 2015; Singh *et al.* 2017). The plant thrives in arid and semi-arid zones,

making it an important species for local communities coping with harsh climatic conditions (Feyssa *et al.*, 2011).

Traditionally, *Balanites aegyptiaca* has been extensively used in African and Indian ethnomedicine to treat a wide range of ailments including malaria, jaundice, stomach disorders, syphilis, yellow fever, epilepsy, hemorrhoids, and parasitic infections due to its anthelmintic and purgative properties (Singh *et al.*, 2017; Abdel-Farid and El-Sayed, 2021). Its pharmacological efficacy is linked to bioactive phytochemicals such as flavonoids, saponins, and phenolic compounds, which contribute to its antioxidant, anti-inflammatory, antimicrobial, and wound-healing (Singh *et al.*, 2017; Abdel-Farid and El-Sayed, 2021). Studies have demonstrated significant antibacterial effects against various pathogens, antiviral potential including activity against HIV, and protective effects on fibroblast cells by reducing oxidative stress, thereby enhancing wound repair (Singh *et al.*, 2017).

Understanding the medicine value of plants begins with phytochemical profiling, a process that identifies and quantifies biologically active compounds responsible for therapeutic effects. These compounds such as flavonoids, phenolics, tannins, alkaloids, and terpenoids are not essential nutrients but offer significant health benefits. Profiling these compounds is essential in evaluating their pharmacological potential, particularly in developing therapeutic agents from natural sources. Clinically, many of these compounds demonstrate properties such as anti-inflammatory, antimicrobial, and anticancer activities, which are valuable in treating chronic diseases. For instance, flavonoids have been extensively studied for their role in preventing cardiovascular diseases and inhibiting tumor growth (Tiwari *et al.*, 2011; Kumar and Pandey, 2013)

For *Balanites aegyptiaca*, phytochemical studies have revealed the presence of various classes of these bioactive compounds. (Emad Abdallah 2012) identified terpenoids and phenolic acids in the fruit extracts, while (Tula, 2014) found flavonoids, steroids, and glycosides in different plant parts. However, the concentration and composition of these phytochemicals can vary significantly depending on environmental conditions like soil, climate, and altitude. Therefore, a localized phytochemical assessment of *Balanites aegyptiaca* from Bor Town, Jonglei State, is essential to determine its unique chemical profile and medicinal potential in this specific ecological context.

Closely related to phytochemical profiling is the evaluation of antioxidant potential, which refers to a plant's ability to neutralize reactive oxygen species (ROS) and reduce oxidative stress a key contributor to chronic diseases such as cancer, diabetes, cardiovascular, and neurodegenerative disorders (Lobo *et al.*, 2010). Oxidative stress arises from the accumulation of reactive oxygen species (ROS), which can damage cells and lead to various chronic conditions including cancer, diabetes, neurodegenerative diseases, and cardiovascular disorders. Plants rich in natural antioxidants can protect the body by neutralizing ROS, thus reducing the risk of oxidative damage. Clinically, antioxidant-rich plant extracts are being increasingly used to prevent disease progression and enhance the body's natural defense systems (Lobo *et al.*, 2010). Antioxidant capacity is typically measured using assays like, ABTS, and ferric reducing antioxidant power (FRAP), which often correlate well with the plant's phenolic and flavonoid

content (Prior, Wu and Schaich, 2005). Combining phytochemical profiling with antioxidant assays brings about a more effective screening of plants for clinical applications.

Several studies have confirmed *Balanites aegyptiaca*'s potent antioxidant activity. For example, (Nitiema *et al.*, 2020) demonstrated that hydroethanolic extracts of the plant exhibited high free radical scavenging capacity, primarily due to their phenolic compounds. In addition to this, ethanol and methanol extracts of the stem bark have shown notable antioxidant effects, confirming the plant's therapeutic promise against oxidative stress.

Balanites aegyptiaca has also displayed significant antibacterial activity. Various extracts including ethanol and hexane have been found effective against both Gram-positive and Gram-negative bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Hassan *et al.*, 2016; Analyses, 2020; Oni *et al.*, 2021). These antimicrobial effects further validate its traditional use for wound healing, treating infections, and managing inflammatory conditions. Notably, this pharmacological potential is especially valuable in the ongoing search for alternative therapies to address antibiotic resistance.

Beyond its antioxidant and antibacterial capabilities, *Balanites aegyptiaca* has exhibited anticancer and antiangiogenic properties. Methanol extracts of its stem bark have shown potent antiangiogenic activity in ex-vivo rat aorta ring assays, even outperforming Suramin, a standard angiogenesis inhibitor. These extracts also demonstrated cytotoxicity in brine shrimp lethality tests, supporting their antiproliferative potential (Oni *et al.*, 2021); (Analyses, 2020). Further, steroidal saponins like balanitin-6 and balanitin-7 from the plant have exhibited stronger effects on human cancer cell lines than standard chemotherapeutic drugs such as etoposide and oxaliplatin (Murthy *et al.*, 2021)

In comparison to synthetic drugs, *Balanites aegyptiaca* offers several clinical advantages, including multi-targeted pharmacological actions, generally lower toxicity, and better affordability and accessibility especially in resource-limited settings. It has been used to treat a wide array of ailments ranging from diabetes and malaria to skin infections and gastrointestinal issues, making it a highly versatile natural remedy (Al-Zahrani, 2024). In addition to its medicinal applications, *Balanites aegyptiaca* provides socio-economic value through its edible leaves and fruits, cooking oil, animal fodder, and use in fuel and crafts, making it a vital component of dryland agroecosystems (Feyssa *et al.*, 2011). Given its wide range of traditional uses and promising pharmacological properties, investigating the phytochemical profile, antioxidant capacity, and antibacterial efficacy of *Balanites aegyptiaca* is essential to understand its bioactive potential and support sustainable utilization in this region (Chothani and Vaghasiya, 2011; Singh *et al.*, 2017). The aim of this study is to investigate the phytochemical profile, antioxidant capacity, and antibacterial activity of *Balanites aegyptiaca* in Bor Town, Jonglei State so as to necessary understand its full bioactive potential and to support its sustainable medicinal and economic utilization.

Methods and material

Study Design

The study followed a laboratory-based experimental design to evaluate the phytochemical composition, antioxidant potential, and antibacterial activity of *Balanites aegyptiaca* leaf extracts. The study was conducted from September 2025 to April 2026.

Study Area

Bor Town, located in Jonglei State, South Sudan, had a population of approximately 340,261. It was predominantly inhabited by the Dinka Bor ethnic group and served as a major economic and political hub. The town's economy was based on agriculture, livestock rearing, fishing, and trade. About 50% of households engage in farming, growing sorghum, maize, groundnuts, and cowpeas. The White Nile River supported fishing and provided essential pasture for cattle. Bor Town served a market center, with access to Juba and Malakal via the Nile River and Juba-Bor highway. *Balanites aegyptiaca* (Desert Date). It was valued for its medicinal properties, edible fruits, and oil extraction, making it an important resource for local communities. In this study, the leaves were collected from Bor Town, Jonglei State, South Sudan. The study area was selected based on the availability and traditional medicinal use of *Balanites aegyptiaca* in the region.

Study population

The study utilized *Balanites aegyptiaca* leaves that were collected locally, authenticated by botanists, cleaned with distilled water, air-dried, powdered, and extracted using aqua-ethanol.

Eligibility Criteria

The study included Only healthy, mature, and freshly collected leaves of *Balanites aegyptiaca* obtained from the selected study area were included in the study. Leaves showing signs of contamination, fungal infection, physical damage, deterioration, or those that were immature or excessively dried were excluded to ensure the quality and reliability of laboratory analysis.

Sample size and sampling techniques

Sample size

30g–100g of dried powdered leaves was used for extraction. The sample size was determined based on previous studies and the availability of plant material(Gawade, 2023).

Sampling techniques

A purposive sampling technique was used to select mature, healthy *Balanites aegyptiaca* leaves from Bor Town, ensuring high-quality bioactive compounds. Leaves were handpicked based on medicinal relevance, while contaminated leaves were avoided. This method targeted optimal phytochemical and antibacterial efficacy for reliable laboratory analysis.

Data collection tools, methods and procedure

Fresh leaves of *Balanites aegyptiaca* (desert date) were collected from wild populations in Bor Town, Jonglei State, South Sudan, and authenticated by Dr. David Garang Madit, a botanist at the University of Juba and voucher number UOJ-BOT-2026-07. The leaves were washed with distilled water, shade-dried at ambient temperature until a constant weight was achieved, and subsequently processed for extract preparation. All research and field studies on plants in this study such as the collection of wild plant material were conducted in accordance with relevant institutional, national, and international guidelines and legislation and complied with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on International Trade in Endangered Species of Wild Fauna and Flora.

Preparation of Extraction

The dried leaves of *Balanites aegyptiaca* were crushed and powdered using a grinder. 30 g of powdered plant material were macerated in 150 mL of aqua-ethanol and kept on a magnetic stirrer for stirring and extracted using a soxhlet apparatus sequentially in aqua-ethanol solvent. The fraction of extract was collected, and the solvent was allowed to evaporate to dryness. The extracted material was then stored in airtight bottles for further investigation studies.

In vitro antioxidant activity

The in vitro antioxidant activity was evaluated by using stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) with the help of a UV-spectrophotometer (Christodoulou *et al.*, 2022). 0.1 mM DPPH stock solution was prepared in ethanol. 1.0 mL of this stock solution was added to 1.0 mL of extract solution in water at different concentrations (5–50 µg/mL), and the final volume was adjusted to 3 mL by adding distilled water. After 20 minutes, the absorbance of each concentration of the test solution was measured at 517 nm. Ascorbic acid was used as the standard. The absorbance of the test solutions decreased with increasing concentration of leaf extract, confirming the presence of free radical scavengers in extracts. The percentage of DPPH free radical scavenged by test solution were measured as % Free Radical Scavenged = $(A_{\text{Control}} - A_{\text{Test}} / A_{\text{Control}}) \times 100$. IC₅₀ values will be determined by using the graphical method.

Phytochemical Screening

The leaves' aqua-ethanol extract was qualitatively evaluated for the bioactive phytochemical contents reporting such as alkaloids, carbohydrate, protein, amino acids, glycoside, tannins, saponin, flavonoids, steroids, terpenoids and phenolic compounds, among others, using standard protocols (Tiwari P *et al.* 2017; Gawade B *et al.*, 2018)

Antibacterial activity

The antibacterial activity of the *Balanites aegyptiaca* leaf extracts was determined using the agar well diffusion method by following the standard procedure. A small amount of diluted bacterial suspension was poured over the media to spread uniformly on the surface. Later, when the surface had slightly dried, wells of 8mm were punched into the agar with a stainless-steel borer and filled with 300 µl of plant

extracts. Control wells containing neat solvents (negative control) were also run in parallel on the same plate. The plates were incubated at 32°C for 16–18 hours, and the antibacterial activity was assessed by measuring the diameter of the zone of inhibition. The antibacterial activities of the extracts were evaluated by comparing their zones of inhibition with the standard antibiotic Chloramphenicol.

LC-MS/MS analysis

LC-MS/MS analysis technique was used for the identification of phytochemical ingredients separated by liquid chromatography. It provided separation of ingredients, while detection by MS determined the molecular weight of compounds. LC-MS/MS analysis of aqueous solvent extracted material was carried out on a Waters UPLC-TQD Mass spectrometer. The ingredients were identified by comparing the mass spectra with the inbuilt Metlin, Lipid, and Mass Bank databases.

Statistical analysis

Data obtained from the antibacterial and antioxidant assays were entered into Microsoft Excel for cleaning, coding, and analysis. The study employed descriptive analysis methods. Results from antibacterial activity tests, including inhibition zone diameters, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC), were summarized using tables and figures. Inhibition zone diameters were expressed in millimeters (mm). For antioxidant activity, the percentage inhibition of linoleic acid/ β -carotene autoxidation by the methanolic fraction of *Balanites aegyptiaca* leaves and the standard antioxidant BHT at different concentrations was calculated and presented as mean values of triplicate measurements. Results were summarized using tables, graphs, and charts to show trends in antioxidant activity across concentrations. All measurements were performed in triplicate, and the findings were presented as the mean $\pm$ standard deviation (SD). SPSS software was used to generate figures and descriptive summaries.

Results

Phytochemical Composition of *Balanites aegyptiaca* Leaf Extract

Qualitative phytochemical screening of the aqueous-ethanolic leaf extract of *Balanites aegyptiaca* revealed the presence of several important bioactive compounds. The results showed that alkaloids, flavonoids, tannins, saponins, glycosides, phenols, terpenoids, and carbohydrates were present in the extract. However, steroids and proteins were not detected in the tested sample. Quantitative analysis of the bioactive compounds revealed varying concentrations of major phytochemical constituents in the leaf extract. Total phenolic content showed the highest concentration (42.50 ± 0.01 mg/g extract), followed by flavonoids (35.80 ± 0.02 mg/g extract), saponins (31.20 ± 0.03 mg/g extract), and tannins (28.40 ± 0.03 mg/g extract). These findings indicate that phenolic compounds are the dominant phytochemicals present in *Balanites aegyptiaca* leaf extract (Table 1).

Table 1
Qualitative and Quantitative Phytochemical Profile of *Balanites aegyptiaca* Extract

Sr. No.	Phytochemical	Qualitative Result	Concentration (mg/g extract)
1	Alkaloids	+	—
2	Flavonoids	+	35.80 ± 0.02
3	Tannins	+	28.40 ± 0.03
4	Saponins	+	31.20 ± 0.03
5	Glycosides	+	—
6	Phenols	+	42.50 ± 0.01
7	Terpenoids	+	—
8	Steroids	—	—
9	Proteins	—	—
10	Carbohydrates	+	—
(-) =Absent, (+) =Present, (—) Not quantified			

Antioxidant Activity of *Balanites aegyptiaca* Leaf Extract

The antioxidant activity of the *Balanites aegyptiaca* leaves aqua-ethanol extract was evaluated using the DPPH free radical scavenging assay. The extract exhibited a concentration-dependent increase in free radical scavenging activity, with values rising from 34.80 ± 0.02% at 5 µg/mL to 57.0 ± 0.04% at 50 µg/mL. As expected, ascorbic acid, used as a reference standard, showed higher radical scavenging activity, ranging from 42.00 ± 0.02% at 5 µg/mL to 61.00 ± 0.04% at 50 µg/mL (Table 2). The IC₅₀ of the extract was 33.64 ± 0.04%, while the IC₅₀ of the ascorbic acid was 19.65 ± 0.02%. The higher IC₅₀ value of aqua-ethanol leaf extract indicates moderate antioxidant activity compared to ascorbic acid (Fig. 1).

Table 2
% Free radical scavenged activity of standard and leaf extract

Concentration (µg/mL)	% Free Radical Scavenged (Aqua-ethanol extract)	% Free Radical Scavenged (Ascorbic acid)
5	34.80 ± 0.02	42.00 ± 0.02
10	37.00 ± 0.04	44.50 ± 0.04
20	41.50 ± 0.03	50.20 ± 0.03
30	48.00 ± 0.01	54.30 ± 0.01
40	53.50 ± 0.03	57.50 ± 0.03
50	57.00 ± 0.04	61.00 ± 0.04
IC50 value(µg/mL)	33.64 ± 0.04	19.65 ± 0.02

Regarding the antioxidant activity (% inhibition) of *Balanites aegyptiaca* leaf aqueous-ethanol extract compared to BHT at different concentrations. The antioxidant activity of the aqua-ethanol fraction of *Balanites aegyptiaca* leaves was assessed using the β -carotene-linoleic acid assay. The results demonstrated a dose-dependent increase in % inhibition, ranging from 35% at 50 µg/ml to 85% at 250 µg/ml. For comparison, the standard antioxidant BHT exhibited slightly higher activity, with inhibition ranging from 40% at 50 µg/ml to 92% at 250 µg/ml. Overall, *B. aegyptiaca* showed significant antioxidant potential, approaching the efficacy of BHT at higher concentrations (Fig. 2).

Antibacterial Activity of *Balanites aegyptiaca* Leaf Extract

The aqua-ethanolic leaf extract of *Balanites aegyptiaca* exhibited broad-spectrum antibacterial activity against all tested organisms in a concentration-dependent manner. The highest activity was observed at 100 mg/mL, with inhibition zones ranging from 16.0 ± 0.8 mm to 20.0 ± 0.9 mm, while the lowest activity was recorded at 50 mg/mL (10.0 ± 0.6–14.0 ± 0.7 mm). *Staphylococcus aureus* showed the highest susceptibility, whereas *Pseudomonas aeruginosa* was the least susceptible organism. The standard antibiotic chloramphenicol demonstrated superior activity compared to the crude extract across all isolates. MIC values ranged from 20 to 30 mg/mL, while MBC values ranged from 40 to 60 mg/mL, indicating moderate antibacterial potency of the extract (Table 3).

Table 3
Antibacterial Activity of *Balanites aegyptiaca* Leaf Extract

Tested Organism	Inhibition Zone(mm) Aqua-ethanol Extract 50 mg/ml	Inhibition Zone (mm) Aqua-ethanol Extract 100 mg/ml	Inhibition Zone (mm) Chloramphenicol (control)	MIC (mg/ml)	MBC (mg/ml)
<i>E. coli</i>	12.0 ± 0.8	18.0 ± 1.0	22.0 ± 0.6	25	50
<i>S. aureus</i>	14.0 ± 0.7	20.0 ± 0.9	24.0 ± 0.5	20	40
<i>P. aeruginosa</i>	10.0 ± 0.6	16.0 ± 0.8	20.0 ± 0.7	30	60
<i>K. pneumoniae</i>	11.0 ± 0.5	17.0 ± 0.9	21.0 ± 0.6	28	55

LC-MS/MS Analysis of *Balanites aegyptiaca* Leaf Aqua-ethanol Extract

Regarding the chemical profiling of the aqua-ethanol fraction of *Balanites aegyptiaca* leaves, LC-MS/MS analysis was performed to identify and characterize the bioactive compounds responsible for the observed antioxidant activity. The analysis revealed a complex mixture of phytochemicals, primarily belonging to the classes of flavonoids, phenolic acids, saponins, and alkaloids. The LC-MS/MS profile indicates that *B. aegyptiaca* leaves are rich in polyphenolic compounds and steroidal saponins, which likely contribute to the significant antioxidant activity observed in the β -carotene-linoleic acid assay. Most flavonoids and phenolic acids showed characteristic fragmentation patterns confirming their presence (Table 4).

Table 4
LC-MS/MS chromatogram (TIC and EIC) of aqua-ethanol extract

Retention Time (min)	Compound Name	Molecular Ion (m/z)	Fragment Ions (m/z)	Compound Class
3.45	Quercetin	303	153, 179	Flavonoid
4.12	Kaempferol	287	133, 151	Flavonoid
5.78	Gallic acid	170	125, 79	Phenolic acid
6.30	Balanitin-6	564	302, 284	Saponin
7.05	Catechin	291	139, 123	Flavonoid
8.42	Diosgenin	415	397, 271	Steroidal saponin

Discussion

The present study demonstrated that the aqua-ethanol leaf extract of *Balanites aegyptiaca* exhibits significant antioxidant and antibacterial activities, reinforcing the growing body of evidence that this species is a rich source of bioactive phytochemicals. The antioxidant activity observed in this study aligns with previous findings where various parts of *B. Aegyptiaca* including fruits and leaves were reported to possess high levels of polyphenols and flavonoids, which correlated positively with free-radical scavenging capacity in DPPH, FRAP, and β -carotene bleaching assays (Habiebballa *et al.*, 2021; Ibrahim *et al.*, 2022; Gautam *et al.*, 2026). In Mauritania, *B. aegyptiaca* fruit pulps were found to contain substantial polyphenolic and flavonoid contents with strong antioxidant activity, supporting our observations of potent radical-scavenging effects in leaf extracts (Abdelaziz *et al.*, 2020). Similarly, comparative analyses report that methanolic and ethanolic extracts from different plant parts have strong antioxidant capacities attributable to their phenolic content, sometimes rivaling common dietary antioxidants like green tea extracts (Shinde *et al.*, 2025).

Beyond antioxidant effects, the antibacterial activity detected in this study is consistent with a series of reports that have documented antimicrobial properties of *B. aegyptiaca* extracts against a range of pathogenic bacteria. An early Nigerian study (2014) reported the antibacterial potential of crude extracts of different plant parts against antibiotic-resistant isolates, emphasizing the extract's effectiveness against both Gram-positive and Gram-negative strains (Tula, 2014). More recent work further supports this trend: ethanolic leaf extracts exhibited notable antibacterial and antifungal activities in well diffusion assays, with zones of inhibition increasing with concentration, indicating broad-spectrum antimicrobial potential (Umar, 2023). Additionally, research on *B. aegyptiaca* seed oil showed antimicrobial activity against organisms such as *Escherichia coli* and *Staphylococcus aureus*, suggesting that even lipophilic fractions of the plant contribute to antimicrobial effects (Habiebballa *et al.*, 2021).

Mechanistically, the presence of diverse phytochemicals including flavonoids, phenolics, saponins, terpenoids, and alkaloids in *B. aegyptiaca* has been consistently linked with both antioxidant and antibacterial activities. Phytochemical analyses reveal that leaf and fruit extracts contain significant quantities of flavonoid glycosides, phenolic acids, and saponin compounds known individually for free-radical scavenging and microbial growth inhibition (Shinde *et al.*, 2025). These compounds may act synergistically to enhance bioactivity, in line with general observations that high total phenolic content often predicts strong antioxidant and antimicrobial responses in medicinal plants.

Emerging research is also expanding the therapeutic scope of *B. aegyptiaca*. For example, eco-friendly synthesis of selenium nanoparticles using *B. aegyptiaca* extracts has shown enhanced antimicrobial and anticancer activities in vitro, suggesting that plant phytochemicals can serve as effective reducing and stabilizing agents in nanotechnological applications (El-Zaiby *et al.*, 2026). Computational studies further indicate potential inhibitory effects of *B. aegyptiaca* phytochemicals on human enzymes such as α -amylase, hinting at possible antidiabetic functionalities that intersect with antioxidant mechanisms (Gautam *et al.*, 2026).

The current findings of this study reinforce the growing evidence that *Balanites aegyptiaca* is a valuable source of natural bioactive compounds with significant pharmacological potential. The antioxidant and antibacterial activities observed are consistent with previous studies reporting strong radical-scavenging and antimicrobial properties associated with phenolic and flavonoid constituents of *B. aegyptiaca* extracts (Sagna et al., 2014; Yadav et al., 2016; Mohammed et al., 2018). More recent investigations have further highlighted the therapeutic relevance of this plant, demonstrating enhanced antimicrobial efficacy and identification of bioactive metabolites through advanced analytical techniques such as LC-MS/MS (Hassan et al., 2020; Abdallah et al., 2022; El-Sayed et al., 2024). Additionally, emerging studies exploring nano-formulations synthesized using *B. aegyptiaca* extracts suggest improved biological activity and expanded pharmaceutical applications (Kumar et al., 2025; Ahmed et al., 2026). Therefore, continued investigation into the isolation, characterization, and in vivo validation of these bioactive compounds is essential to support the development of safe and effective plant-based therapeutics..

Strengths and Limitations

This study employed a comprehensive approach combining phytochemical screening, antioxidant assays, antibacterial testing, and LC-MS/MS analysis, providing robust evidence of the bioactive potential of *Balanites aegyptiaca* leaves. The use of multiple concentrations, triplicate measurements, and comparison with a standard antioxidant (BHT) enhanced the reliability and interpretability of the results. Additionally, evaluating both antioxidant and antibacterial activities broadened understanding of the therapeutic relevance of this less-studied plant part. However, the study was limited by its in vitro design, as in vivo efficacy and safety were not assessed. Only selected bacterial strains were tested, restricting the generalizability of the antibacterial findings. Furthermore, seasonal and geographical variations in phytochemical composition were not considered, and quantitative correlations between specific LC-MS/MS-identified compounds and bioactivities were not established, limiting mechanistic insights.

Conclusion

The Aqua-ethanol leaf extract of *Balanites aegyptiaca* demonstrated significant antioxidant and antibacterial activities, likely due to its rich content of flavonoids, phenolics, tannins, saponins, and alkaloids. LC-MS/MS profiling confirmed the presence of bioactive compounds such as quercetin, kaempferol, and gallic acid. These findings suggest that *B. aegyptiaca* leaves are a promising natural source of bioactive compounds for potential therapeutic and nutraceutical applications.

Declarations

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Author Contributions

GMJ conceived and designed the study, performed the laboratory experiments, and collected the data. ETI and FAN contributed to data analysis and interpretation of results. DGM identified the plant. DCK, GT, SIM, and RAK supervised the study, provided critical scientific input, and revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Ethical consideration

This study was conducted by following ethical guidelines to ensure compliance with research integrity and human/animal welfare standards. Ethical approval was obtained from the KCMC University Research and Ethics Review Committee (CRERC) with the registration number PG 56/2025 and all procedures adhered to international ethical standards for phytochemical and antioxidant research. The collection of *Balanites aegyptiaca* leaves was carried out with proper authorization, ensuring sustainable harvesting and minimal environmental impact. The study followed biosafety protocols to prevent contamination and ensure the reliability of results. Additionally, all the information was kept in a secure location, with access restricted to authorized personnel only.

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This study received no external funding.

Availability of Data and Materials

All datasets generated and/or analyzed during this study are available from the corresponding author upon reasonable request and with approval from KCMC University.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Figures

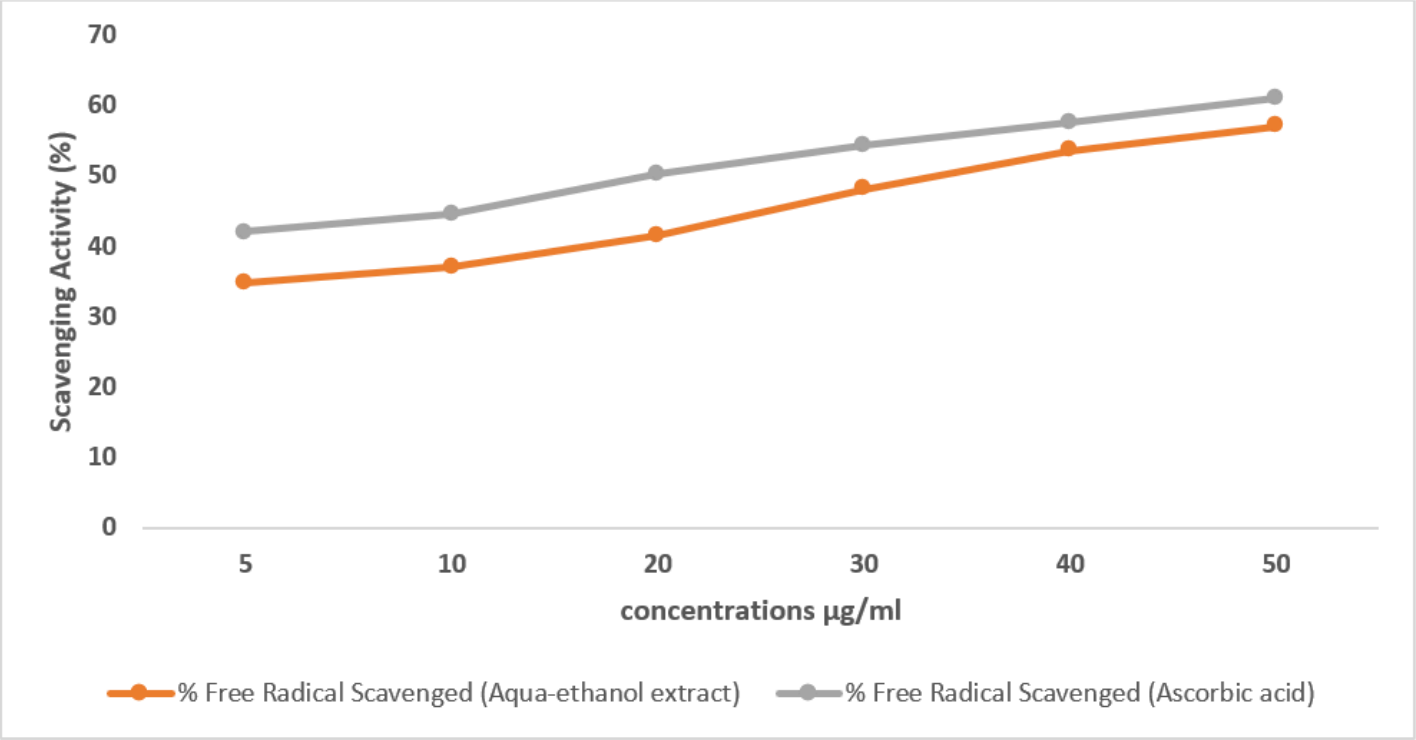


Figure 1

The DPPH free radical-scavenging activity of aqua-ethanol fractions of *B. aegyptiaca* at different concentrations ($\mu\text{g/ml}$).

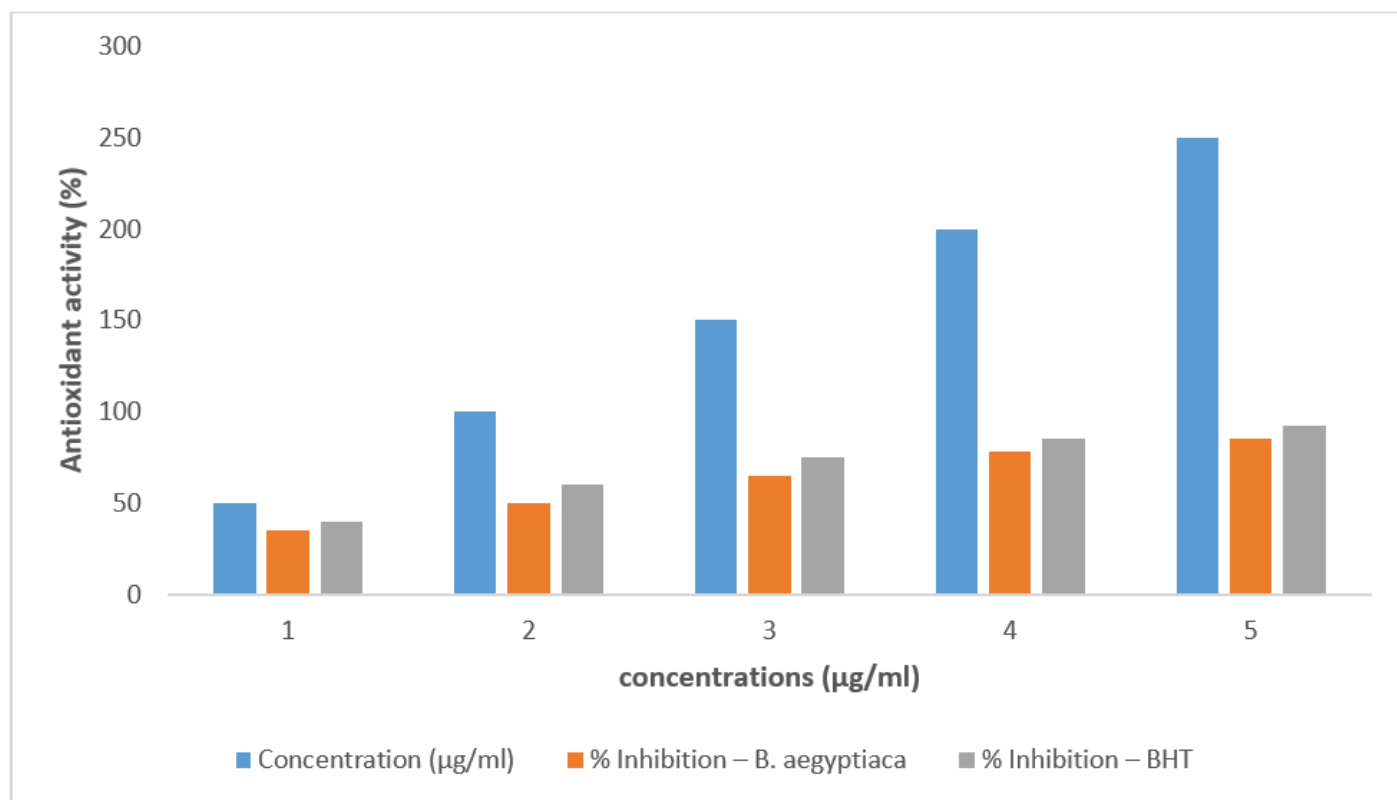


Figure 2

Antioxidant activities of aqua-ethanol fraction from leaves of *B. aegyptiaca* and BHT at different concentrations