

The Antibacterial Activity of Aqueous and Ethanolic Leaf Extracts of *Balanites aegyptiaca* (L.) Del Plant on Some Selected Clinical Human Pathogens

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Authors' contributions

This work was carried out in collaboration among all authors. Authors FPC and GCE contributed in the course of conception, design and manuscript writing. Authors FPC and JCA supervised the work, contributed during analysis and interpretation of results. Author GCE wrote the protocol, conducted the research work, literature searches, managed the study and laboratory analysis and wrote the first draft of the manuscript. The three authors managed the analysis of the study, read and approved the final manuscript.

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ABSTRACT

The antibacterial activity of the leaf extract of *Balanites aegyptiaca* plant was investigated on five selected clinical common human pathogens: *Staphylococcus aureus*, *Streptococcus pneumonia*, *Salmonella typhimurium*, *Shigella dysenteriae* and *Escherichia coli*, *in vitro*. The phytochemical screening, susceptibility testing and Minimal Inhibitory Concentrations were determined. Preliminary phytochemical screening of the leaf extracts indicated the presence of alkaloids, cardiac glycosides, flavonoids, glycosides, resins, saponins, steroids, tannins, and carbohydrates in varying concentrations. Ethanolic leaf extract was more effective. The extracts inhibited the growth of

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isolates with increasing concentrations, ranging from 8-12 mm zones of inhibitions, showing the susceptibility of the test organisms. *S. dysenteriae* had the highest susceptibility for both extracts with 12 mm and 13 mm zones of inhibition for aqueous and ethanolic extracts respectively at the highest concentration of 400 mg/ml. *S. aureus* showed the least susceptibility with 8 mm for aqueous extract while *S. typhimurium* showed the least susceptibility for the ethanolic extract with 9mm zones of inhibition. The analysis of variance results on zones of inhibition revealed a significant difference for both extract treatments, concentrations, and interactions between the treatments and concentrations. The Minimal Inhibitory Concentrations determined from the corresponding concentration-response curves showed that *S. dysenteriae* had the least value of 79.433 mg/ml while the aqueous extracts against *S. aureus* and *S. typhimurium* were the largest with 125.893 mg/ml value. The activity index and activity were also deduced to measure activity. The antimicrobial activity of leaf extracts of *B. aegyptiaca* on bacteria of the tested isolates have been established in this study and justify the claims by the traditional healers in its use to treat infectious diseases.

Keywords: *Balanites aegyptiaca*; antibacterial activity; activity index; total activity.

1. INTRODUCTION

Infectious diseases account for approximately one-half of all deaths in tropical countries. The death rate has increased by 58% from 1981 to 1992 and it's still on the increase worldwide till date. Recently, the continuous increase in antibiotic resistance of human is reaching critical levels [1]. This is exacerbated by the inadequacy and inequitably provided health care, as well as human behavior. This implies an increase in morbidity in the healthy population and an imminent risk for hospitalized patients and the immunocompromised [2]. In the pharmaceutical industry, past and current strategies to combat resistance have not been very effective, coupled with the existing short life expectancies of antibiotics [3].

As a result of these threats to man and animals, there has been continuous search for effective alternative treatment and control of antibiotic resistance of pathogenic microorganisms [4,5]. Traditional medicine has been described by the World Health Organization WHO, as one of the surest means to achieve total health care coverage of the world's population [6]. Therefore, many plants are constantly being screened for their medicinal properties, by carrying out scientific researches on them to ascertain, validate and verify their potential [7,8]. Phytochemistry and pharmacological studies of some of these plants have already resulted in the discovery and development of many important drugs [8].

Consequently, this research is centered on the plant *B. aegyptiaca* (L.) Del. which belongs to the family Balanitaceae or Zygophyllaceae. According to [9], the tree was figured and

described in 1592 by Prosper Alpinus under the name 'Agihalid' but the genus was founded by Delile in 1813. It is a multi-branched, evergreen tree, native to much of Africa and parts of the Middle East. A few of its numerous names are Aduwa in Hausa, Eduwa in Yoruba and thorn tree/desert date (fruit) in English. The tree reaches 10 m (33 ft) in height generally narrow form, thorny, branched, dark green compound leaves made up of two leaflets of variable size and shape with single seeded edible fruit [10]. The tree produces several forms of inflorescence [11].

Various parts of the plants are used in different folk medicines for the treatment of different ailments such as syphilis, yellow fever, jaundice, liver and spleen problems, skin boils, wound dressing, pain, epilepsy, cough, malaria, leukoderma and aches [12,13]. They also found uses in food preparations [14,15], non-medicinal products [16], and believed to have magical-religious properties [17].

It was found to be a rich source of saponins, furanocoumarins and flavonoids, alkaloids, metabolites, glycosides, and nutrients such as proteins, lipids, carbohydrates, organic acids amongst others [13,18-20]. The plant was reported to have antibacterial [21-24], antifungal [25,26], antiviral [12], anti-parasitic [27], anticancer [28]; antihelmintic and molluscicidal activities [20,29] and others such as hepatoprotective, cardioprotective, antioxidant, vermifugal, anti-insecticidal, wound healing, spermicidal, antidiabetic properties [20,21].

In the present scenario of antibiotic resistance of human disease-causing microorganisms, it has become obligatory to explore other potential

alternatives to source for new antimicrobial substances such as in plants. Although works have been done to evaluate the medicinal properties of *B. aegyptiaca*, not much antimicrobial activity studies have been documented. Therefore this research is to test the antimicrobial effects of the leaf extract on *S. aureus*, *S. pneumonia*, *E. coli*, *S. typhimurium* and *S. dysentriae*, *In vitro*.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The plant was selected based on their ethno medical and use for conditions related to bacterial infections. Fresh, pesticide free leaves of the plant, *B. aegyptiaca* were collected from around British America in Jos North Local Government Area, Plateau State, between July and August (Plate I). The plant material was labeled, numbered, noted with the date of collection, locality, and their medicinal recorded.

2.1.1 Identification

The plant was identified and authenticated by the herbarium, Federal School of Forestry, Jos, and given the voucher specimen number FHJ 995.

2.1.2 Preparation of the leaves

The fresh leaves were washed with distilled water to remove dirt; air dried under shade at room temperature to a constant weight for 5 days



Plate I. *Balanites aegyptiaca*

(Plate II). It was blended to fine powder using a manual blender and sieved. The powder was then stored in a seal, well labelled and airtight container prior to use [30,31].

2.2 Aqueous and Ethanolic Extraction (by Maceration)

Extraction of plant parts was carried out using modified procedures described [32]. The extracts were prepared by adding 100 g of the powder to 250 ml of sterile distilled water and also to ethanol (95%) for 72 hours and filtered using Watman no 1 filter pare. The filtrate of ethanolic extract was concentrated under vacuum to dryness under reduced pressure using rotary evaporator to obtain the crude extracts while water extract filtrate was gently concentrated by evaporation on water bath at 45°C to dryness, not exceeding the boiling point of the solvent (water) [30] The resultant samples were stored in well labelled sterile sample bottles and kept in the desiccator prior to use (Plate III).

2.3 Phytochemical Screening

The extracts were tested for the presence of bioactive compounds at the Biochemistry Laboratory of Federal College of Veterinary, Jos, by using the standard methods [33,34].

Test for tannins: Each of the crude extracts were mixed with 2 ml of 2 % solution of FeCl₃. A blue green precipitation was taken as evidence for tannins.



Plate II. Dried leaves of *Balanites aegyptiaca*

Test for saponins: Each of the crude extracts of about 0.5g was mixed with 5 ml of distilled water in test tubes and shaken vigorously. The formation of stable foam was taken as indication of the presence of saponins.

Test for flavonoids: To 2 ml of 2 % solution of NaOH, 0.5 g each of plant extract was dissolved followed by a few drops of concentrated H₂SO₄. A resultant colourless solution indicated the presence of flavonoids.

Test for cardiac glycosides (Keller-Kilani Test): To 2 ml of glacial acetic acid containing 1-2 drops of 5% solution of FeCl₃, 0.1 g of extracts were dissolved. These mixtures were then poured into other test tubes containing 2 ml of concentrated H₂SO₄. A brown ring contained at the interface indicated the presence of cardiac glycosides.

Test for alkalodes: About 0.5 g of each plant extract was stirred with 3 ml of 1 % aqueous HCL in a steam bath, 1 ml of the filtrate was treated with few drops of Dragendorff's precipitate's reagent. Turbidity of the resulting precipitate was taken as evidence of alkaloids.

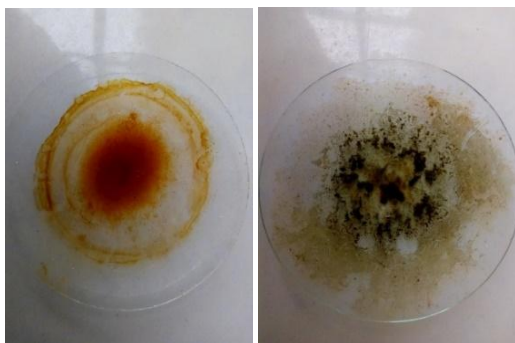


Plate III. Aqueous and ethanolic leaf extracts of *Balanites aegyptiaca*

Tests for steriodes (Salkowski test): about 0.1 g of the extracts was dissolved in 2 ml of chloroform. H₂SO₄ was carefully added to form a lower layer. A red colour produced in the lower chloroform layer and greenish yellow fluorescence in the acid layer indicated a positive result.

Test for resins: To 5 ml of boiling ethanol, 0.5 g of the plant extracts, filtered through Watsman no 1 filter paper was mixed, and then diluted with 2 ml of 1 % aqueous HCL. The formation of resinous precipitate indicated the presence of resins.

Test for carbohydrates (Iodine Test): To 2 ml of iodine solution, 0.5 g each of the plant extracts was added and mixed. A dark blue coloration indicated the presence of carbohydrates.

Test for anthraquinones (Borntrager's test): 3 ml of the extracts were shaken with 3ml of benzene and then filtered. Further 5ml of 10% ammonia solution was added to the filtrate. The mixture was shaken vigorously for 30 seconds. A pink, red or violet coloration in the ammonia (lower) phase indicated the presence of Anthraquinones.

Test for Terpenoides: To 2 ml of the extracts, 2 ml of chloroform was dissolved and evaporated to dryness. Concentrated Sulphuric acid (2 ml) of was then carefully added and heated for about 2 min. A greyish colour indicated the presence of terpenoides.

2.4 Preparation of Culture Media

The commercial media (Nutrient broth and agar, Mueller Hinton Broth and agar, Salmonella Shigella agar (Oxoid Ltd, London, UK) were all prepared according to standard manufacturing's instructions.

2.5 Test Organisms

The test organisms were identified clinical isolates obtained from the Bacteriology Unit of National Veterinary Research Institute, Vom. It comprised of two gram positive bacteria (*S. aureus* and *S. pneumoniae*) and three gram negative bacteria (*E. coli*, *S. typhimurium* and *S. dysenteriae*).

2.5.1 Preparation of test bacteria isolates

Fresh isolates of nutrient agar plates of test organisms were made from the stock cultures obtained on agar slants incubated for 24 hours at 37°C. Several colonies of the different bacteria isolates were picked and suspended in 5ml of broth in sterile bijou bottles and grown under same condition.

2.5.2 Characterization and confirmatory identification test of bacteria isolates

Subcultures were produced and discrete colonies were identified by bacteriological analysis using selective media method, morphological

characterization on the basis of simple staining and gram staining, and set of biochemical characterization such as catalase, coagulase, bile solubility and triple sugar iron medium tests by standard methods as described [35-37].

2.6 Preparation of the Stock Solution of Extracts

The stock solution of the extracts were prepared by dissolving 8 g of each extract in 5 ml of sterile distilled water to a concentration of 1600 mg/ml, and was labelled appropriately [38].

2.7 Purity (Sterility) Test

Loopfuls of extracts were streaked on a prepared Nutrient Agar and incubated for 24 hours at 37°C for possible growth [39].

2.8 Determination of Antimicrobial Activity of Extracts

The agar well diffusion method as described by [40] was used. McFarland turbidity (0.5) standard equivalent to 1.5×10^8 CFU/ml of each test bacteria was spread on Mueller-Hinton agar plates so as to achieve even growth, allowed to dry and a sterile cork borer (5.0 mm diameter) was used to bore holes in the agar plates. The concentration of the stock was made to 400 mg/ml and subsequent concentration gradients of the extracts were prepared by double dilution to concentration of 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml. Subsequently, 0.1 ml of each concentration of the extracts was introduced in wells earlier bored. Ciprofloxacin (0.625 mg/ml) was used as positive control. The plates were then incubated at 27°C for 24 hours. Zones on inhibition of ≥ 7 mm would be considered positive [38].

2.9 Determination of the Inhibitory Concentration (MIC)

A plot of the square of radius diameter of the zones of inhibition against log concentration of the dilutions was done and a suitable curve drawn from the plots of each extracts. Extrapolation of the curves was done to determine the log of MIC. From this log the MIC was calculated as the antilog [41].

2.10 Determination of Activity Index (AI)

The activity index of the plant material was derived using the formula described by [42].

Activity index = Inhibition Zone of the sample/
Inhibition Zone of the standard

2.11 Determination of Total Activity (TA)

The total activity of the plant material extracted from one gram of dried plant material was derived using the formula described by [42].

$$\text{Total activity (ml/g)} = \frac{\text{Amount extracted from 1 g (mg g}^{-1}\text{)}}{\text{MIC (mg mL}^{-1}\text{)}}$$

2.12 Statistical Analysis

All the experiments of antimicrobial susceptibility testing were performed in triplicate. Data analyzed statistically by one way analysis of variance (ANOVA) and $P \leq 0.05$ were taken as statistically significant.

3. RESULTS AND DISCUSSION

3.1 Percentage Yield

Out of the initial weight of powdered plant of 100 g, 24.06 g of extract was obtained using distilled water for aqueous extract giving 24.065 percentage yield while 22.61 g (with 22.61 percentage yield) was obtained from 100 g powdered plant leaves using absolute ethanol to give the ethanolic extract. The percentage extract yield of the aqueous extract was greater than that of ethanolic extract, by about 1.06 times as presented (Table 1). Physically, the colour appeared as brownish (aqueous extract) to greenish (ethanolic extract) (Plate III).

3.2 Preliminary Phytochemical Analysis

The result of the preliminary phytochemical screening of the aqueous and ethanolic leaf extracts of *B. aegyptiaca* is summarized (Table 2). The results revealed the presence of metabolically active compounds such as alkaloids, cardiac glycosides, flavonoids, glycosides, resins, saponins, steroids, tannins, and carbohydrates in varying concentrations. Anthraquinones and terpenoides were absent among the phytochemicals. Most phytochemicals are common to both aqueous and ethanolic leaf extracts except for alkaloides, which were only present in the ethanolic leaf extract.

Table 1. Percentage yields of crude extracts

Extract	Weight of powdered plant (g)	Weight of extracts obtained (g)	Percentage of extract value (% w/v)	Physical appearance
Aqueous	100	24.06	24.06	Brownish
Ethanollic	100	22.61	22.61	Greenish

Table 2. Phytochemical screening of the leaf extract of *Balanites aegyptiaca*

Phytoconstituents	Aqueous	Ethanollic
Alkaloids	-	++
Anthraquinones	-	-
Carbohydrate	+++	+++
Cardiac glycosides	++	+++
Flavonoids	+++	++
Glycosides	+++	+++
Resins	+	+
Saponins	+++	++
Steroids	+	+
Tannins	++	+++
Terpenoids	-	-

Key

+ = present

++ = present in low concentration

+++ = present in moderate concentration

- = present in high concentration

- = absent

Table 3. Confirmatory identification test of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Salmonella typhimurium*, *Shigella dysenteriae* bacteria isolates

Organism	Gram stain	Macroscopic	Microscopic	Biochemical
<i>S. aureus</i>	+ve	Golden yellow colonies on nutrient agar	Grape-like clusters	Coagulase +ve Oxidase +ve Catalase +ve
<i>E.coli</i>	-ve	Large, circular, low convex, grey, white, moist, smooth and opaque colonies on nutrient agar Small, pink to red on SS agar Beta hemolytic on blood agar	Rod shape Appear singly or in pairs	Catalase +ve Yellow slop and butt gas in Triple Sugar Iron Medium
<i>S. typhimurium</i>	-ve	Colourless transparent, with black center colonies on SS agar	Rod shaped	Red slop red butt with hydrogen Sulphur gas in Iron Medium Formation of H ₂ S
<i>S. pneumoniae</i>	+ve	Alpha haemolytic on blood agar	Lancet shaped cocci Appear singly or in pairs	+ve for Bile Solubility Test
<i>S. dysenteriae</i>	-ve	Clear, colourless, transparent on SS agar	Rod shaped	Red slop, yellow butt on Triple Sugar Iron Medium

3.4 Confirmatory Identification Test of Bacteria Isolates

The macroscopical, microscopical and biochemical confirmatory tests of the clinical isolates are summarized (Table 3).

3.5 Antimicrobial Activities

The antibacterial activity of the extracts were accessed by the inhibition of growth by the bacteria isolates, observed as zones of inhibition near the wells (Plate IV). With respect to the microbial sensitivity of the test organisms, both aqueous and ethanolic leaf extracts showed zones of inhibition to the concentrations used (Table 4). The analysis of variance result on zones of inhibition determined, revealed a significant difference for both extract treatments, concentrations and the interactions between the treatments and concentrations. The result thus shows that the values recorded for each treatment and control were in the following order of performance; control > ethanol extract > aqueous extract. The mean effects and standard error of treatment and concentrations on zones of inhibitions of test organisms are shown (Tables 5 and 6 respectively).

Test organisms were more susceptible to the ciprofloxacin standard drug (positive control) with

zones of inhibition ranging from 24-30 mm at constant concentration of 0.625 mg/ml compared to both plant crude extracts. Among the five tested organisms, the highest zone of inhibition (susceptibility) was seen with *S. dysenteries*, where diameters for aqueous extracts were 12 mm, 10 mm, and 6 mm at concentrations of 400 mg/ml, 200 mg/ml, and 100 mg/ml respectively while that of ethanolic extracts were 13 mm, 11 mm and 8 mm respectively at the same concentrations. The test organism with averagely lowest susceptibility for both extracts was *S. typhimurium* with diameters of 9 mm, 6 mm and 0 mm for aqueous and 9 mm, 7 mm, and 0 mm for ethanolic extract at concentrations of 400 mg/ml, 200 mg/ml and 100 mg/ml respectively. At concentrations of 50 mg/ml to 6.25 mg/ml, no zones of inhibition were observed.

The activities of the extracts for all groups against the test isolates were concentration – dependent, therefore the order of performance were 400 mg/ml > 200 mg/ml > 100 mg/ml > 50 mg/ml > 25 mg/ml > 12.5 mg/ml > 6.25 mg/ml.

The trend graph on mean effects of treatment and concentrations on zones of inhibitions of test organisms are shown (Figs. 1 and 2 respectively).

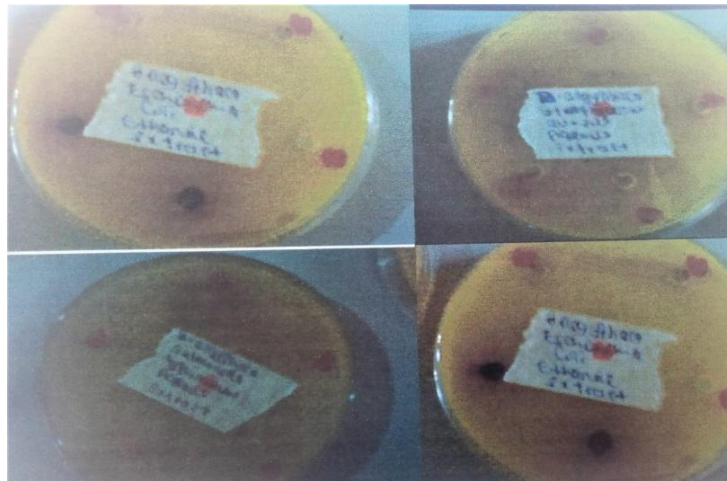


Plate IV. Susceptible testing of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Salmonella typhimurium*, *Shigella dysenteriae* and *Escherichia coli*, *In vitro* against aqueous and ethanolic extracts of *Balanites aegyptiaca*

Table 4. Susceptibility of test organisms to aqueous and ethanolic leaf extract of *Balanites aegyptiaca*

Test organisms	Zones of inhibition															
	Aqueous extract							Ethanolic extract								
	Con (mg/ml)	Crude extract						Cif	Crude extract						Cif	
	400	200	100	50	25	12.5	6.25	0.625	400	200	100	50	25	12.5	6.25	0.625
<i>S. aureus</i>	8±1.037	6±0.500	-	-	-	-	-	28±0.007	10±0.000	8±1.693	6±0.080	-	-	-	-	27±2.000
<i>S. pneumonia</i>	12±0.080	9±1.620	1±0.320	-	-	-	-	27±0.500	12±1.805	10±0.180	7±0.180	-	-	-	-	28±0.180
<i>E. coli</i>	11±0.080	9±0.500	7±0.500	-	-	-	-	25±0.500	12±0.500	9±0.000	6±0.080	-	-	-	-	24±0.180
<i>S. typhimurium</i>	9±0.320	6±0.500	-	-	-	-	-	25±0.007	9±0.207	7±1.805	-	-	-	-	-	27±0.000
<i>S. dysenteriae</i>	12±0.000	10±0.020	6±0.020	-	-	-	-	29±1.805	13±0.500	11±0.000	8±2.420	-	-	-	-	30±2.000

Key- = no zone of inhibition

Cif =ciprofloxacin standard drug (the positive control)

Table 5. Mean effects and standard error of treatment on zones of inhibitions of test organisms

Mean Effects and SE on Zones of Inhibitions of Test Organisms						
VARIABLES	Extract	<i>S. aureus</i>	<i>S. pneumonia</i>	<i>E. coli</i>	<i>S. typhimurium</i>	<i>S. dysenteriae</i>
Treatment	Aqueous	2.00 ^c	3.86 ^c	3.86 ^b	2.14 ^b	4.00 ^c
	Ethanol	3.42 ^b	4.14 ^b	3.86 ^b	2.31 ^b	4.57 ^b
	Control	27.42 ^a	27.43 ^a	24.43 ^a	25.84 ^a	29.43 ^a
	SE	0.113	0.087	0.066	0.057	0.135

Each value is a mean of three replicates. Mean followed by the same superscripts in a column are not significantly different from each other

Table 6. Mean effects and standard error of concentration on zones of inhibitions of test organisms

SD + Mean effects and SE on zones of inhibitions of test organisms						
VARIABLES	Concentrations	<i>S. aureus</i>	<i>S. pneumonia</i>	<i>E. coli</i>	<i>S. typhimurium</i>	<i>S. dysenteriae</i>
Concentration	400 mg/ml	15.32 ^a	17.00 ^a	16.00 ^a	14.37 ^a	18.00 ^a
	200 mg/ml	13.99 ^b	15.33 ^b	14.33 ^b	12.66 ^b	16.67 ^b
	100 mg/ml	11.32 ^c	13.33 ^c	12.67 ^c	8.32 ^d	14.33 ^c
	50 mg/ml	9.00 ^d	9.00 ^d	8.00 ^d	8.32 ^d	9.67 ^d
	25 mg/ml	9.00 ^d	9.33 ^d	8.00 ^d	9.00 ^c	10.00 ^d
	12.5 mg/ml	9.00 ^d	9.33 ^d	8.00 ^d	9.00 ^c	10.00 ^d
	6.25 mg/ml	9.00 ^d	9.33 ^d	8.00 ^d	9.00 ^c	10.00 ^d
	SE	0.173	0.133	0.101	0.087	0.21

Each value is a mean of three replicates. Mean followed by the same superscripts in a column are not significantly different from each other

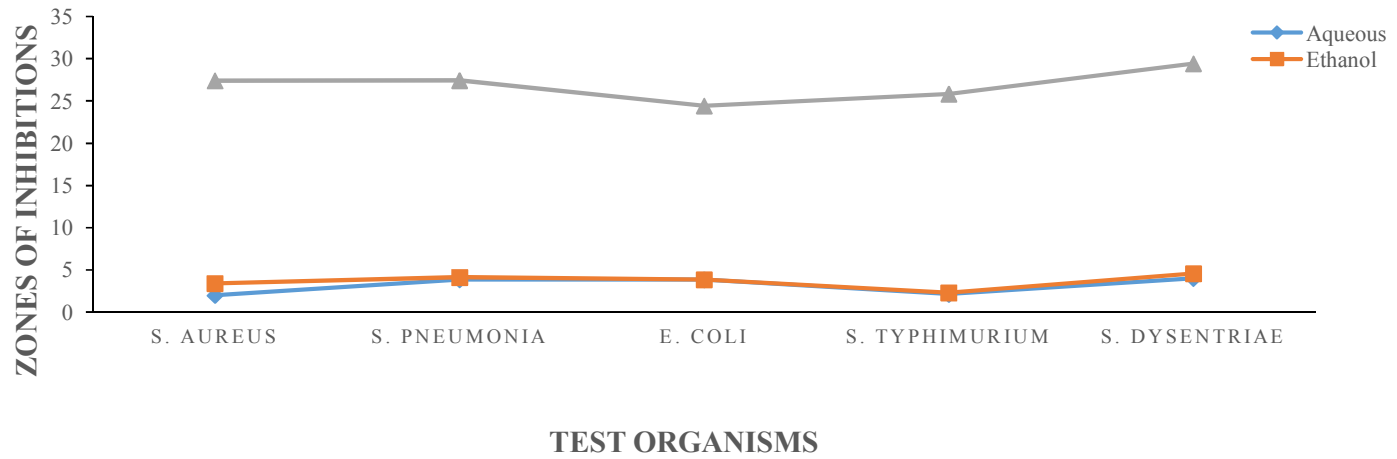


Fig. 1. Trend graph on mean effects of treatment on zones of inhibitions of test organisms

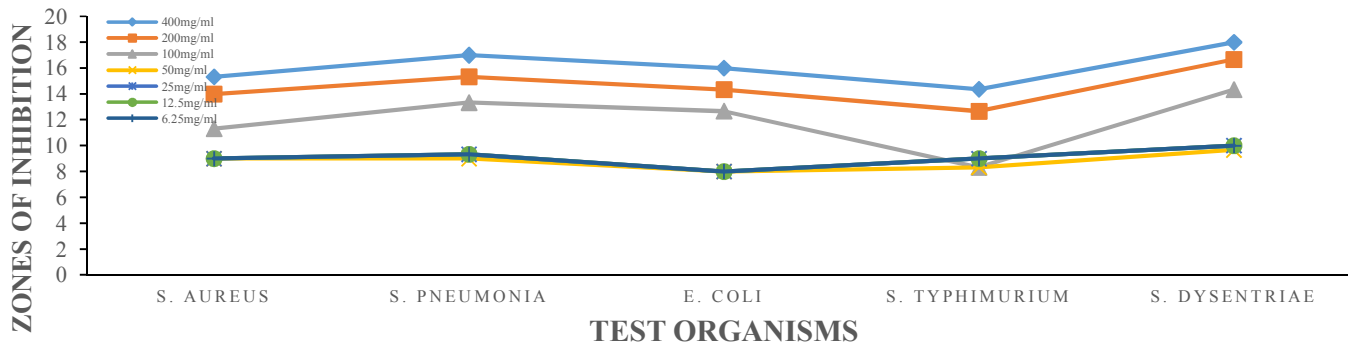


Fig. 2. Trend graph on mean effects of different concentrations on zones of inhibitions

3.6 Minimal Inhibitory Concentration (MIC)

The lowest concentrations that will prevent the growth of the test organisms, mics were determined from the corresponding concentration-response curves. The values of the mean radius R^2 (mm²) and corresponding antilog of concentrations used are shown (Table 7). The resultant excel plots of logs against R^2 (mm²) for the crude extracts and Ceftriaxone (Figs. 3-4). The X-intercepts were obtained from equations of the trend line from excel plots of R^2 against log concentration of crude extracts

(leaving out the coordinates with 0 values of R^2) and used to calculate the MIC in the table. The values of X (Intercepts) from the graphs and its antilog values representing the MICs (Table 8). The MIC values of the extracts ranged from 79.433 to 125.893 mg/ml for all tested isolates. The ethanolic extract against *S. dysenteriae* had the least value of 79.433 mg/ml while the aqueous extracts against *S. aureus* and *S. typhimurium* were the largest. Ethanolic extract against *S. aureus*, *E. coli*, *S. typhimurium* and aqueous extract against *S. dysenteriae* all had the same value of 100 mg/ml.

Table 7. Different log concentration of the aqueous and ethanolic extracts and their corresponding square of the radii zones of inhibition

Test organism	Zones of inhibition (R^2)															
	Plant Extracts								Cif (Control)							
	A	E	A	E	A	E	A	E	A	E	A	E	A	E	A	E
Con (mg/ml)	400		200		100		50		25		12.5		6.25		0.625	
Log con	2.602		2.301		2.000		1.699		1.398		1.097		0.795		-0.204	
<i>S. aureus</i>	2.25	6.25	0.25	2.25	-	0.25	-	-	-	-	-	-	-	132.25	121.00	
<i>S. pneumoniae</i>	12.25	12.25	4.00	6.25	-	1.00	-	-	-	-	-	-	-	121.00	132.25	
<i>E. coli</i>	9.00	12.25	4.00	4.00	1.00	0.25	-	-	-	-	-	-	-	100.00	90.25	
<i>S. typhimurium</i>	4.00	4.00	0.25	1.00	-	-	-	-	-	-	-	-	-	100.00	121.00	
<i>S. dysenteriae</i>	12.25	16.00	6.25	9.00	0.25	2.25	-	-	-	-	-	-	-	144.00	156.25	

Key

A=Aqueous Extract

E=Ethanolic Extract

Where $R^2 = \text{mean radius (mm)} - \{(\text{mean diameter inclusive of well diameter (mm)} - \{\text{well diameter (5mm)}\}) / 2\}$

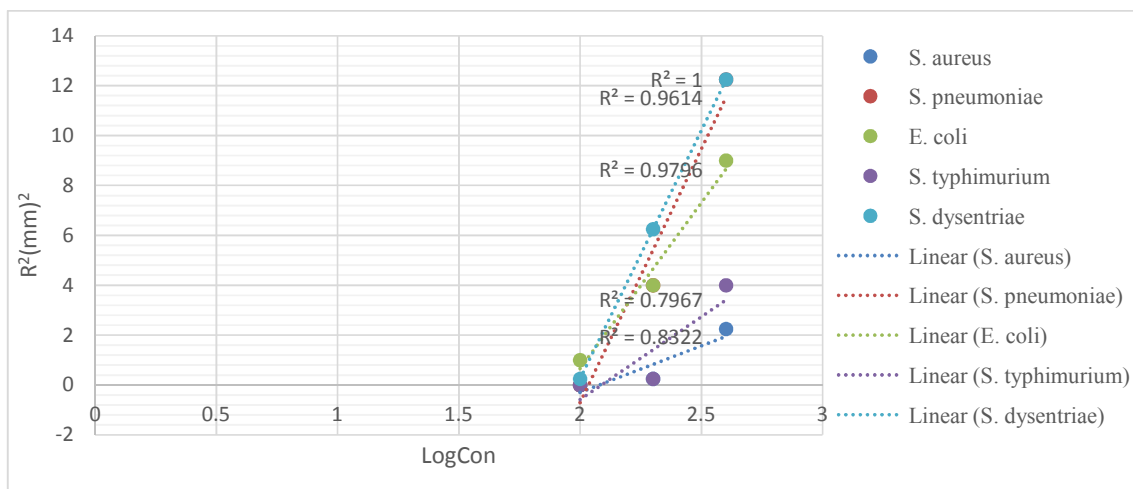


Fig. 3. Excel plots of logs concentration against R^2 (mm²) for the Aqueous Extracts of *Balanites aegyptiaca* against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Salmonella typhimurium* and *Shigella dysenteriae*

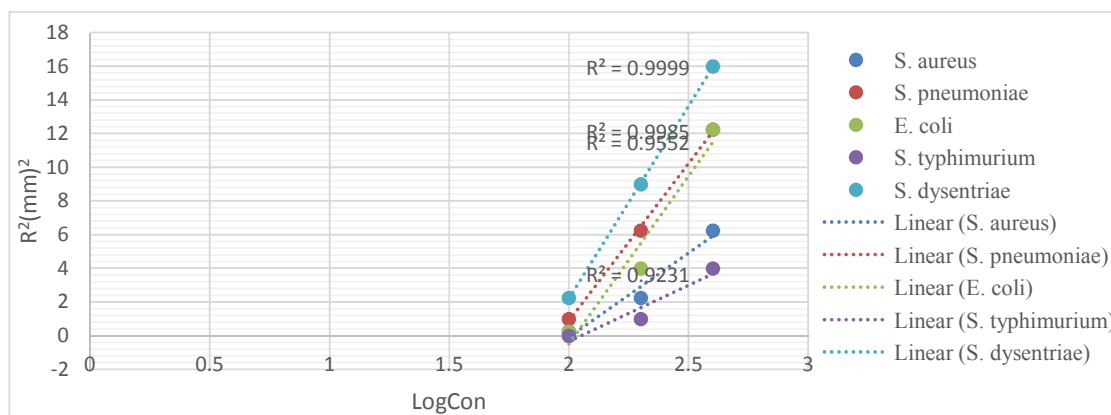


Fig. 4. Excel plots of logs concentration against R² (mm²) for the Ethanolic Extracts of *Balanites aegyptiaca* against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Salmonella typhimurium* and *Shigella dysenteriae*

Table 8. Minimal Inhibitory Concentration and X (Intercept)

Extracts	<i>S. aureus</i>		<i>S. pneumoniae</i>		<i>E. coli</i>		<i>S. typhimurium</i>		<i>S. dysenteriae</i>	
	X	MIC	X	MIC	X	MIC	X	MIC	X	MIC
Aqueous	2.10	125.893	2.05	112.202	1.95	89.125	2.10	125.893	2.00	100.000
Ethanolic	2.00	100.000	1.95	89.125	2.00	100.000	2.00	100.000	1.90	79.433

Key:

X=Intercept of the extracts

(MIC) (mg/ml) = antilog X to the nearest 3 degrees

3.7 Activity Index

Apart from those of the positive control drugs that had the highest values of 1, the ethanolic extract of *B. aegyptiaca* against *E. coli* had the highest value of 0.5000 at 400 mg/ml, while the least (0.2069) was aqueous extracts against *S. dysenteriae* at 100 mg/ml. *E. coli* for all extracts had the highest range of values, the least were those of *S. aureus*. The values of the activity index have been summarized (Table 9).

3.8 Total Activity

The total activity showed that the ethanolic extract of *B. aegyptiaca* against *S. aureus* and *S. typhimurium* had the lowest value of 0.001911 ml/g while the highest was of 0.002846 ml/g followed by 0.002700 ml/g were of ethanolic extract against *S. dysenteriae* and aqueous extract against *E.coli*. Aqueous (0.001911 ml/g) and ethanolic extract (0.002261 ml/g) against *S. aureus* and *S. typhimurium* were the same. The results are summarized (Table 10).

3.9 Discussion

The preliminary investigation of the leaf of *B. aegyptiaca* revealed the presence of some

phytochemical compounds that are known to exhibit medicinal as well as physiological activities. These secondary metabolites (or active principles) are linked to the antimicrobial activity of the plant. Some of these phytochemicals have earlier been reported in the leaf extract of the plant material [19,20], although Anthraquinones was reported in work of [21]. This might be due to the extraction method. Similar phytochemical have been found from other parts of the plant and also parts of related species [18,20]. These phytochemical constituents therefor account for the various properties of *B. aegyptiaca* plant already mentioned in the literature, such as antimicrobial, hepatoprotective, cardioprotective and antioxidant, antihelminthic, wound healing, spermicidal, insecticidal, anthelminthic, molluscicidal and contraceptive activities [13].

Environmental factors such as seasons and geographic regions have been reported to strongly affect the metabolism, accumulation and fluctuation of Phytoconstituents [43,44]. As a result, therapeutic efficacy varies during different times [45]. Appropriate period of collection of plant part has been mentioned as at the herbs peak of maturity and concentration [46]. Other linked factors affecting the quality of the herbs

Table 9. Activity index of test organisms to aqueous and ethanolic leaf extract of *Balanites aegyptiaca*

Test organisms	Concentrations of Aqueous and Ethanolic Leaf Extract (mg/ml)														
	400		200		100		50		25		12.5		6.25 Cif(0.625)		
	A	E	A	E	A	E	A	E	A	E	A	E	A	E	
<i>S. aureus</i>	0.2857	0.3704	0.2143	0.2963	0.2222	0	0	0	0	0	0	0	0	1	1
<i>S. pneumoniae</i>	0.4444	0.4286	0.3333	0.3571	0.2500	0	0	0	0	0	0	0	0	1	1
<i>E. coli</i>	0.4400	0.5000	0.3600	0.3750	0.2800	0.2500	0	0	0	0	0	0	0	1	1
<i>S. typhimurium</i>	0.3600	0.3333	0.2400	0.2592	0	0	0	0	0	0	0	0	0	1	1
<i>S. dysenteriae</i>	0.4138	0.4333	0.3449	0.6667	0.2069	0.2667	0	0	0	0	0	0	0	1	1

Key
 - = no growth
 + = growth
 A = aqueous extract
 E = ethanolic extract

Table 10. Total activity of test organisms to aqueous and ethanolic leaf extract of *Balanites aegyptiaca*

Extract	Qty (mg/g)	Total Activity (ml/g)				
		<i>S. aureus</i>	<i>S. pneumoniae</i>	<i>E. coli</i>	<i>S. typhimurium</i>	<i>S. dysenteriae</i>
Aqueous	0.2406	0.001911	0.002144	0.002700	0.001911	0.002406
Ethanolic	0.2261	0.002261	0.002537	0.002261	0.002261	0.002846

Key:
 Qty (mg/g): quantity of extract of extract mg/g dried plant part
 Total activity per gram dried plant parts: MIC

have been stated as period of harvesting, age, stage of development, soil, rainfall, altitude, collecting person and post-harvest conditions [47]. The influence of altitude has been studied on plant, higher altitude revealed higher amounts of phytochemicals and better antioxidant activity in plant leaves [48].

Both aqueous and ethanolic leaf extracts exhibited variable antibacterial activity against the isolates. The results reveal that the extracts obtained from ethanol solvent with lower polarity showed more antibacterial activity to the test organisms than the more polar water solvent, and which are in line with many works like [21], and [49], despite the higher yield of aqueous extract. [50] also reported that ethanolic and aqueous extracts of *B. aegyptiaca* were more potent than petroleum ether extract of *Phyllanthus reticulatus* against *S. aureus*, *E. coli*, amongst others. Ethanol and methanol (alcoholic) extracts were more active than aqueous extract for all the plants as reported by [51]. This might be explained by the ability of the ethanol to dissolve multifarious types of compounds; polar and non-polar, simple and complex chemical structures compared with water and the observation in the phytochemical results which revealed ethanolic extract to have

more and higher concentration of phytoconstituents.

Based on the traditional claims, there has been need on evaluation and characterization of various plant constituents against many diseases. The extraction of the bioactive components of plants have always been challenging processes at concentrations and constitutes advantages and disadvantages [52,53].

The difference in activities may also suggest one of the following: the active principles responsible for the antibacterial activity could be identical in structures in both extracts, only that they could be more soluble in ethanol (more lipophilic) than water due the following reasons, the active principles could be different in structure and those extracted by ethanol maybe more potent compared to those extracted by water; there could be impurities that are more soluble in water than ethanol which may be responsible for lower activity; or there could be different impurities with different antagonisms against the active principles and those impurities dissolved by the ethanol extract could be less antagonistic compared to those taken up by water. This inconsistency between yield and activity could

also be due to the reduction in potency of the aqueous extract due to several factors such as be the storage condition, moisture content, environmental factors, chemical and biochemical interactions and presence of deteriorating microorganisms [54].

The results obtained showed that the activities of the extracts increased with increasing concentration and showed antimicrobial activity to both gram positive and negative test organisms [55], suggesting that they may have a broad spectrum activity. This trend was seen in other works where different extracts and parts of the plants, and in combination with other plants have shown activity against *S. aureus* and *E. coli* [56], *S. aureus*, *E. coli*, *S. typhimurium* and *S. dysenteriae* [55], *S. aureus*, *E. coli*, *S. typhimurium* and *S. dysenteriae* [55], *S. aureus* [50], *S. E. coli* [57], *S. typhimurium* ATCC 23564 and *E. coli* [49] and others [22,28,50]. The plant extracts have also been reported to have antifungal activity against *Aspergillus niger* and *C. albicans*, with minimal fungicidal concentration, MFC of 250 mg/ml [25]. The synergic activity with other medicinal plants has shown to have strong activity against tested isolates [21,28,58].

Ciprofloxacin (0.625 mg/ml) showed high microbial activity with zones of inhibition ranging from 24-30 mm in both extract compared to the crude extracts, indicating that the test organisms were susceptible, compared to the crude extracts. This is because the antibiotics is in pure state and has gone refining processes that has established it as a standard. The zones of inhibition of the standard, though of same volume varied slightly in both extracts duplicates, this might be due to uneven distribution of test organisms on agar surfaces or slight differences in temperature and volume of the standard as the time it was used.

From the susceptibility test and MIC assay, *S. aureus* showed least susceptibility amongst the test clinical isolates, and also less susceptible compared to *E. coli*, this findings is in line with [50] but contrary to [22] where *S. aureus* was more susceptible, same trend seen in [49] where *S. aureus* was found most susceptible among other *Staphylococcus* species when screened, though under slightly different experimental conditions. In other findings, *S. typhimurium* and *E. coli* was said to be most resistant species amongst other tested organisms. This agrees with the present study where *S. typhimurium*

showed the least susceptibility for the ethanolic extract with 9 mm zones of inhibition and corresponding high MIC value.

Activity index and total activity was used as tools in the study to also determine the antibacterial activity of the extracts, alongside the MICs. The susceptibility test results of *E. coli* was 9 mm at 200 mg/ml for aqueous extract, is in line with the work of [58], whose zone of inhibition was 10.66 mm, but that of *S. aureus* was 6 mm under the same condition slightly contradicts the work of [58] which was 10 mm and whose MIC range was from 50 mg/ml to 1.563 mg/ml for the aqueous and methanolic root extracts of the plant, in an experiment that included other organisms, against 79.433 mg/ml - 125.893 mg/ml MIC from the present study. The test organisms were all susceptible to the crude extracts since the values were ≥ 7 mm. The higher MIC values despite rich composition of phytochemicals could be attributed to slight differences in the active component in various parts of the plant, method and solvent used, possible resistance in the strains use or possible interference with the bioactive components by intrinsic or extrinsic factors.

The organisms used for this purpose of investigation are associated with various forms of infections: *S. aureus* (skin infection, pneumonia, meningitis, osteomyelitis, toxic shock syndrome, bacteremia, wound infection, sepsis and food poisoning [1], *S. pneumonia* (invasive pneumococcal diseases such as sinusitis, otitis media, meningitis, bacteremia, sepsis, osteomyelitis, septic arthritis, cellulitis and brain abscess [59]; *E. coli* (food poisoning, enteric/diarrhea disease, urinary tract infection and sepsis/meningitis [60]; *S. typhimurium* (fever, osteomyelitis, gastroenteritis in humans and other mammals [61]; and *S. dysenteriae* (colitis, malnutrition, rectal prolapse, tenesmus, reactive arthritis and central nervous system problems [62], all having long history of pathogenicity and drug resistance. Result of this investigation has therefore shown that the plant is a potential source of antibiotic substances for use against these test organisms. The usefulness of these extracts as antimicrobial compounds, however can further be predicted after toxicity testing.

In regards to all the limitations and constraints of this research, the recommendations are to isolate, purify and characterize the active constituents responsible for the activity of the plant material; determine the toxicity of the

leaf and the plant both in vivo and in vitro; the plant against more pathogenic microbes as well as other classes of microbes such as viruses, fungi, protozoa and helminthes using different solvents and different extraction methods.

4. CONCLUSION

The antimicrobial activity of leaf extracts of *B. aegyptiaca* of the test isolates have been established in this study and justified the claims by the traditional healers in its use to test infectious diseases. It could be used in the treatment of diseases caused by the tested *S. aureus*, *S. pneumoniae*, *E. coli*, *S. typhimurium*, *S. dysenteriae* clinical isolates used in the study as well as other various infectious diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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