



**Phytochemistry, Antimicrobial Potency and Safe Dose of *Balanites aegyptiaca*
(Desert Date) Methanolic Stem bark Fractions**

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ABSTRACT

This study investigated the phytochemical composition, antimicrobial potency, and acute toxicity of *Balanites aegyptiaca* (desert date) methanolic stem bark extracts and its fractions, addressing the critical need for novel therapeutic agents amidst rising antimicrobial resistance. The research aimed to assess the antifungal potential against *Aspergillus flavus*, *Trichophyton spp.*, *Aspergillus niger*, and *Penicillium spp.*, and establish the safety profile of its fractions. Stem bark samples were collected from Bayo Local Government Area, Borno State, Nigeria, a region known for its savanna vegetation and traditional use of the species. Qualitative analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, and phenols, with quantitative analysis highlighting phenols (13.06 mg/g) and flavonoids (10.24 mg/g) as dominant. While the crude extract showed moderate antifungal activity (MICs 25-100 mg/mL), fractionation significantly enhanced potency. Notably, Fraction 3 (F3) demonstrated the strongest antifungal efficacy with MICs ranging from 6.25 mg/mL to 12.5 mg/mL, particularly effective against *A. flavus* and *Penicillium spp.* (6.25 mg/mL). All fractions exhibited fungistatic rather than fungicidal effects (MFCs >100 mg/mL). Acute toxicity tests confirmed the fractions are practically non-toxic, with an estimated LD₅₀ of 8,776.67 mg/kg body weight in albino rats, supporting the plant's traditional medicinal use. These findings suggest *B. aegyptiaca* stem bark fractions, especially F3, are promising candidates for developing new and safe antifungal therapies.

Keywords: Antimicrobial, *Balanites aegyptiaca*, Fractions, Phytochemistry and Safe dose

INTRODUCTION

Natural products are both the base of traditional medicine and important sources of lead molecules in drug discovery (Thomford *et al.*, 2018). It is known that many herbal extracts (traditional medicines) act as chemosensitizers or resistance modulators (Tinoush *et al.*, 2020). Natural chemicals used by plants for their defense against pests and

disease organisms have recently become the choice for integrated pest management and disease control for plants. Due to their eco-friendliness and safety compared to synthetic compounds, they have the potential to solve worldwide agricultural, public health, population, and environmental challenges (Oguh *et al.*, 2019). Medicinal plants are known to have a wide range of bioactive

compounds that have antimicrobial, antifungal, anticancer, anti-inflammatory, and antioxidant activities (Konappa *et al.*, 2020 and Jagannath *et al.*, 2021). Many researchers have documented the potent activity of plants' bioactive compounds on drug-sensitive and resistant bacteria (Shakeri *et al.*, 2018). Although plants contain a very large number of bioactive compounds, few have been discovered (Konappa *et al.*, 2020). The development of extraction methods and the use of molecular spectroscopic techniques such as Gas chromatography and mass spectrum (GC-MS) and fourier-transform infrared (FTIR) has led to the discovery and characterization of new plant bioactive compounds (Konappa *et al.*, 2020, Abubaker and Haque, 2020).

Badal and Delgoda (2017) have defined the term Traditional Medicine (TM) as the total of knowledge skills and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illness. It is a system of health practice based on indigenous knowledge (also called folk knowledge, indigenous knowledge or ancestral knowledge) that has over a long span of time been passed on from generation to generation (Mukhwana, 2021).

Balanites aegyptiaca is a plant that belongs to the *Balanitaceae* family. It is commonly known as the 'desert date palm' (Abdulhamid and Sani, 2016), the specie is an indigenous tree that is highly exploited for its fruits and leaves, its edible fruit is used in the manufacturing of soap and cosmetic products, while the pulp is processed into various consumable and commercial products (Ouédraogo *et al.*, 2019). *B. aegyptiaca*, has been used traditionally in the management of different diseases such as jaundice, intestinal worm infection, wound healings, malaria, syphilis, epilepsy, dysentery, stomach aches, constipation, diarrhea, haemorrhoid and asthma (Al-Thobaiti and Abu Zeid, 2018), and for ages it has been used in the treatment of several disorders and diseases (Charity *et al.*, 2018).

The increasing prevalence of antimicrobial resistance and oxidative stress-related

diseases poses significant global health challenges, necessitating the exploration of novel therapeutic agents from natural sources. Traditional medicinal plants such as *Balanites aegyptiaca*, have been reported to contain bioactive compounds with potential antimicrobial and antioxidant properties, attributed to secondary metabolites like saponins, flavonoids, tannins, and phenolic compounds (Dogara, 2022; Taher-Bufarwa *et al.*, 2023). However, the efficacy of these plant extracts and their fractions against specific species of bacteria and fungi, as well as their antioxidant capabilities, remains insufficiently characterized. The aim of this study is to assess anti-microbial and anti-oxidant potentials of *Balanitis aegyptiaca* stem bark extracts and fractions on some species of bacteria and fungi. The investigation of this study is critical due to the escalating global challenge of antimicrobial resistance and oxidative stress-related diseases. This study was revealing the best concentration of stem bark extracts and fractions that inhibit the growth of bacteria, fungi and also the bioactive constituents that exhibit such control. This enhances place of these species on the ladder of pharmaceutical industries and ethno medicinal as well as enhance studies in microbial.

MATERIALS AND METHODS

Study Area

The study area is Bayo Local Government Area situated in the southern part of Borno State, northeastern Nigeria, located between Latitude 10°25'21" N to 10°42'25" N and between Longitude 11°42'22" E to 11°70'62" E in an elevation of 950 ft (290 m) above sea level. The study area is within the Sudan Savanna ecological zone. The region is characterized by distinct semi-arid climatic conditions with two prominent seasons: the rainy season typically occurs between June and September, with an average annual rainfall of 600–1000 mm and the dry season spanning from October to May, the dry season is dominated by the Harmattan winds, which blow from the northeast across the Sahara Desert, bringing dust-laden and dry air (Adebayo and Tukur, 2020b). This season is characterized by very low humidity and prolonged dry spells. The Temperature of the study area remain consistently high, with peak

temperatures exceeding 40°C between March and May, and cooler months (December to January) averaging 25°C to 30°C (Nigerian Meteorological Agency (NiMet), 2025). And the vegetation type is savanna vegetation, typical of northern Nigeria's transition between the Sahel and Guinea Savannah belts (Adedire et al., 2020). This vegetation type is

adapted to seasonal rainfall and extended dry periods. Scattered Trees: The landscape is dominated by drought-resistant trees, including: *Balanites aegyptiaca* (Desert Date), *Acacia* species, *Parkia biglobosa* (African Locust Bean), *Azadirachta indica* (Neem), and *Adansonia digitata* (Baobab) (Adebayo and Tukur, 2020b).

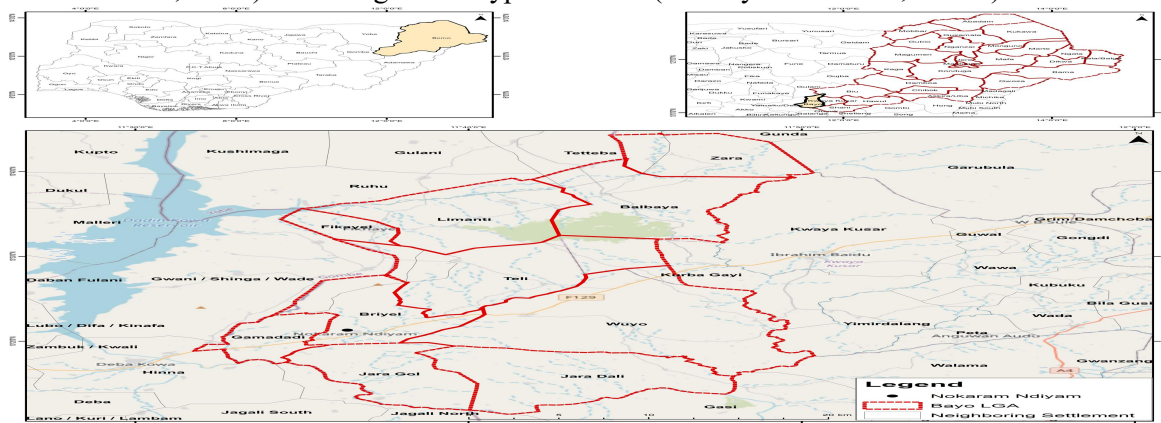


Figure 1: Map of the Study Area

Source: www.earth.google.com (2024)

Data Collection

The plant samples of *Balanites aegyptiaca* were collected from the study area, an area well-known for its diverse savanna vegetation where the species is commonly found in the wild and utilized traditionally for medicinal purposes. The selection of the study site was based on the natural abundance and ethnobotanical relevance of the species within the local communities. A purposive sampling technique was employed to identify mature *B. aegyptiaca* plants that exhibited no visible signs of disease or pest infestation. This method was chosen to ensure that only healthy specimens were selected for phytochemical and antimicrobial analysis. A systematic sample collection procedure was followed in which three replicate samples from each stem bark were collected from multiple mature plants within different sites in the study area to ensure representativeness. The samples were pooled, cleaned, and shade-dried to preserve bioactive compounds before being pulverized for extraction. These procedures ensured that the collected plant materials were of high quality, free from contamination, and sufficiently representative of the species' characteristics in the region.

The stem bark was collected based on their traditional medicinal usage and reports of potential bioactivity from previous studies (Badal and Delgoda, 2017; Yakubu et al., 2020). To ensure sample authenticity, the collected plant materials were identified and authenticated at the Herbarium of the Department of Plant Science, Faculty of Life Sciences, Modibbo Adama University (MAU), Yola, where a voucher specimen number MAU/PLS/0020 was assigned for future reference. The research activities, including sample preparation, extraction, phytochemical screening, antimicrobial testing, and toxicity evaluation, were carried out at the Biochemistry and Microbiology laboratories of Modibbo Adama University, **Extraction of Stem Bark of *Balanites aegyptiaca***

The extraction of the stem bark of *Balanites aegyptiaca* was carried out following the method described by Jean-Bosco et al. (2020) with slight modifications. The collected stem bark samples were first shade-dried to prevent the degradation of heat-sensitive bioactive compounds. Once completely dried, the samples were ground into fine powder using an electric milling machine to increase the surface area for effective solvent penetration.

The powdered stem bark was then subjected to solvent extraction using the maceration method. In this process, 350g of the powdered sample was immersed in 750 mL of 80% methanol. The mixture was allowed to stand for 72 hours with intermittent shaking, facilitating the dissolution of phytochemical constituents into the solvent. After maceration, the extract was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated to dryness under reduced pressure using a rotary evaporator. The concentrated extract was further air-dried to remove residual solvent and then stored in airtight containers at 4°C until required for further phytochemical and antimicrobial analyses. This method ensures the efficient extraction of bioactive compounds and is widely adopted for preparing plant extracts for pharmacological studies.

Fungal Strains Acquisition and Maintenance

The fungal strains used in this study were obtained from the Department of Microbiology, Modibbo Adama University, Yola, Nigeria. The species were initially isolated and maintained on sterile Nutrient Agar (NA) slants under aseptic conditions. The cultures were incubated at an appropriate temperature of $28 \pm 2^\circ\text{C}$ to promote optimal growth and were subsequently stored at 4°C for preservation until required for experimental analysis. This method of isolation and storage ensured the viability and purity of the fungal cultures throughout the research period, in line with standard microbiological practices (Osuntokun *et al.*, 2018). The fungi used in the study were *Aspergillus flavus*, *Aspergillus niger*, *penicillium chrysogenum*, and *Trychophyton tonsurans*.

Antimicrobial Efficacy of *B. aegyptiaca* Stem Bark Extracts Using Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The antimicrobial activity of the stem bark extract of *Balanites aegyptiaca* was evaluated using the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) methods, following the standard procedures outlined by the Clinical

and Laboratory Standards Institute (CLSI, 2012).

Minimum Inhibitory Concentration (MIC)

The MIC was determined by the broth dilution method. Serial dilutions of the stem bark extract were prepared in sterile broth media to achieve varying concentrations of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml was introduced into each dilution tube. The inoculated tubes were incubated at $28 \pm 2^\circ\text{C}$ for 48-72 hours, after which they were examined for turbidity or visible growth. The lowest concentration of the extract that inhibited visible growth was recorded as the MIC. The *Fulcin* were use as control of fungi according to Ekhuemelo *et al.* (2018).

Minimum Fungicidal Concentration (MFC)

The MFC was then determined by sub-culturing samples from tubes that showed no growth in the MIC test onto Potato Dextrose Agar (PDA) plates. The plates were incubated under the same conditions. The MFC was defined as the lowest concentration of the extract that resulted in no fungal growth on the agar plates, indicating a fungicidal effect. These methods allowed for a quantitative assessment of the antifungal efficacy of the stem bark extract of *Balanites aegyptiaca* (Ekhuemelo *et al.*, 2018).

Thin Layer Chromatography of Crude Extracts

The Analytical thin layer chromatographic technique was done according to the method described by (Oloke *et al.*, 1988) to spot, separate and determine the R_f (Retention factors) values and a suitable solvent system for fractionation of the phytochemical components by column chromatography on the crude extracts. This was achieved by using the TLC silica gel 60 F254 Aluminum sheet made by Merck KGaA, Millipore Corporation Germany was used as the stationary phase for the most active extracts. The solvent system that gave the best separation based on the R_f values were used to fractionate the crude extracts by column chromatography, while the number of spots seen was recorded.

Fractionation of the *Balanites aegyptiaca* Stem Bark Extract Using Column Chromatography

The methanolic extract of the stem bark of *Balanites aegyptiaca* was subjected to column chromatography for fractionation. The procedure was carried out following the method described by Ighodaro *et al.* (2016), with slight modifications to suit the extract characteristics.

Phytochemical Analysis of *Balanites aegyptiaca* Stem bark Fractions

Qualitative and Quantitative phytochemical test were carried out using Standard Phytochemical Screening Methods of Okoli *et al.* (2023).

Acute Toxicity Tests of *Balanites aegyptiaca* Fraction

The acute toxicity and lethality of *Balanites aegyptiaca* fractions were assessed using Lorke's Method for Acute Toxicity Testing described by Lorke (1983).

RESULTS AND DISCUSSION

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of stem bark extracts of *B. aegyptiaca* against *Aspergillus flavus*, *Trichophyton spp.*, *Aspergillus niger*, and *Penicillium spp*

The data presented in Table 1 shows the antifungal activity of *Balanites aegyptiaca* stem bark extracts against four species of fungi: *Aspergillus flavus*, *Trichophyton spp.*, *Aspergillus niger*, and *Penicillium spp*. MIC of *B. aegyptiaca* stem bark extract against *A. flavus* and *Penicillium spp* showed effectiveness at 25mg/ml, *Trichophyton spp* showed effect at 50mg/ml, while *A. niger* was greater than 100 mg/ml. MFC for all fungi was greater than 200 mg/ml. These findings are consistent with previous reports that highlight the moderate antifungal potential of *B. aegyptiaca* stem bark extracts. Hassan *et al.* (2022), who reported MICs of 20 to 50 mg/ml against *A. flavus* and *Trichophyton spp*, attributed to saponins and alkaloids. The lack of seed extract activity is consistent with Musa *et al.* (2022), who found no antifungal effects at concentrations up to 200 mg/ml.

Table 1: Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of *Balanites aegyptiaca* Stem bark Extracts against *A. Flavours*, *Trychophaton spp*, *A. Niger*, and *Penicillium spp*

Plant Part	Fungi	Concentration (Mg/ml)						MIC Mg/mL	MFC Mg/mL
		200	100	50	25	12.5	6.25		
Stem bark	<i>A. Flavours</i>	-	-	-	-	+	+	25	> 200
	<i>Trychophyton spp</i>	-	-	-	+	+	+	50	> 200
Stem bark	<i>A. Niger</i>	-	-	+	+	+	+	100	> 200
	<i>Penicillium spp</i>	-	-	-	-	+	+	25	> 200

Note: + Growth, - No Growth, > Greater than

Thin-Layer Chromatography (TLC) Screening of *Balanites aegyptiaca* Stem bark extracts

The table 2 shows the results obtained using a specific solvent system (Methanol, Ethyl Acetate:N-Hexane in a 2:1:2 ratio). This solvent system was chosen for its ability to effectively separate the compounds in the *B. aegyptiaca* extract. Previous findings support

the utility of TLC. For instance, Lubis *et al.* (2024) highlight how "different R_f values of the phytochemicals add very important data regarding their polarity and also provide guidance for the selection of a proper solvent system." Similarly, El-Aziz *et al.* (2016) used TLC to confirm the presence and diversity of secondary metabolites like flavonoids and tannins, relating their presence to observed biological activities.

Table 2: Thin-Layer Chromatography (TLC) Screening of *Balanites aegyptiaca* Stem Bark Extracts

Solvent system	NS	DMS cm	DMF cm	RF Cm
Methanol: ethyl acetate: N-hexane 2:1:2		5.7	4.5	0.789
	F1	5.7	1.5	0.298
	F2	5.7	3.4	0.596
	F3	5.7	4.4	0.772

Note: NS= Number or – Spot, DMS = Distance more by solvent DMF = Distance move by fraction, RF = Retention factor.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of *B. aegyptiaca* stem bark fractions against *A. Flavours*, *Trychophaton spp*, *A. Niger*, and *Penicillium spp*

Table 3 presents the antifungal activity of three fractions (F1, F2, F3) of *Balanites aegyptiaca* stem bark against fungal species: *Aspergillus flavus*, *Trichophyton spp.*, *Aspergillus niger*, and *Penicillium spp.*, assessed using Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC). Fraction 3 (F3) exhibited the strongest antifungal activity across all tested fungi, with MICs ranging from 6.25 mg/mL to 12.5 mg/ml, demonstrating notable potency especially against *A. flavus* and *Penicillium spp.* with MIC of 6.25 mg/ml. Fraction 2 (F2) also showed good antifungal effects, particularly against *Trichophyton spp.* and *Penicillium spp.* with MIC of 6.25 mg/ml. Fraction 1 (F1) had comparatively lower antifungal efficacy, with MICs ranging from 12.5 mg/mL to 50 mg/ml. The MFC values for all fractions against the tested fungi were >100 mg/mL, suggesting that the extracts exert fungistatic rather than fungicidal effects within the concentration range tested. This study is in line with the findings of Okello *et al.*, (2020) who documented the antifungal properties of *Balanites aegyptiaca* seed and stem bark extracts, reporting significant inhibition of *Aspergillus* and *Penicillium* species, albeit at relatively higher concentrations. The stronger activity observed in F3 suggests that bioactive compounds responsible for antifungal effects are more concentrated or bioavailable in this

fraction, supporting the idea of fractionation enhancing bioactivity (Ezekiel *et al.*, 2019). Notably, *Trichophyton spp.*, a dermatophyte causing skin infections, was inhibited at low MICs by F2 and F3 (6.25 - 12.5 mg/mL), corroborating findings by Mahmoud *et al.* (2017) who reported the sensitivity of dermatophytic fungi to plant-derived saponins and phenolics. The MFC values were higher than 100 mg/mL suggests that while the fractions can inhibit growth, they are less effective in completely eradicating fungal cells within the tested doses. This points to the potential of combining these fractions with conventional antifungals for synergistic effects, as recommended by Singh *et al.*, (2022) in exploring plant-drug interactions.

Determination of Phytochemical Screening of *Balanites aegyptiaca* Stem Bark Fractions

The qualitative phytochemical screening of *Balanites aegyptiaca* stem bark fractions revealed the presence of diverse bioactive compounds, including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenols. However, steroids and glycosides were absent across the examined fractions as presented in table 4. These results suggest that *B. aegyptiaca* stem bark is rich in secondary metabolites known for their pharmacological activities, particularly antimicrobial properties. The presence of these bioactive compounds aligns with previous reports on *B. aegyptiaca*. For instance, Obi *et al.* (2022) documented similar phytochemical constituents in the stem bark, linking these compounds to the plant's therapeutic properties, such as antimicrobial, antifungal.

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of *B. aegyptiaca* Stem bark Fractions against *A. Flavours*, *Trychophyton spp*, *A. Niger* and *Penicilium Spp*.

Plant Part	Fungi	Concentration (Mg/mL)						MIC Mg/mL	MFC Mg/mL
		100	50	25	12.5	6.25	3.12		
F ₁ (0.29)	<i>A. Flavours</i>	-	-	-	-	+	+	12.5	>100
	<i>Trychophyton spp</i>	-	-	-	+	+	+	2.5	> 100
	<i>A. Niger</i>	-	-	-	+	+	+	25	> 100
	<i>Penicilium spp</i>	-	-	+	+	+	+	50	> 100
F ₂ (0.59)	<i>A. Flavours</i>	-	-	-	-	+	+	12.5	> 100
	<i>Trychophyton spp</i>	-	-	-	-	-	+	6.25	> 100
	<i>A. Niger</i>	-	-	-	+	+	+	25	> 100
	<i>Penicilium spp</i>	-	-	-	-	-	+	6.25	> 100
F ₃ (0.77)	<i>A. Flavours</i>	-	-	-	-	-	+	6.25	> 100
	<i>Trychophyton spp</i>	-	-	-	-	+	+	12.5	> 100
	<i>A. Niger</i>	-	-	-	-	+	+	12.5	> 100
	<i>Penicilium spp</i>	-	-	-	-	-	+	6.25	> 100

Key: + Growth, - No Growth, > Greater than

Table 4: Qualitative Phytochemical Screening of *B. Aegyptiaca* Stem Bark Fractions

S/N	Phytochemical	<i>B. aegyptiaca</i>
1.	Saponin	+
2.	Tannins	+
3.	Alkaloids	+
4.	Flavonoids	+
5.	Phenols	+
6.	Steroids	-
7.	Terpenoids	+
8.	Glycoside	-

Source: Field Survey (2025)

Key: + = present and - = absent.

Quantitative Phytochemical Screening of *Balanites aegyptiaca* Stem Bark Fractions

The quantitative phytochemical analysis of *Balanites aegyptiaca* stem bark fractions revealed varying concentrations of bioactive compounds, as presented in Table 5. Notably, phenols (13.06 mg/g) and flavonoids (10.24 mg/g) were present in the highest amounts, indicating significant antifungal activity. Tannins (4.06 mg/g), saponins (1.74 mg/g), alkaloids (1.67 mg/g), and terpenoids (0.21 mg/g) were also detected, while steroids and glycosides remained undetected. The high content of phenols and flavonoids aligns with findings by Zida *et al.* (2021), who emphasized their role in antifungal activities. The presence of tannins and saponins also corroborate with the findings by (Mahmoud *et al.*, 2019) that supports the plant's traditional use in antimicrobial treatments, as these

compounds disrupt microbial membranes. And the absence of steroids and glycosides in the stem bark is consistent with earlier reports on specific organ-based metabolite distribution in *Balanites aegyptiaca* by Adamu *et al.*, (2020).

Acute Toxicity Test of *Balanites aegyptiaca* Fraction on Albino Rats

Table 6 shows the acute toxicity assessment of *Balanites aegyptiaca* stem bark fractions was conducted in two phases according to the method of Lorke (1983). The objective was to determine the median lethal dose (LD₅₀) and evaluate the safety profile of the plant fractions. The phase I results show the doses administered are 500, 1000, 2000, and 4000 mg/kg bw body weight and it is observed that there was no clinical signs of toxicity or mortality were recorded in any of the treatment groups, including the control group.

Table 5: Quantitative Phytochemical Screening of *Balanites aegyptiaca* Stem Bark Fractions

S/N	Phytochemical	Value (mg/g)
1.	Saponins	1.74
2.	Tannins	4.06
3.	Alkaloids	1.67
4.	Flavonoids	10.24
5.	Phenols	13.06
6.	Steroids	-
7.	Terpenoids	0.21
8.	Glycosides	-

Source: Field Survey (2025)

This suggests that the fractions are safe up to 4000 mg/kg bw in rats. In other hand the phase II results shows that the doses administered are 5000, 6000, 8000, and 10000 mg/kg bw and it is observed that at 5000 mg/kg bw rats showed no mortality but mild signs marked as "S" (likely sedation or slight behavioral changes), while doses of between 6000 mg/kg bw to 8000 mg/kg bw produced symptoms of sleep and exhaustion (E), with mortality. The LD₅₀ of fraction of *B. aegyptiaca* were calculated to be 8,776.67mg/kg bw body weight, classifying

the extract as practically non-toxic since it exceeds the 5000 mg/kg bw threshold for oral toxicity in rats. This finding aligns with studies by Jean-Bosco *et al.*, (2020) who found that *B. aegyptiaca* stem bark extract exhibits a high safety margin in animal models. Akinmoladun *et al.*, (2021) also reported that medicinal plants with LD₅₀ values greater than 5000 mg/kg are considered to have low acute toxicity, making them safer candidates for therapeutic applications.

Table 6: Acute Toxicity Test of *Balanite aegyptiaca* Fraction on Albino Rats

Phase I Group of rats	No of rats per group	Dose (mg/kg bw)	Dose Difference (a)(mg/kgbw)	Clinical signs	Mortality	Mean Mortality (b)	Product (a×b)
1	3	500	500	—	0	0	0
2	3	1000	500	—	0	0	0
3	3	2000	1000	—	0	0	0
4	3	4000	2000	—	0	0	0
Control group	3			—	0	0	0
Phase II Group of rats	No of rats per group	Dose (mg/kg)	Dose Difference (a) (mg/kg)	Clinical signs	Death	Mean Death (b)	Product (a×b)
1	3	5000	5000	S	0	0	0
2	3	6000	1000	S and E	1	0.33	330
3	3	8000	2000	S and E	2	0.67	1340
4	3	10000	2000	E	3	1	2000
Control group	3			—	0	0	0

Source: Field Survey (2025)

Key: mg/kg b.w.t = milligram per kilogram body weight, - No response, E = Exhaustion, S = Sleep.

CONCLUSION

This study investigated the phytochemical composition, antimicrobial potency, and acute toxicity of *Balanites aegyptiaca* (desert date)

methanolic stem bark extracts and its fractions, addressing the critical need for novel therapeutic agents amidst rising antimicrobial resistance. The research aimed to assess the antifungal potential against

Aspergillus flavus, *Trichophyton spp.*, *Aspergillus niger*, and *Penicillium spp.*, and establish the safety profile of its fractions. Qualitative analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, and phenols, with quantitative analysis highlighting phenols (13.06 mg/g) and flavonoids (10.24 mg/g) as dominant. While the crude extract showed moderate antifungal activity (MICs 25-100 mg/mL), fractionation significantly enhanced potency. Notably, Fraction 3 (F3) demonstrated the strongest antifungal efficacy with MICs ranging from 6.25 mg/mL to 12.5 mg/mL, particularly effective against *A. flavus* and *Penicillium spp.* (6.25 mg/mL). All fractions exhibited fungistatic rather than fungicidal effects (MFCs >100 mg/mL). Acute toxicity tests confirmed the fractions are practically non-toxic, with an estimated LD₅₀ of 8,776.67 mg/kg body weight in albino rats, supporting the plant's traditional medicinal use. These findings suggest *B. aegyptiaca* stem bark fractions, especially F3, are promising candidates for developing new and safe antifungal therapies.

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