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Evaluation of Antimicrobial Activity and Phytochemical Analysis of Ethanolic Leave Extract of *Balanites Aegyptiaca*

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***Abstract - Balanites Aegyptiaca* is a medicinal plant used in Africa's folk medicines to treat wide range of diseases. Phytochemical analysis of ethanolic extract of balanites aegyptiaca revealed the presence of flavonoids, Glycosides, Alkaloids, Tannins, Anthraquinones, Terpenoids, phenols, saponins. However, steroids was found to be absent. The plant was evaluated for potential antimicrobial activity against medically important bacterial and fungal strains. The antimicrobial activity was determined in the extracts using agar well diffusion method. The antibacterial and antifungal activities of extracts in different concentrations 5, 10, 15, and 20 mg/ml were tested against two Gram-positive *Staphylococcus aureus, Staphylococcus Epidermis*; two Gram-negative, *Escherichia coli, Pseudomonas aeruginosa* and two fungal strains, *Aspergillus flavus, Candida albicans*. The zones of inhibition of the extracts were compared with that of different standards like ampicillin 1mg/ml for antibacterial activity and fluconazole 1mg/ml for antifungal activity. The results showed that the extract exhibited a remarkable inhibition of the microbial growth against the tested organisms.**

Keywords: Phytochemical, *Balanites Aegyptiaca*, Antimicrobial Activity, Medicinal plant, Ethanolic extract.

I. Introduction

Traditionally people use plant materials to cure diseases and infections, because plants contain components of therapeutic values. These medicinal plants are known to owe their curative potentials to certain biological active substances which exist in parts of the plants known as phytochemicals. Phytochemicals are bioactive non-nutrients compounds naturally found in plant parts such as flower, buds, roots, leaves, fruits, seeds and barks.

The compounds work as a defensive mechanism for the plants against diseases and many external attacks and they also provide characteristic colour, aroma and flavour to the plants [1]. The fact that plants extract exhibits bioactivity of interest, modern pharmaceutical industry itself still relies largely on the diversity of phytochemicals obtained from plants [2].As a result of the development of antimicrobial drug resistance and often the occurrence of undesirable side effect of some antibiotics, many researchers have focused on the investigation of plants as potential antimicrobial agents [3]. *Balanites aegyptiaca* also known as 'Desert dates' in English, belongs to the genus balanites, which consists of nine species and eleven intra-specific taxa. It is an arid and semi-arid multi branch evergreen tree reaching up to 10 metres in height and is widely distributed in the Sudano-Sahielian region of Africa, the Middle East and South Asia [4]. *Balanites aegyptiaca* has good adaptive mechanisms to grow and thrive under different soil types, salinity stresses and climatic moisture levels [5]. It is also a tree with a wide range of products and values such as food, fodder, shade, oil and traditional medicine. The pharmacological investigation of different part of the plant has several usefulproperties such as insecticidal, antimicrobial, antioxidant and anti-inflammatory activity [6]. Balanites 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 [7,8]

II. Materials and Methods

2.1 Plant Material

The leaves of *balanites aegyptica were* collected in Wuro Harde area of Mubi-North, Adamawa State Nigeria. And was identified and authenticated in the Botany Department, Adamawa State University, Mubi. The fresh leaveswere dried under a shed and powdered with a pestle and mortar. The powder was then passed through a sieve and stored in an airtight container until extraction.

2.2 Preparation of Extracts

100 g of the leaf powder was dissolved in 500ml of ethanol and subjected to maceration for 48 hours, which was performed five times with continuous stirring, filtration using

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filter paper, and a combination of the five portions. The final extract was then concentrated under reduced pressure in a rotary evaporator at 40 °C, and further dried for 48 h in an oven at 40 °C to produce dry powder, packed in a vial, and kept in a desiccator containing silica until the in vitro experiments were performed.

2.3 Phytochemical Analysis

Standard phytochemical test was carried out on the plant samples using the method of described by [9,10] to determine presence of saponins, flavonoids, glycosides, alkaloid, tannins, anthroquinone, terpenoid, steroids and phenols.

2.4 Test Organism

The clinical strains of the microrganisms were obtained from microbiology Department Adamawa State University, Mubi. Two gram-positive (*Staphylococcus aureus* and *staphylococcus epidermis*) and two gram-negative (*Escherichia coli*, and *Pseudomonas aeruginosa)*. As well as two fungal organism (*Candida Albicans* and *Aspergillus flavus*).

2.5 Antimicrobial Assay

Agar well diffusion method was employed for the antimicrobial activity of balanites aegyptiaca plant extracts as described by [11]. The cultures of all the bacterial strains were grown in Muller Hinton agar plates at 37 °C for 24 h. The colonies of all bacteria were dissolve in normal saline with a sterilized inoculating loop, and the turbidity of the test suspension was compared with McFarland turbidity standard. A serialized swab was then used to streak a sample of each inoculum of the standard bacteria taken from the liquid medium into agar plates to assure formation of uniform growth throughout incubation. A sterile cork borer was then used to form bore wells of 6mm size on the agar plates. The different concentrations of plant extracts as well as the extract of the control drugs Ampicillin 1mg/ml for bacteria, Fluconazole 1mg/ml forfungal organisms were dispensed into the wells using a micropipette. All the plates containing the test extract and the control drug were then kept at room temperature for 1h for diffusion. Finally, the measurement of the diameter of zones of inhibition was performed for each plate after 24 h incubation at 37°C for Bacteria and 48 hours for the fungal organism.

III. Results and Discussions

The phytochemical analysis of the balanites aegyptiaca ethanolic extract revealed the presence of flavonoids, Glycosides, Alkaloids, Tannins, Anthraquinones, Terpenoids, phenols, saponins. And steroids was found to be absent as shown in table 1. The positive test results for alkaloids, saponins, and flavonoids are supported by the results of another study on balanites aegyptiaca plant [12]. However confirmatory test results for anthraquinones in this study contradict the same study. The variation may be due to the difference in climate, soil, and other environmental conditions for plant growth in the current study and solvent effect.

Table 1: The Qualitative Phytochemical Analysis of Ethanolic Leave Extract of Balanites Aegyptiaca

S/N	Phytochemicals	Inferences
1	Flavonoids	++
2	Steroids	-
3	Glycosides	+
4	Alkaloids	++
5	Tannins	+
6	Anthraquinone	+
7	Terpenoids	+
8	Phenols	+
9	Saponins	++

Key: + = Slightly present, ++ = Moderately present, - = Absent

Inhibitory Effect of the Ethanolic Extract of Balanites Aegyptica on Test Organism

Plant extracts having chemicals with antimicrobial activity usually belong to alkaloids, .determination of antimicrobial activity in the current study were the common causes of illness in the community. The antimicrobial activity of the extracts of *balanite Aegyptica* were studiedin different concentrations 5, 10, 15, and 20 mg/ml against four pathogenic bacterial strains, two Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermis*) and two Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*), and two fungal strains (*Aspergillus flavus*, *Candida albicans*). The antimicrobial potential of extracts were assessed in terms of zone of inhibition of the microbial growth.

The results of the antimicrobial activities are presented in Tables 2 to 4. The activities of the extracts against the test organism increased linearly with increase in concentration of extracts (mg/ml). The results revealed the extracts possess significant activity against most of the test organisms. The extract exhibited antibacterial activity against gram positive bacteria as shown in table 2. *Staphylococcus aureus* were more sensitive with zone of inhibition 15.7 mm at conc. 20 mg/ml as compared with *Staphylococcus epidermis* 12.5mm. and for gram negative bacteria is shown in table 3, *Pseudomonas aeruginosa* were more sensitivewith inhibitory

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zone 16.5mm at conc. 20 mg/ml as compared with *Escherichia coli* with 15.5mm. The results in table 4 showed the antifungal activity of the extracts, *Candida albicans* shows good result with inhibitory zone of 15.8mm as compare to Aspergillus *flavus* which showed the lowest degree of sensitivity at 9.3mm.The presence of flavonoids, alkaloids, phenols, terpenoids and tannins in the leaves extracts of *Balanite aegyptica* has medicinal implications. These phytochemicals are known to be biologically active, and were found to play a role in antifungal, antibacterial activities [14, 15].

Table 2: Inhibitory effect of Ethanolic extract of Balanites Aegyptica and Ampicillin against the gram positive bacterial organism

Samples	Conc. mg/ml	Zones of Inhibition (mm)	
		Staphylococcus Epidermis	*Staphylococcus Aureus*
Ethanol Extract	5	8.4±0.32	11.5±0.25
	10		9.5±0.35
13.4±0.35			
	15		10.6±0.28
14.8±0.34			
	20		12.5±0.35
15.7±0.41			
Ampicillin	(1mg/ml)		15.2±0.25
17.8±0.28			

Key: Results are presented as mean ± SD of triplicate MeasurementsAmpicillin (control).

Table 3: Inhibitory effect of Ethanolic extract of Balanite Aegyptica and Ampicillin against the gram negative bacterial organism

Samples	Conc. mg/ml	Zones of Inhibition (mm)	
		Pseudomonas Aeruginosa	*Escherichia Coli*
Plant Extract	5		13.5±0.32
12.7±0.40			
	10	14.4±0.25	13.5±0.35
	15	15.8±0.28	14.6±0.28
	20		16.5±0.35
15.5±0.25			
Ampicillin	(1mg/ml)		15.0±0.25
16.8±0.28			

Key: Results are presented as mean ± SD of triplicate Measurements Ampicillin (control).

Table 4: Inhibitory effect of Ethanolic Extract of Balanite Aegyptica and Fluconazole against Fungal Organism

Samples	Conc. mg/ml	Zones of Inhibition (mm)	
		Candida albicans flavus	*Aspergillus*
Plant Extract	5	12.3±0.25	0 ±0.00
	10	13.5±0.27	0±0.00
	15	14.2±0.32	8.7±0.35
	20	15.8±0.38	9.3±0.26
Fluconazole	(1mg/ml)18.5±0.25		13.4±0.40

Key: Results are presented as mean ± SD of triplicate Measurements, 0±0.00 = No Inhibition, Fluconazole (control).

IV. Conclusion

The results obtained from this study revealed the presence of important phytochemicals in the ethanolic leaves extract of *balanites aegyptiaca*. The significant zones of inhibition exhibited by the extract on the tested organisms indicated that it has a broad spectrum antimicrobial property. This justifies the use of this plant in traditional medicine practices. The plant may be of great use for the development of medicines by pharmaceutical industries as a therapy against various diseases.

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