

## Pharmacognostical, phytochemical, Pharmacological Evaluation of Leaves of Plant *Balanites aegyptiaca* (L.) Delile

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### Abstract :

Medicinal plants play a crucial role in the health of individuals and communities, largely due to their chemical constituents that elicit specific physiological effects on the human body. Traditional systems of medicine, deeply rooted in cultural practices, remain prevalent across the globe, highlighting the enduring significance of herbal remedies in healthcare. The use of plants as medicine is humanity's oldest healthcare practice, underpinning the universal reliance on phytotherapeutic approaches throughout history. Phytochemistry, the study of plant-derived chemicals, seeks to elucidate the structures and functions of secondary metabolites, many of which exhibit potent antioxidant properties. These antioxidants, primarily sourced from fruits, vegetables, and whole grains, are vital for protecting health by neutralizing free radicals. Helminthic infections, which are among the most widespread infectious diseases globally, particularly threaten public health in developing countries, contributing to conditions like anemia and malnutrition. Anthelmintics, or anti-parasitic drugs, have been essential in managing these infections, highlighting the historical importance of antimicrobial therapies since their inception in the early twentieth century. This study focuses on *Balanites aegyptiaca* Del., a tree belonging to the *Balanitaceae* family, known for its wide availability and potential medicinal benefits. The investigation aims to evaluate the Pharmacognostic, phytochemical, and pharmacological properties of *B. aegyptiaca* leaf extracts. Methodologies employed include thin layer chromatography, spectral analysis, and in-vitro assessments of antioxidant, anthelmintic, antibacterial, and antifungal activities. The findings will contribute to the understanding of this plant's therapeutic potential and underscore the importance of traditional medicinal practices in modern healthcare.

**Keywords :** Pharmacognostic Screening, Anthelmintics, Pharmacological Screening, Phytochemistry

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### INTRODUCTION

Medicinal plants have long been integral to human health, serving as a foundation for both traditional and modern medical practices. Their significance extends beyond individual well-being to encompass community health, making them crucial to the cultural and therapeutic landscape of societies around the globe. The medicinal value of these plants is primarily attributed to their phytochemicals—biologically active compounds that can exert specific physiological effects on the human body. This intricate relationship between plants and health is central to the field of phytochemistry, which focuses on understanding these compounds, their structures, and their potential benefits. The use of plants for medicinal purposes is arguably the oldest form of healthcare known to humanity, with roots that trace back to ancient civilizations. Historical records from diverse cultures—ranging from Ayurvedic practices in India to traditional Chinese medicine—highlight the universality of herbal remedies. Such practices have persisted across generations, adapting to contemporary medical paradigms while retaining their foundational beliefs. Traditional systems of medicine continue to thrive today,

largely due to their holistic approach to health, emphasizing prevention and the natural balance between the body and environment. In many regions, especially in developing countries, access to modern healthcare can be limited. Consequently, traditional medicine often remains the primary source of healthcare, underscoring the importance of medicinal plants in addressing health challenges. For instance, herbal remedies are commonly employed to treat a wide array of conditions, from minor ailments to more severe diseases. This reliance on plant-based treatments is not merely a relic of the past; it reflects a continued search for effective, accessible, and culturally relevant healthcare solutions. Phytochemistry plays a pivotal role in bridging traditional knowledge and modern science. By studying the diverse range of secondary metabolites produced by plants, researchers aim to uncover the underlying mechanisms that confer medicinal properties. These compounds can be categorized into various classes, including alkaloids, flavonoids, terpenoids, and phenolics, each possessing distinct biological activities. Antioxidants, in particular, have garnered significant attention due to their role in protecting the body from oxidative stress, which is linked to various chronic diseases, including cancer and cardiovascular disorders. Natural antioxidants are primarily derived from fruits, vegetables, and whole grains, making them essential components of a health-promoting diet. The capacity of antioxidants to neutralize free radicals—unstable molecules that can cause cellular damage—is fundamental to their protective effects. Despite advances in modern medicine, helminthic infections remain a pressing public health issue worldwide, particularly in developing nations. These infections, caused by parasitic worms, affect millions of people, leading to significant morbidity and contributing to issues such as anemia, malnutrition, and respiratory complications. The prevalence of helminthiasis is exacerbated by socio-economic factors, including poor sanitation and limited access to healthcare services. As a result, communities affected by these infections face a dual challenge: the immediate health impact and the long-term socio-economic consequences. Anthelmintics—medications used to treat helminthic infections—are vital in managing these diseases. The development of effective anthelmintic therapies is crucial for public health, as these drugs can help reduce the burden of disease and improve overall health outcomes. However, there is a growing concern regarding the efficacy of conventional anthelmintics due to issues like drug resistance. This situation necessitates exploring alternative treatments, including those derived from medicinal plants, which have shown promise in traditional medicine.

Among the various medicinal plants, *Balanites aegyptiaca* Del., commonly known as the desert date tree, stands out for its extensive use in traditional medicine and its potential therapeutic benefits. Belonging to the *Balanitaceae* family, this open-branching tree can reach heights of up to 12 meters, and it is widely available in various regions, particularly in Africa and parts of the Middle East. The different parts of *B. aegyptiaca*, including its leaves, fruits, and seeds, have been utilized for a range of medicinal purposes, such as treating digestive disorders, skin ailments, and respiratory conditions. Research into the pharmacognostic, phytochemical, and pharmacological properties of *B. aegyptiaca* is crucial for validating its traditional uses and identifying its active compounds. Such investigations can provide insights into the plant's efficacy and safety, paving the way for its integration into modern therapeutic practices. In this context, understanding the extraction and standardization of the crude drug material is essential to ensure consistent quality and potency.

The present study aims to systematically investigate the leaves of *Balanites aegyptiaca*, focusing on several key objectives:

**Selection of the Plant:** This study begins with the selection of *B. aegyptiaca* based on its traditional and ethno medicinal uses, which have been documented across various cultures.

**Collection and Authentication:** Proper collection and authentication of the plant material are crucial to ensure the accuracy of the subsequent analyses. This step involves verifying the identity and quality of the plant samples.

**Pharmacognostic Investigation:** A thorough Pharmacognostic evaluation was conducted, encompassing the study of the plant's morphology, anatomy, and histological characteristics. This foundational knowledge is vital for understanding the plant's medicinal properties.

**Extraction and Standardization:** The extraction process will focus on optimizing methods to obtain the bioactive compounds from the leaves of *B. aegyptiaca*. Standardization of the crude drug material will ensure consistent quality for further studies.

**Phytochemical Investigation:** A detailed phytochemical analysis was conducted, including the development of thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), and column chromatography. These techniques will aid in identifying and quantifying the phytochemicals present in the extracts.

**Assessment of Bioactivity:** The extracts were evaluated for their in-vitro antioxidant, antibacterial, antifungal, and anthelmintic activities. Such assessments are essential to establish the therapeutic potential of *B. aegyptiaca* and its relevance in addressing contemporary health challenges.

By focusing on these objectives, the present study seeks to contribute to the growing body of knowledge surrounding medicinal plants and their applications in healthcare. The exploration of *Balanites aegyptiaca* not only aims to validate its traditional uses but also to provide a scientific basis for its incorporation into modern medicinal practices. Ultimately, this research endeavors to bridge the gap between traditional herbal medicine and contemporary pharmacotherapy, offering new insights into the potential of plant-based treatments in the fight against prevalent health issues.

## **Materials and Methods:**

### **1. STANDARDISATION OF PLANT MATERIAL**

#### **Collection:**

Authentication: PHARMACOGNOSTIC STUDY

Macroscopic evaluation Microscopic evaluation Equipments

Microscopical Powder Analysis

1. Leaf surface Area
2. Vein- islet number
3. Vein islet termination
4. Stomatal number
5. Stomatal Index
6. Determination of Loss on Drying
7. Determination of Ash Value
  - 7.1. Determination of Total Ash Value

7.2. Determination of Water – Soluble Ash Value

7.3. Determination of Acid – Insoluble Ash Value

8. Determination of Extractive Value

8.1. Determination of water Soluble Extractive value

8.2. Determination of Alcohol – Soluble Extractive value

#### EXTRACTION METHODOLOGY

1. Petroleum Ether Extraction

2. Chloroform Extraction

3. Methanolic Extraction

4. Aqueous Extraction

Preliminary Phytochemical Screening For Various Extracts:

a) Test for Carbohydrates

i) Molisch test

b) Test for reducing sugars

i) Benedict's test

ii) Fehling's test

c) Test for monosaccharides

i) Barfoed's test

d) Test for Proteins

i) Biuret test

ii) Million's test

iii) Xanthoprotein test

e) Test for amino acids

- i) Ninhydrin test
- f) Test for Steroids
- i) Salkowski test
- ii) Liebermann – Burchard reaction
- iii) Liebermann’s reaction
- g) Test for Cardiac Glycosides
- i) Test for deoxysugars (Keller - Killiani test)
- ii) Legal’s test (Test for cardenoloids)
- h) Test for Anthraquinone Glycosides.
- i) Borntrager’s test
- ii) Modified Borntrager’s test
- i) Test for saponin Glycoside Foam test
- j) Test for Alkaloids
- i) Dragendorff’s Test
- ii) Mayer’s test
- iii) Wagner’s test
- iv) Hager’s test
- k) Test for Tannins and Phenolic compounds
- i) Ferric Chloride test
- ii) Lead acetate test
- iii) Dilute Iodine test
- iv) Dilute nitric acid test
- v) Dilute potassium permanganate solution test

- 1) Test for Flavonoids
  - i) Lead acetate test
  - ii) Ferric choride test
  - iii) Sodium Hydroxide test
- 5 Chromatographic Separation
  - 5.1. Thin Layer Chromatography
  - 5.2. Column Chromatography
- 6 Characterization of Isolated compound
  - 6.1 Physical properties
  - 6.2 Chemical tests
  - 6.3 Melting Point
- 7 Spectroscopic Analysis
  - 7.1 Ultraviolet Spectrophotometer (U.V.Spectrum)
  - 7.2 Fourier transform infrared spectroscopy(FTIR)
- 8 Quantitative Estimation Of Phytoconstituents
  - 8.1 Total phenolic Content
  - 8.2 Total Flavonoid Content
- 9 Evaluation Of Pharmacological Activity
  - 9.1 Evaluation of Antioxidant activity
  - 9.2 Evaluation of Antibacterial Activity
  - 9.3 Antifungal Activity
- 6.4 Anthelmintic Activity

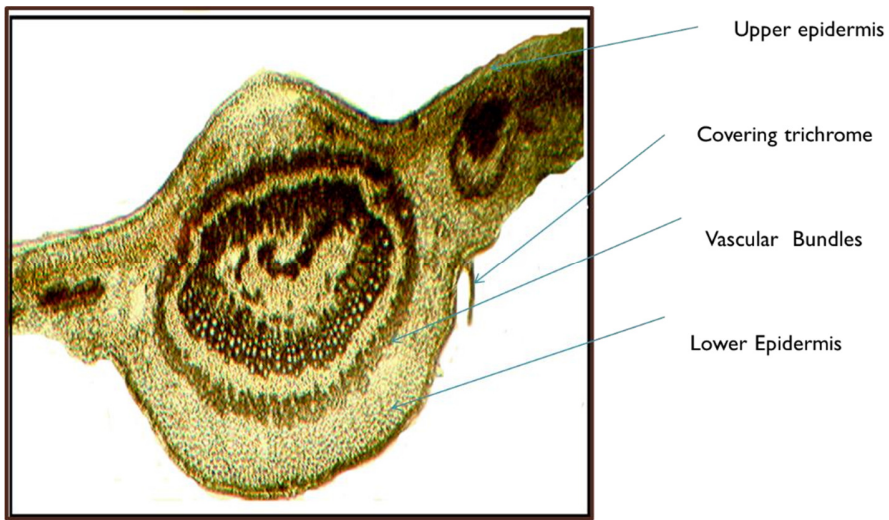
***1. Organoleptic characteristics of leaf of Plant *Balanites aegyptiaca****

<b>Characteristics</b>	<b>Observation</b>
Colour	Bright Green
Odour	Characteristics
Taste	Characteristics

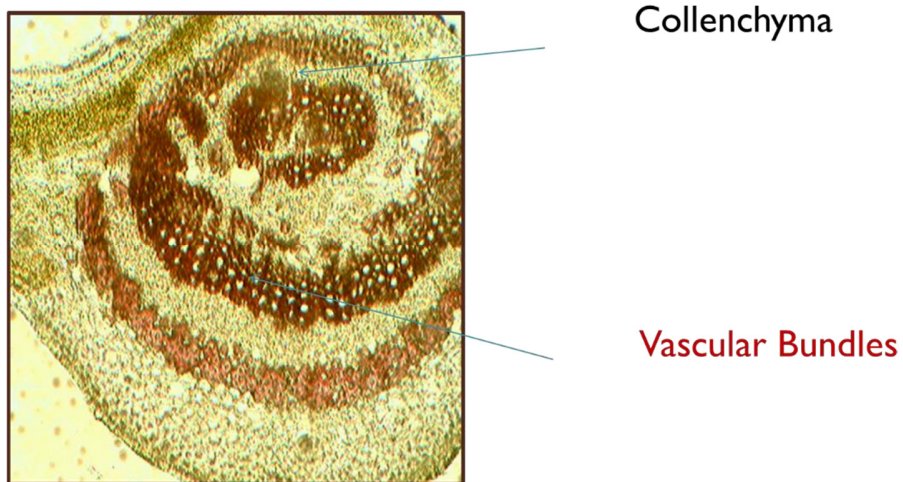
**2. Morphological characteristics of the Leaf of Plant *Balanites aegyptiaca***

<b>Characteristics</b>	<b>Observation</b>
Size	2.5 cm x 6 cm
Shape	Obovate
Apex	Acute
Venation	Reticulate
Margine	Entire
Lamina	Thin
Petiole	Petiolate
Leaf	Alternate, Simple,
Base	Asymmetrical

**3. Microscopical Evaluation of the leaf**



**Figure:** T.S. Of leaf of Plant *Balanites aegyptiaca* stained with Phloroglucinol + Conc. HCL



**Figure** T.S. Of leaf of Plant *Balanites aegyptiaca* stained with Dil. sulphuric acid



**4. Microscopical Powder Characteristics:**

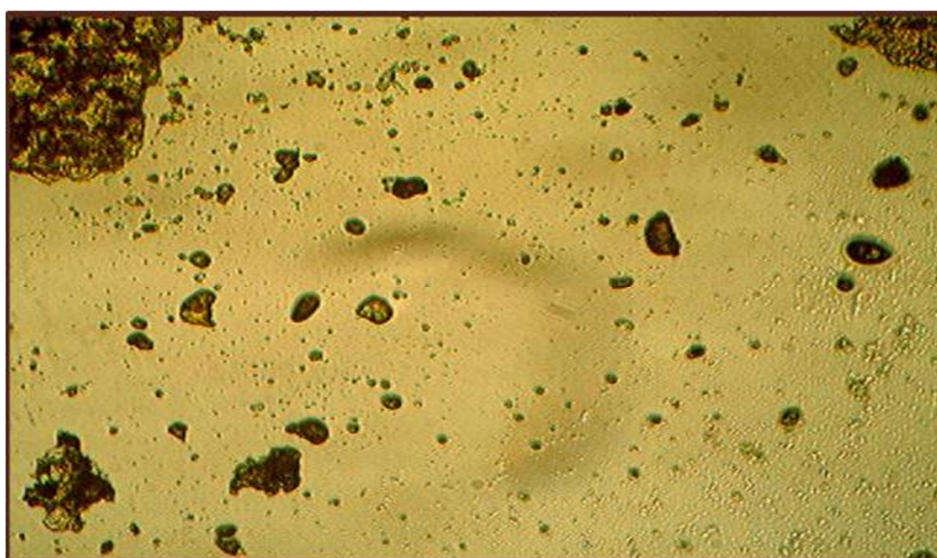


*Figure : Starch grains stained with dil. iodine solution*

**Figure : Starch grains stained**



**Figure Fiber Stained with Phloroglucinol +conc.HCL (1:1)**



*Figure Calcium oxalate crystals stained with Dil. Sulphuric acid*

**5. Microscopical Powder Characteristics of Leaf of Plant *Balanites aegyptiaca***

<i>Sr. No</i>	<i>Test</i>	<i>Result</i>
1.	Powder + Dilute iodine solution	Starch grains observed.

2.	Powder + Phloroglucinol + Conc. hydrochloric acid (1:1).	Lignified fibers observed.
3.	Powder + Dilute sulphuric acid	Calcium oxalate crystals observed.

**6. Microscopical Characters of leaves of Plant *Balanites aegyptiaca***

<i>Sr. No</i>	<i>Reagents</i>	<i>Observation</i>	<i>Inference</i>
1.	Phloroglucinol + Conc. hydrochloric acid (1:1)	Pink	Presence of Vascular Bundles Covering trichome
2.	Dil. Sulphuric acid	Insoluble	Calcium Oxalate Crystals

**7. Determination of Leaf constant of plant *Balanites aegyptiaca***

**7.1 Observation of Determination of Leaf constant**

<i>Sr. No</i>	<i>Particulars</i>	<i>Value</i>
1.	Vein islet number	4.1 - 4.35
2.	Vein termination number	1.9 - 4.45
3.	Stomatal number	13
4.	Stomatal index	48

**7.2 Determination of loss on drying**

<i>Sr. No.</i>	<i>Parameters</i>	<i>Values (%w/w)</i>
1.	Loss on drying	61.56

**7.3 Determination of Ash value**

<i>Sr. No</i>	<i>Parameters</i>	<i>Values (% w/w)</i>
<i>1</i>	<i>Total ash</i>	<i>5.8</i>
<i>2</i>	<i>Water- soluble ash</i>	<i>3.85</i>
<i>3</i>	<i>Acid insoluble ash</i>	<i>5.2</i>

**7.4 Determination of extractive value**

<i>Sr. No</i>	<i>Extractive</i>	<i>Extractive value (%w/w)</i>
<i>1</i>	<i>Alcohol soluble</i>	<i>78.4</i>

<i>2</i>	<i>Water soluble</i>	<i>82.6</i>
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**7.5 Yield of various extracts obtained**

<i>Sr. No.</i>	<i>Extract</i>	<i>Nature</i>	<i>Chloroform extract</i>	<i>Yield (%w/w)</i>
<i>1</i>	Petroleum ether	Very sticky	Dark green	5.62
<i>2</i>	Chloroform	Sticky	Dark green	3.78
<i>3</i>	Methanolic	Thick and sticky	Dark green	47.2
<i>4</i>	Aqueous	Semi solid	Green	17.82

**8. Preliminary Phytochemical Screening**

<i>Sr. No</i>	<i>Chemical test</i>	<i>Petroleum Ether Extract</i>	<i>Chloroform Extract</i>	<i>Methanolic Extract</i>	<i>Aqueous Extract</i>
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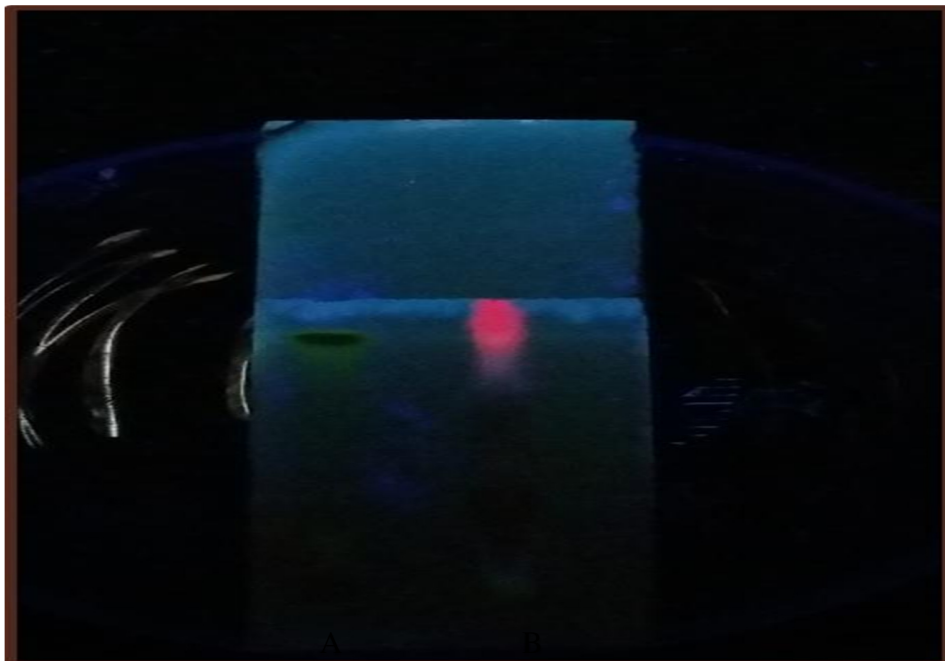
1.	<i>Test for Carbohydrates</i>	-	-	-	-
	<i>a) Molisch Test</i>	-	-	-	-
	<i>b) Fehilings Test</i>	-	-	-	-
	<i>c) Benedicts Test</i>	-	-	-	-
	<i>d) Barfoed's Test</i>	-	-	-	-
2.	<i>Test for Proteins</i>				
	<i>a) a) Biuret Test</i>	-	+	-	-
	<i>b) b) Millions Test</i>	-	+	-	-
	<i>c) c) Xanthoprotien Test</i>	-	+	-	-
3.	<i>Test for Amino Acids</i>	-	-	-	-

	<i>a) a) Ninhydrin Test</i>				
4.	<i>Test for Steroids</i>				
	<i>a) a) Salkowski Test</i>				
	<i>b) b) Liebermann -</i>	+	+	-	-
	<i>c) Burchard reaction</i>	+	+	-	-
	<i>c) Liebermann's reaction</i>				

5.	<i>Test for Glycosides</i>				
	a) <i>a) Deoxysugares</i>				
	b) <i>(Killer-KillaniTest)</i>	-	-	+	+
	c) <i>b) Legal's Test</i>	-	-	+	+
	d) <i>c) Brontrager's Test</i>	-	-	-	-
	e) <i>d) Modified Brontrager's Test</i>	-	-	-	-
6.	<i>Test for Alkaloids</i>				
	a) <i>a) Drogendroff's Test</i>	-	+	+	+
	b) <i>b) Mayers Test</i>	+	+	+	+
	c) <i>c) Hagers Test</i>	-	-	+	+
d) <i>Wagners Test</i>					
7.	<i>Test for Flavonoids</i>				
	a) <i>a) Lead Acetate</i>	-	+	++	+
	b) <i>b) Sodium Hydroxide</i>	-	-	+	+
	c) <i>c) Ferric Chloride Test</i>	-	-	++	+

8.	<p><i>Test for Tannins</i></p> <p><i>a) 5% Ferric Chloride Test</i></p> <p><i>a) b) Lead Acetate Test</i></p> <p><i>b) c) Dilute Iodine Test.</i></p> <p><i>c) d) Dilute Nitric acid Test.</i></p> <p><i>e) Potassium Permanganate Solution.</i></p>	<p>-</p> <p>-</p> <p>-</p> <p>-</p> <p>-</p>	<p>+</p> <p>-</p> <p>+</p> <p>-</p> <p>-</p>	<p>++</p> <p>++</p> <p>++</p> <p>++</p> <p>++</p>	<p>+</p> <p>++</p> <p>++</p> <p>-</p> <p>++</p>
9.	<p><i>Test for Triterpenoids</i></p> <p><i>a) Libermann Burchard's reaction</i></p>	<p>++</p>	<p>++</p>	<p>-</p>	<p>-</p>
10.	<p><i>Test for saponins</i></p> <p><i>a) Foam test</i></p>	<p>-</p>	<p>-</p>	<p>+</p>	<p>+</p>

**9. Observation Under U.V Light**



**Fig:** Under UV light

**10. Column chromatography**

<i>Sr. No.</i>	<i>Fractions</i>	<i>No. of spots</i>	<i>Color</i>	<i>Rf value</i>
<i>1</i>	1-5	No spot	-	-
<i>2</i>	6-9	No spot	-	-
<i>3</i>	10-18	Single spot	Bluish green	0.56
<i>4</i>	19-28	Single spot	Bluish green	0.58

**10.1 Chemical test of isolated compound**

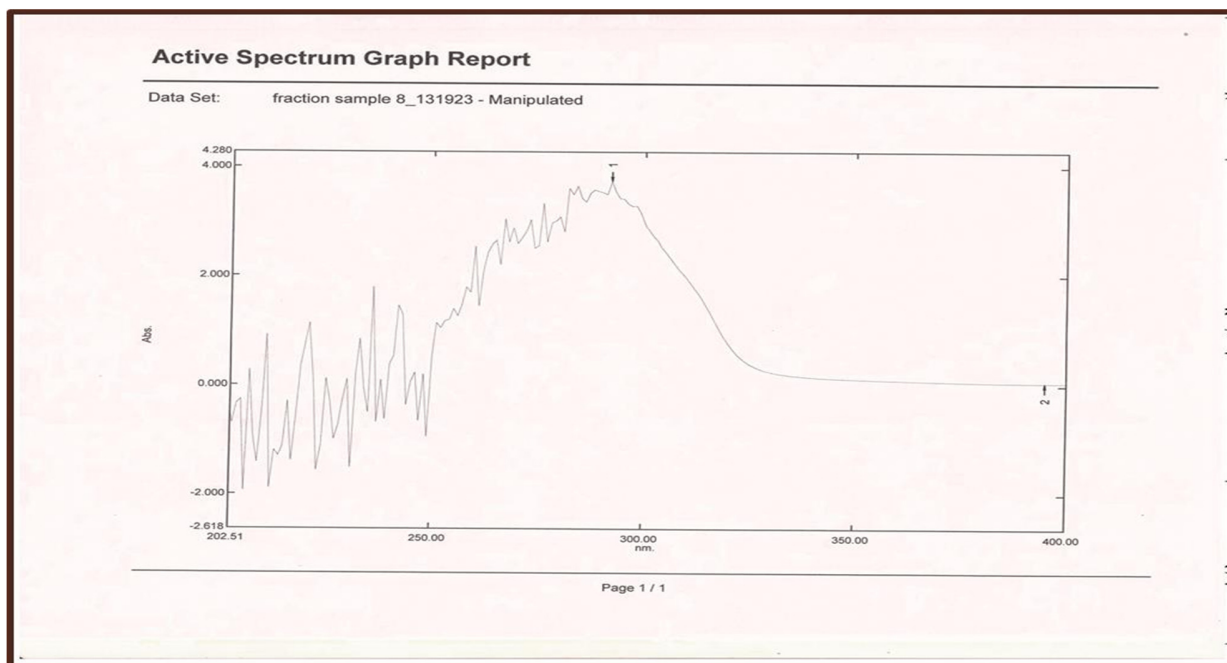
<i>Sr. No.</i>	<i>Chemical test</i>	<i>Absorbance</i>	<i>Inference</i>

<b>1</b>	Ferric Chloride Test	Deep blue color	Tannins may be present
<b>Sr. No.</b>	<b>Isolated compound</b>		<b>Yield from column</b>
<b>1</b>	<b>Compound A</b>		<b>0.52 mg</b>

**10.2 Parameters of isolated compounds**

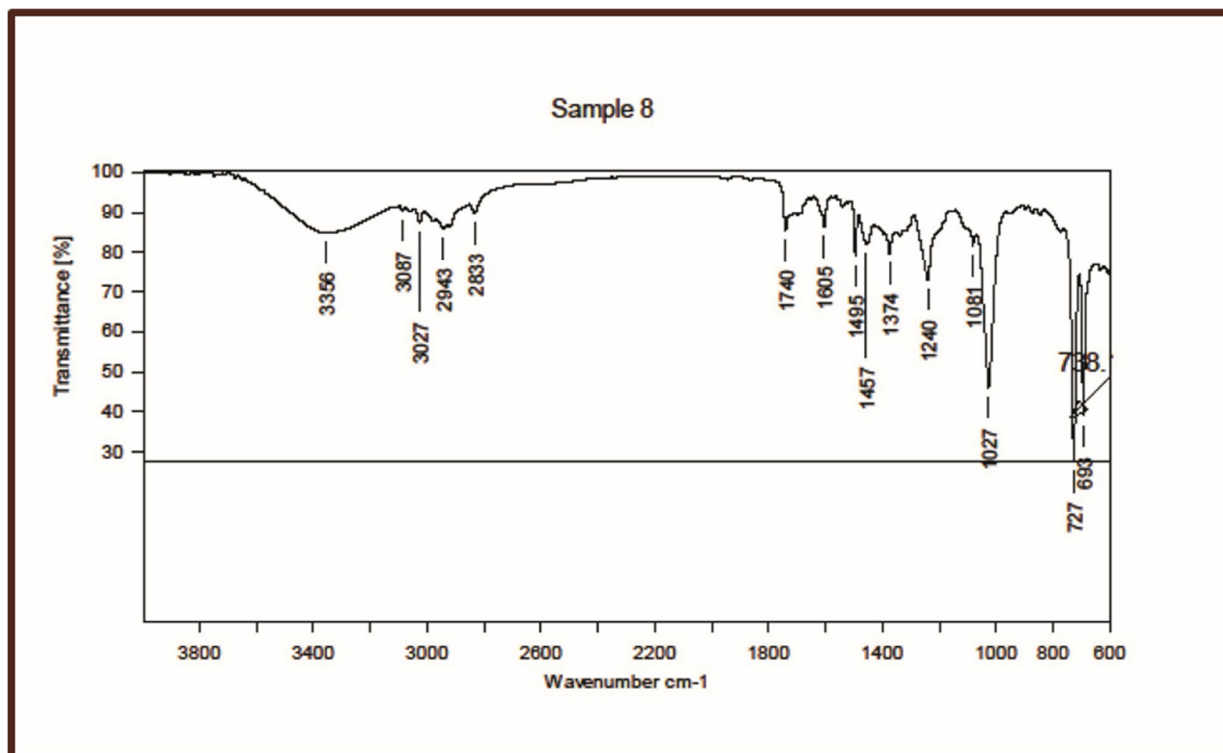
<b>Sr. No.</b>	<b>Parameters</b>	<b>Isolated compounds</b>
<b>1</b>	Physical state	Solid crystalline
<b>2</b>	Color	Bluish green
<b>3</b>	Solubility	Methanol, Ethanol
<b>4</b>	Melting point	258-264 °C

**10.3 Spectroscopy Analysis**





Graph : UV spectra of isolated compound



Graph : FT-IR of isolated compound

<i>Spectra</i>	<i>Characters</i>
U.V	Two peak with $\lambda$ max at 255 & 369nm
FT-IR	Peaks at following wave number are observed Wave number( $\text{cm}^{-1}$ ) 3037.02 C-H Stretching 1699.34 C=O Stretching 3354.32 O-H Stretching 1606.76 C=C Stretching 1291.39 O-H bending 1119.71 C-O-C Stretching

Table: Results of plant *Balanites aegyptiaca*.

**11. QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS**

**Total Phenolic Content**

<i>Sr. No.</i>	<i>Concentration (ug/ml)</i>	<i>Absorbance (nm)</i>
<i>1</i>	10	0.480
<i>2</i>	20	0.84
<i>3</i>	30	0.990
<i>4</i>	40	1.365
<i>5</i>	50	1.548
<i>6</i>	60	2.148
<i>7</i>	70	2.366
<i>8</i>	80	3.054
<i>9</i>	90	3.228
<i>10</i>	100	3.683

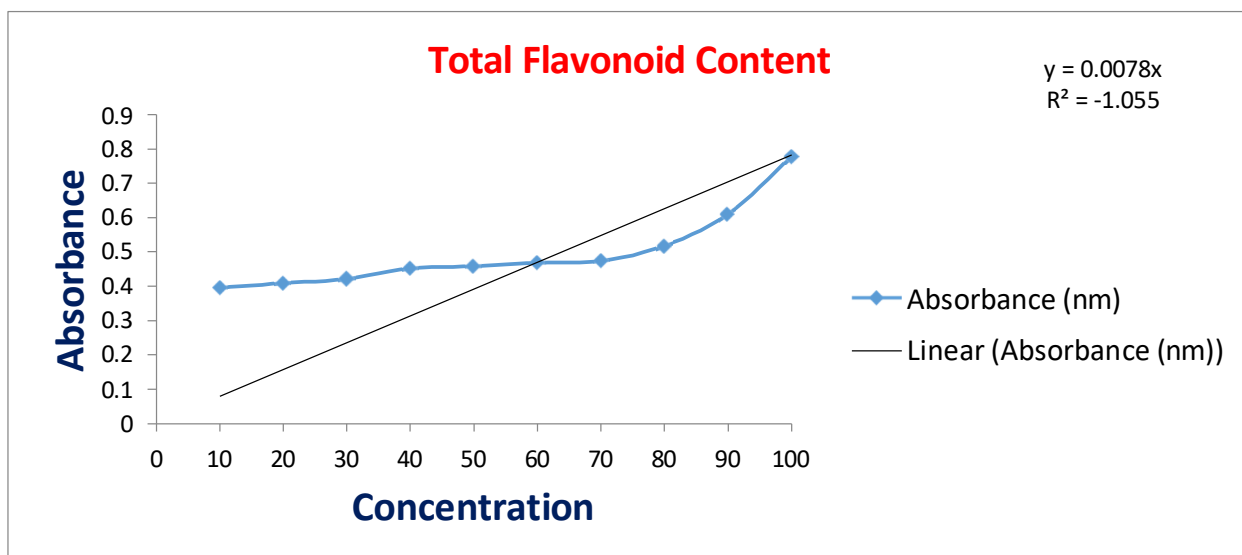
**Table:** Absorbance of standard Gallic acid at different concentration

<i>Sr. No.</i>	<i>Sample</i>	<i>Absorbance</i>	<i>Concentration (ug/ml)</i>
<i>1</i>	<i>Methanolic extract</i>	<i>0.525</i>	<i>15</i>
		<i>0.752</i>	<i>21.48</i>
<i>2</i>	<i>Aqueous extract</i>	<i>0.496</i>	<i>14.17</i>
		<i>0.518</i>	<i>14.80</i>

**Table:** Result of Total phenolic content of *Balanites aegyptiaca* leaves extract

### 11.1 Total Flavonoid Content:

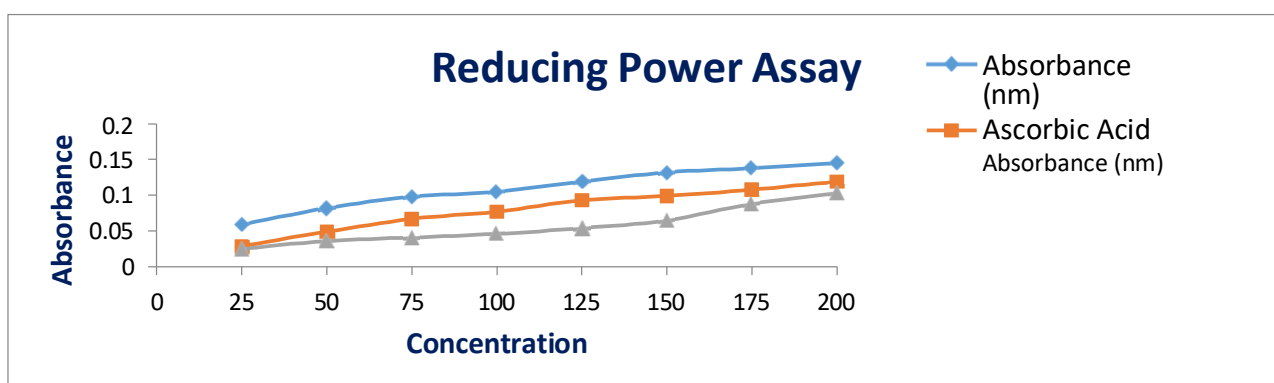
Sr. No	Concentration (ug/ml)	Absorbance (nm)
1	10	0.395
2	20	0.409
3	30	0.421
4	40	0.452
5	50	0.458
6	60	0.468
7	70	0.473
8	80	0.518
9	90	0.610
10	100	0.778



**Graph:** Concentration response curve for Standard Quercetin At different Concentration

**11.2 Determination of reducing power of Standard Ascorbic acid, Methanol and Aqueous extract**

Sr. No	Conc. ( $\mu\text{g/ml}$ )	Absorbance (nm)		
		Ascorbic Acid Standard	Methanolic extract	Aqueous extract
1	25	$0.059 \pm 0.0098^*$	$0.029 \pm 0.0008^*$	$0.025 \pm 0.0008^*$
2	50	$0.082 \pm 0.0003^*$	$0.049 \pm 0.0005^*$	$0.036 \pm 0.0012^*$
3	75	$0.098 \pm 0.0003^*$	$0.067 \pm 0.0008^*$	$0.041 \pm 0.0014^*$
4	100	$0.105 \pm 0.0003^*$	$0.077 \pm 0.0008^*$	$0.046 \pm 0.0008^*$
5	125	$0.119 \pm 0.0003^*$	$0.093 \pm 0.0012^*$	$0.054 \pm 0.0015^*$
6	150	$0.132 \pm 0.0003^*$	$0.099 \pm 0.0014^*$	$0.065 \pm 0.0026^*$
7	175	$0.138 \pm 0.0003^*$	$0.108 \pm 0.0012^*$	$0.088 \pm 0.0014^*$
8	200	$0.145 \pm 0.0003^*$	$0.119 \pm 0.0008^*$	$0.103 \pm 0.0012^*$

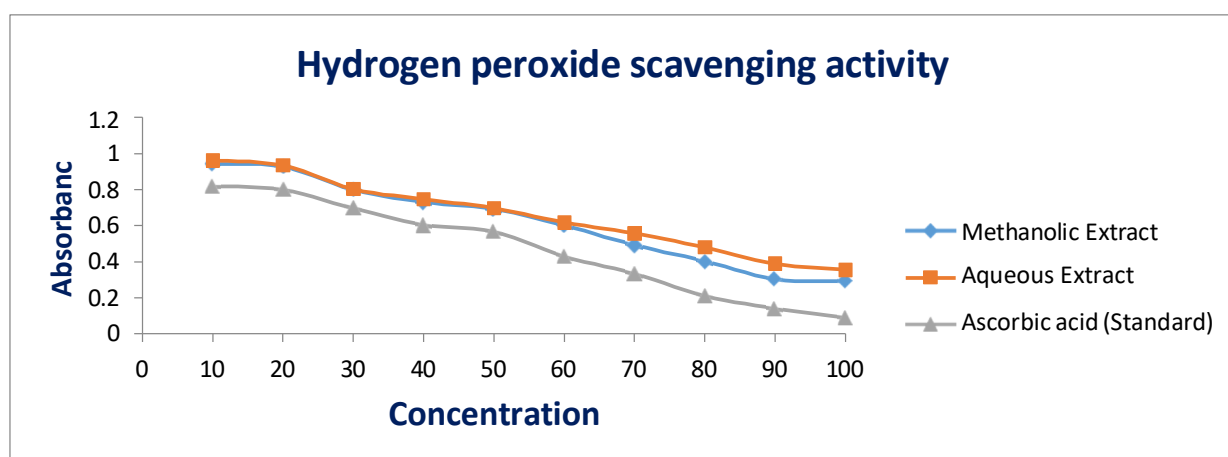


**Graph :** Concentration response curve of Reducing Power determination for Standard Ascorbic acid, Methanol and Aqueous extract

### 11.3 Hydrogen peroxide scavenging activity

Sr. No.	Conc. ( $\mu\text{g/ml}$ )	Methanolic Extract	Aqueous Extract	Ascorbic acid (Standard)
1	10	$0.943 \pm 0.001^*$	$0.962 \pm 0.0006^*$	$0.820 \pm 0.001^*$
2	20	$0.927 \pm 0.0006^*$	$0.934 \pm 0.0006^*$	$0.798 \pm 0.0003^*$
3	30	$0.799 \pm 0.0013^*$	$0.802 \pm 0.0006^*$	$0.696 \pm 0.001^*$
4	40	$0.731 \pm 0.0006^*$	$0.747 \pm 0.0016^*$	$0.604 \pm 0.0006^*$
5	50	$0.692 \pm 0.0006^*$	$0.697 \pm 0.0003^*$	$0.567 \pm 0.0003^*$
6	60	$0.601 \pm 0.0006^*$	$0.618 \pm 0.001^*$	$0.428 \pm 0.001^*$
7	70	$0.492 \pm 0.0006^*$	$0.557 \pm 0.001^*$	$0.333 \pm 0.0003^*$
8	80	$0.403 \pm 0.0006^*$	$0.478 \pm 0.0006^*$	$0.209 \pm 0.001^*$
9	90	$0.302 \pm 0.0006^*$	$0.388 \pm 0.0005^*$	$0.140 \pm 0.0003^*$
10	100	$0.292 \pm 0.0003^*$	$0.355 \pm 0.0006^*$	$0.088 \pm 0.0013^*$

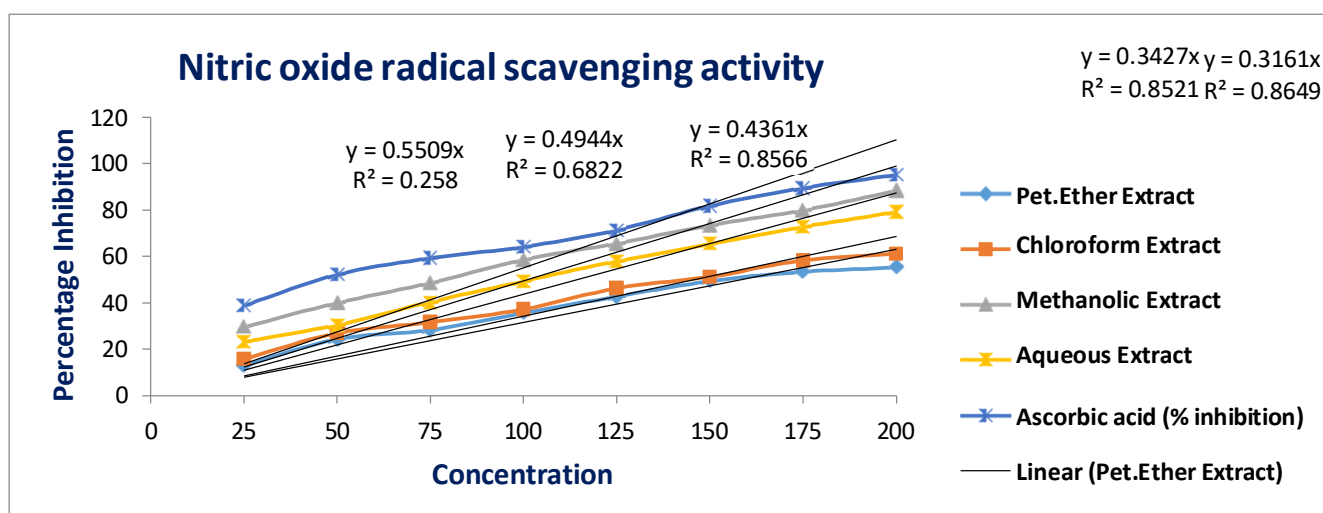
**Table** Hydrogen peroxide scavenging activity of extracts of plant *Balanites aegyptiaca* with Standard Ascorbic acid (Absorbance)



**Graph :** Concentration response curve of Hydrogen peroxide scavenging activity

11.4 Nitric oxide radical scavenging activity

Sr. No.	Conce. ( $\mu\text{g/ml}$ )	Pet Ether Extract (nm)	Chloroform Extract (nm)	Mehanolic Extract (nm)	Aqueous Extract (nm)	Ascorbic Acid (nm)
1	25	$0.124 \pm 0.0008^*$	$0.120 \pm 0.0008^*$	$0.096 \pm 0.0008^*$	$0.110 \pm 0.0008^*$	$0.088 \pm 0.0012^*$
2	50	$0.108 \pm 0.0012^*$	$0.104 \pm 0.0011^*$	$0.086 \pm 0.0006^*$	$0.097 \pm 0.0015^*$	$0.068 \pm 0.0008^*$
3	75	$0.103 \pm 0.0011^*$	$0.098 \pm 0.0008^*$	$0.074 \pm 0.0005^*$	$0.085 \pm 0.0003^*$	$0.058 \pm 0.0008^*$
4	100	$0.092 \pm 0.0008^*$	$0.093 \pm 0.0014^*$	$0.060 \pm 0.0003^*$	$0.073 \pm 0.0012^*$	$0.051 \pm 0.0008^*$
5	125	$0.081 \pm 0.0012^*$	$0.075 \pm 0.0014^*$	$0.049 \pm 0.0008^*$	$0.061 \pm 0.0012^*$	$0.041 \pm 0.0008^*$
6	150	$0.072 \pm 0.0014^*$	$0.068 \pm 0.0005^*$	$0.039 \pm 0.0003^*$	$0.049 \pm 0.0012^*$	$0.026 \pm 0.0008^*$
7	175	$0.065 \pm 0.0001^*$	$0.057 \pm 0.0012^*$	$0.027 \pm 0.0008^*$	$0.039 \pm 0.0017^*$	$0.015 \pm 0.0015^*$
	200	$0.062 \pm 0.0003^*$	$0.054 \pm 0.0008^*$	$0.017 \pm 0.0008^*$	$0.028 \pm 0.0008^*$	$0.007 \pm 0.0003^*$



**Graph:** Nitric oxide radical scavenging activity of various extracts of leaves of *Balanites aegyptiaca* with standard Ascorbic acid.

<i>Sr. No</i>	<i>Extract</i>	<i>IC<sub>50</sub></i>
1	<i>Petroleum ether</i>	158.22 $\mu\text{g/ml}$
2	<i>Chloroform</i>	146.19 $\mu\text{g/ml}$
3	<i>Methanolic</i>	101.22 $\mu\text{g/ml}$
4	<i>Aqueous</i>	114.67 $\mu\text{g/ml}$
5	<i>Ascorbic Acid</i>	90.90 $\mu\text{g/ml}$

**Table:** IC<sub>50</sub> result Nitric oxide radical scavenging activity of various extracts

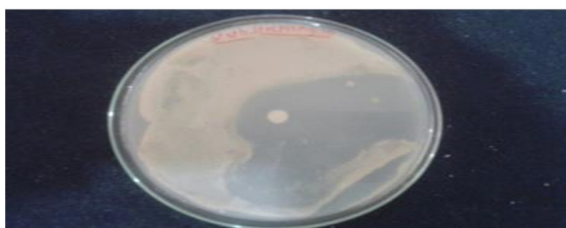
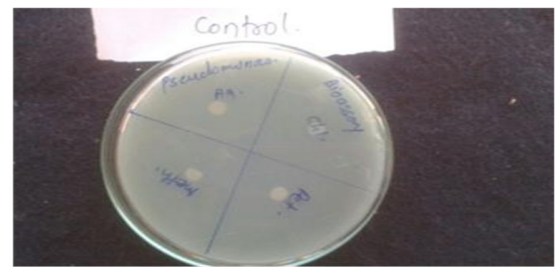
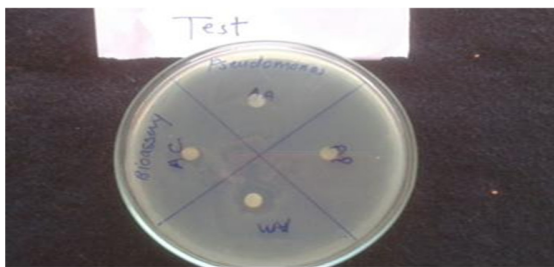
### 11.5 Antibacterial Activity

<b>Extract</b>	<b>Zone of inhibition diameter in mm.</b>			
	<i>E Coli</i>	<i>Pseudo. Aerugi</i>	<i>Staph. aureus</i>	<i>Bacillus subtilis</i>
<b>Petroleum ether extract of <i>Balanites aegyptiaca</i></b>	18	14	15	14
<b>Control</b>	–	–	–	–
<b>Choloroform extract of <i>Balanites aegyptiaca</i></b>	11	12	10	10
<b>Control</b>	–	–	–	–
<b>Methanol extract of <i>Balanites aegyptiaca</i></b>	–	–	–	–
<b>Control</b>	–	–	–	–
<b>Standard Streptomycin</b>	28	29	27	23

**Table :** Result of Antibacterial activity of *Balanites aegyptiaca* plant

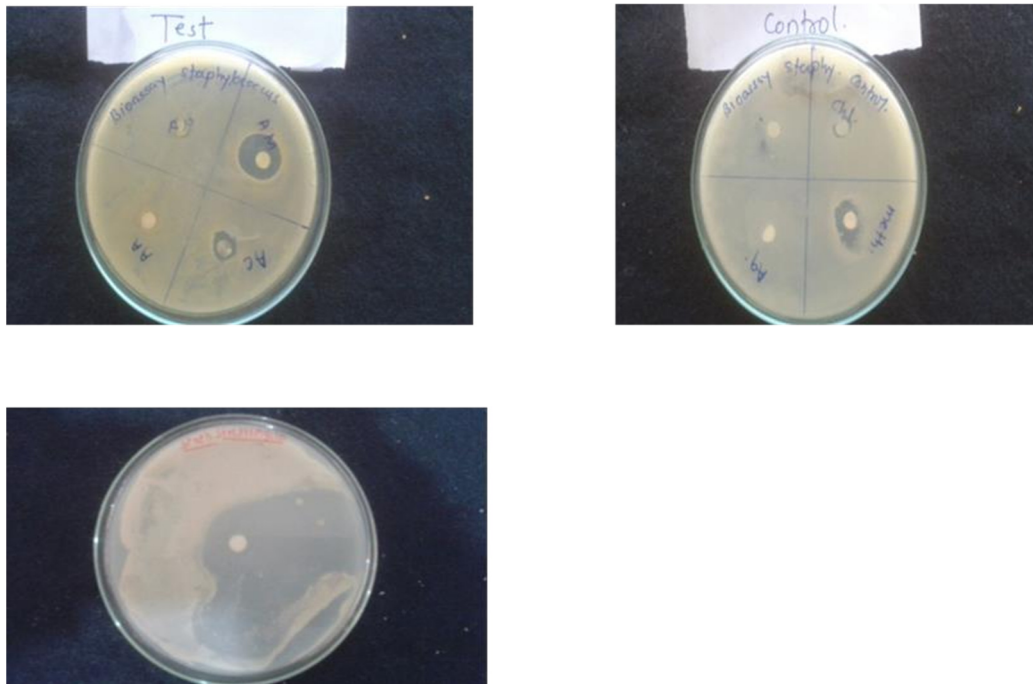


Figure : Antibacterial activity against E.coli

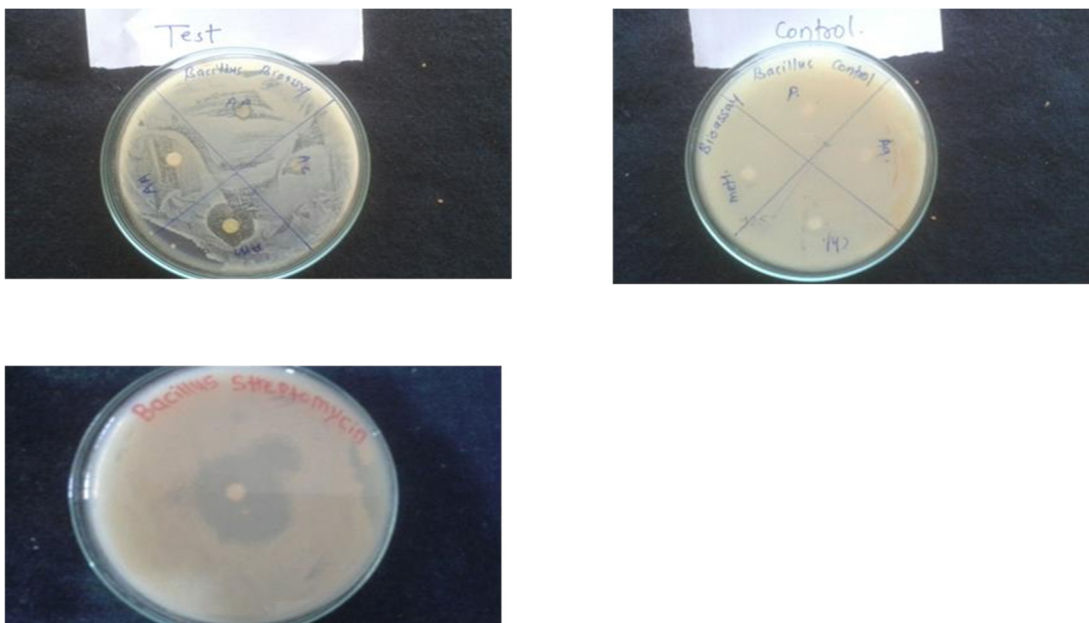




**Figure :** Antibacterial activity against *Pseudomonas aeruginosa*



**Figure :** Antibacterial activity against *Staphylococcus aureus*



**Figure :** Antibacterial activity against *BacillusSubtilis*

### 11.6 Antifungal Activity

Extract	Zone of inhibition diameter in mm.
	<i>Aspergillus niger</i>
Petroleum ether extract of <i>Balanites aegyptiaca</i>	07
Choloroform extract of <i>Balanites aegyptiaca</i>	08
Methanol extract of <i>Balanites aegyptiaca</i>	11
Aqueous extract of <i>Balanites aegyptiaca</i>	12
Standard Amphotericin B	17

Table: Result of Antifungal activity of *Balanites aegyptiaca* plant

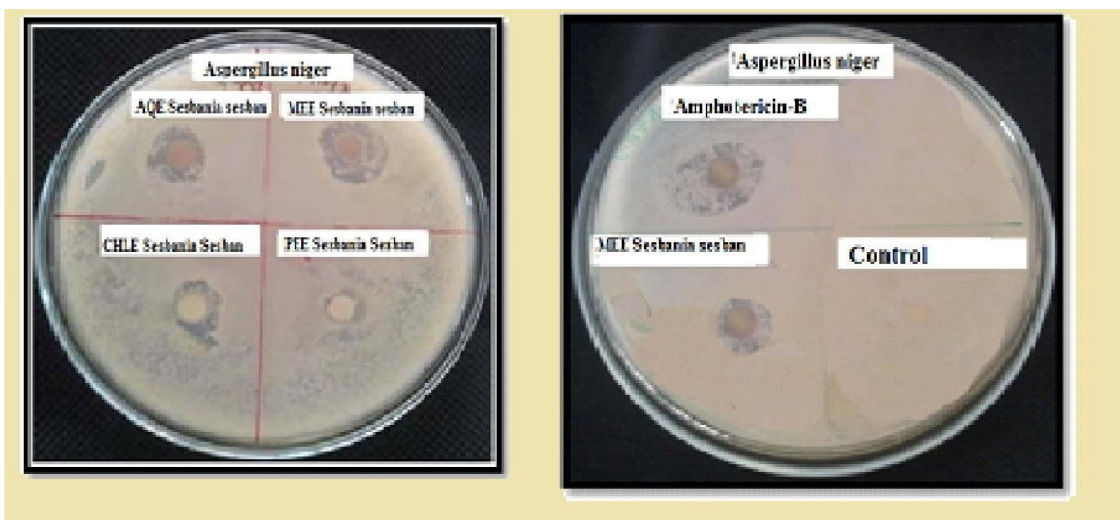
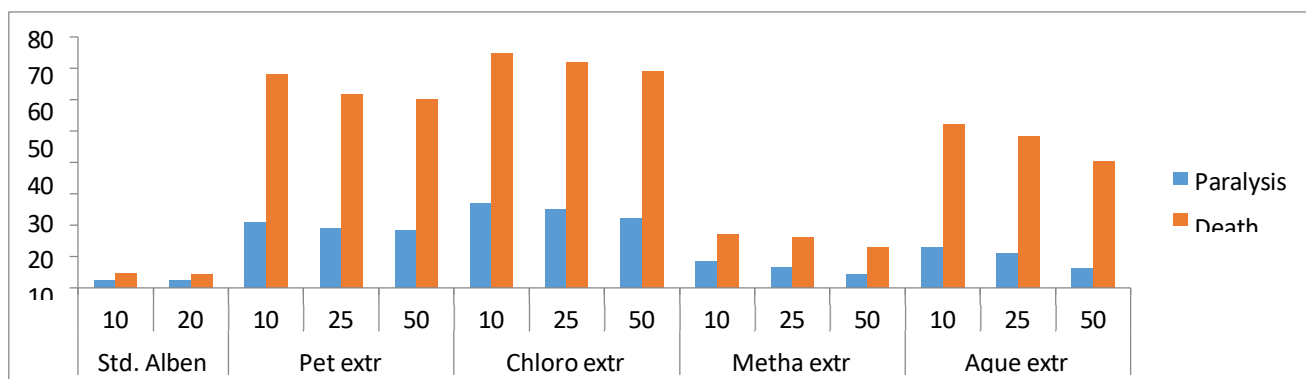


Figure Antifungal activity against *Aspergillus niger*

### 11.7 Anthelmintic activity

Group	Treatment	Concentration (mg/ml)	Time taken by <i>Pheretima posthuma</i> for Paralysis (P) and death (D) of worms in min	
1	Std. Albendazole	10	2.28 ± 0.025*	4.39 ± 0.06*
		20	2.21 ± 0.01*	4.20 ± 0.025*
2	Pet extract	10	23.00 ± 0.51*	66.00 ± 0.50*
		25	18.00 ± 0.30*	64.00 ± 0.50*
		50	19.30 ± 0.10*	61.00 ± 0.50*
3	Chloroform extract	10	26.00 ± 0.40*	71.00 ± 0.50*
		25	23.00 ± 0.60*	69.00 ± 0.50*
		50	21.10 ± 1.00*	65.00 ± 0.50*



**Graph:** Anthelmintic activity of leaves of plant *Balanites aegyptiaca* on *Pheretima posthuma* earthworm

## Discussion:

The present research focuses on the pharmacognostic, phytochemical, and in-vitro

pharmacological evaluation of extracts from the plant *Balanites aegyptiaca*. This plant, commonly known as the desert date tree, has significant traditional medicinal uses, making it an important subject for scientific inquiry. Understanding the pharmacognostic characteristics, including morphology and microscopy, alongside phytochemical properties and pharmacological effects, provides a comprehensive insight into its potential therapeutic applications.

#### Pharmacognostic Investigation

##### *Morphological and Organoleptic Evaluation*

In pharmacognosy, morphological and organoleptic evaluations are fundamental for identifying plant materials. Such evaluations utilize sensory observations to describe the characteristics of the plant. For *Balanites aegyptiaca*, the leaves exhibit distinct traits:

- **Color:** Bright green, which is indicative of healthy foliage and rich chlorophyll content.
- **Odour:** Characteristic scent, contributing to its identification.
- **Taste:** Characteristic taste, suggesting active compounds that may contribute to its medicinal properties.
- **Size:** The leaves measure approximately 2.5 cm x 6 cm.
- **Shape:** Obovate, which aids in species identification.
- **Apex:** Acute, affecting the leaf's overall appearance.
- **Venation:** Reticulate, a feature common in many dicotyledons, indicating complex vascular networks.
- **Margin:** Entire, without lobes or serrations, which affects the leaf's texture and appearance.
- **Lamina:** Thin, facilitating efficient photosynthesis.
- **Petiole:** Petiolate, indicating how the leaf is attached to the stem.
- **Base:** Asymmetrical, which can influence the plant's overall growth and development.

##### *Microscopy*

Microscopy is a vital tool for the identification of plant materials, allowing researchers to discern specific cellular structures that may indicate purity or adulteration. The microscopic examination of *Balanites aegyptiaca* leaves revealed several key features:

- **Epidermis:** A protective layer that helps to prevent water loss and offers resilience against environmental factors.
- **Vascular Bundles:** These structures facilitate the transport of nutrients and water, essential for plant health.
- **Covering Trichomes:** Hair-like structures that may serve various functions, including reducing water loss and protecting against herbivory.
- **Collenchyma and Parenchyma:** These types of cells contribute to the structural integrity and metabolic functions of the leaf.

#### Leaf Constants and Powder Microscopy

The study also included an evaluation of leaf constants, which are essential for the identification of plant materials. In powder microscopy, the leaves of *Balanites aegyptiaca* were observed under different staining techniques to highlight various components:

- **Starch Grains:** Identified when stained with dilute iodine solution, indicating the presence of carbohydrates that may have nutritional or therapeutic benefits.
- **Calcium Oxalate Crystals:** Detected using dilute sulfuric acid, which may play roles in the plant's defense mechanisms.
- **Fibers:** Revealed through staining with phloroglucinol and concentrated hydrochloric acid, contributing to the leaf's structural support.

#### Physicochemical Parameters

The physicochemical evaluation is crucial for assessing the quality and purity of plant materials. Several parameters were investigated:

##### *Ash Value*

The ash value provides insights into the inorganic composition of the plant material, revealing the presence of earthy matter and potential impurities. For the leaves of *Balanites aegyptiaca*, the high total ash content indicates a significant presence of inorganic substances, which is critical for evaluating the drug's quality.

- **Total Ash:** Reflects the total mineral content, providing clues about soil composition and nutrient availability.

- **Water-Soluble Ash:** Indicates the amount of ash soluble in water, highlighting the presence of certain minerals that might be bioavailable.
- **Acid-Insoluble Ash:** Represents the fraction of ash that remains after treatment with acid, typically indicating siliceous impurities.

#### *Extractive Values*

Extractive values serve as indicators of the active components in the plant and are instrumental in assessing the potential for adulteration. The extraction process helps determine the soluble components within the plant material:

- **Water and Alcohol Soluble Extractives:** These values indicate the total amount of active constituents soluble in different solvents. They are particularly useful for identifying exhausted or adulterated drugs, as low extractive values may suggest a lack of active compounds.

The extractive values for *Balanites aegyptiaca* showed promising results, suggesting a rich profile of soluble compounds, which may contribute to its therapeutic efficacy.

#### *Loss on Drying*

Loss on drying is another critical parameter that quantifies the moisture content in the plant material. The leaves of *Balanites aegyptiaca* revealed a moisture content of approximately 61.56% w/w. This high moisture level suggests that the leaves must be carefully processed to prevent spoilage and degradation of active compounds during storage.

### **Conclusion**

The pharmacognostic, phytochemical, and in-vitro pharmacological investigations conducted on *Balanites aegyptiaca* highlight its potential as a valuable medicinal plant. The morphological and organoleptic evaluations provide foundational data for identification, while microscopy reveals the structural characteristics essential for quality assessment. The ash and extractive values serve as important metrics for determining the purity and efficacy of the plant material, crucial for both traditional and contemporary medicinal applications. Furthermore, understanding the moisture content is vital for storage and processing decisions. These findings collectively support the ethnomedicinal claims surrounding *Balanites aegyptiaca* and pave the

way for further exploration of its pharmacological properties. The study reinforces the significance of integrating traditional knowledge with scientific research to uncover the full therapeutic potential of medicinal plants.

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