

**EFFECTS OF THE ETHANOL EXTRACTS OF THE ROOT, STEM AND LEAVES OF
BALANITES AEGYPTIACA AGAINST SELECTED MICROBES**

ABSTRACT

Balanites aegyptiaca has been used in many traditional treatments of microbial infections. This work was carried out with the aim of determining the phytochemical compounds present in the ethanol extracts of the root, stem and leaves of *Balanites aegyptiaca* and their effects against selected microorganisms. Phytochemical screening was undertaken to determine presence of secondary metabolites in the stem-bark, roots and leaves of the test plant. Well diffusion method was used to determine sensitivity of test organisms to the test plant. Result indicated the presence of Alkaloids, Tannins, flavonoids, Saponins, Steroid, and anthraquinones, although anthraquinones were absent in ethanol extracts of root and stem bark of *Balanites aegyptiaca* but present in the ethanol extract of the leaf. Antibacterial effects were tested against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Results obtained from the ethanol extract of the root stem and leaves showed significant zones of inhibition against *Klebsiella pneumoniae* and *Staphylococcus aureus* at all concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.125mg/ml. The positive control performed better than the extracts in terms of zone of inhibition. The results of the minimum inhibitory concentration (MIC) indicated that the leaf ethanol extract had the greatest activity against *K. pneumoniae* with MIC value of 12.5mg/ml, while the results from the minimum bactericidal concentration (MBC) showed that the leaf ethanol extract had the greatest activity against *K. pneumoniae* with an MBC value of 12.5mg/ml.

Key words: *Balanites aegyptiaca*, phytochemical compounds, microorganisms, plant extracts

1. INTRODUCTION

The antimicrobial activities of plant oils and extracts have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies. Moreover, the increasing use of plant extracts in the food, cosmetics and pharmaceutical industries suggests that in order to find active compounds, a systematic study of medicinal plants is very important

The plant (*Balanites aegyptiaca* Delile) has long history of traditional uses for wide ranges of disease [1].

The kernel oil is used for the treatment of wounds in Nigeria [2]. Also, in Nigeria, a mixture of dried leaves powder of *Balanites aegyptiaca* Delile and *Ricinus communis* in water is used for contraception [3]

35 [4]. Traditional medicine has remained the most affordable and easily accessible source of treatment in
36 the primary health care system of resource poor communities and local therapy is the only means of
37 medical treatment for such communities [5]. According to Alaribe [6] about 80% of Nigerian homes,
38 maintain some sort of private family traditional medicine practitioner. Existing data and contemporary
39 researchers seem to authenticate the assumption for general health improvement of the masses by
40 traditional healers. Plants have broader uses than just food and genetic reservoirs. Medicinal plants
41 have been used for centuries to treat a wide variety of ailments [7]. The presence of secondary
42 metabolites in plants has been associated in most of their therapeutic activities [8]. Herbal medicines are
43 now considered a part of complementary and Alternative medicine (CAM) and are gaining popularity due
44 to their potent antioxidant activity, minimal side effects and economic viability [9]. Many research efforts
45 have been directed towards the provision of empirical proofs to back up the use of plants species in trade
46 and medicinal practices in recent years [10]. This work intends to study specific microorganisms'
47 response to extracts of *B. aegyptiaca*

48 **2.0 MATERIALS AND METHODS**

49 **Source and preparation of plant materials**

50 The plant materials were collected from Gombe State University botanical garden Biological science
51 department; Gombe State University, it located in Gombe between the latitude 10°00N to 10°20N and
52 longitude 11°0E to 11° E. These were brought and identified by a taxonomist with voucher number
53 900191 at the Herbarium unit of the Department of Biological Sciences, Gombe State University. The
54 plant parts were air-dried for two weeks at room temperature (25°C) in the laboratory and then ground to
55 powder.

56 **2.1 Extraction procedures**

57 The ground plant parts were extracted at the Department of Pharmacognosy, Faculty of
58 Pharmaceutical Sciences, Gombe State University, following the methods of Sofowora [11].

59 **2.2 Preparation of Ethanol Extraction of *Balanites aegyptiaca***

60 Approximately 800 g of the dried leaves and roots of *B. aegyptiaca* were extracted with 10 litres of 80%
61 (v/v) ethanol by maceration at (25°C) for 3days. The total mixture then is strained and filtered. The filtrate
62 was concentrated to dryness on a water bath at 100° C so as to obtain the dry extract after which was
63 stored at -20°C for further studies.

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66 **2.3 Phytochemical analysis**

67 The method of Sofowora [12] was employed for the test of the presence of the phytochemical properties.

68 **2.4 Source and preparation of test microorganisms**

69 The stock cultures of the test microorganisms were obtained from the Department of Microbiology,
70 Gombe State University. Their validity was determined by sub culturing onto nutrient agar and confirmed
71 by standard cultural, morphological and biochemical techniques as described by [13]. The inocula of the
72 test organisms were standardized by the method of Bauer and Thornsberry [14]. This was done by
73 suspending each test organism in 5ml of nutrient broth and the turbidity was compared with that of 0.5
74 McFarland standard. McFarland standard was prepared by adding 0.6ml of 1% barium chloride (BaCl₂)
75 to 99.4ml of 1% sulphuric acid (H₂SO₄) solution. The turbidity of the 0.5 McFarland standards was used
76 for estimation of the number of bacteria in broth culture (culture for 24 hours at 37°C) to pour into 5ml of
77 distilled water in order to obtain a standard bacterial suspension of 1 x 10⁵ cfu/ml [15].

78 **2.4 Preparation of concentration of extracts**

79 Approximately 1g of each extract was dissolved in 5mls of distilled water to yield 200mg/ml. 1ml of the
80 200mg/ml was taken and added to 1ml of distilled water to give a concentration of 100mg/ml. 1ml of the
81 100mg/ml extract concentration was also taken and added to 1ml of distilled water to get a concentration
82 of 50mg/ml. The procedure was repeated twice to give concentrations of 25mg/ml and 12.5mg/ml.

83 **2.6 Antibacterial susceptibility testing**

84 The antibacterial activity of the fractions of *B. aegyptiaca* was determined using the well method (Kirby-
85 Bauer Methods) as described by Abalaka [16] Standard aseptic Microbiological methods were followed
86 throughout this antibacterial study.

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88 2.7 Well method for antibacterial activity

89 The well method was employed to assay the plant fractions for antibacterial activity. Petri dishes were
90 poured with nutrient agar and allowed for 30 minutes to solidify (This was done in duplicate for each
91 fraction and test organism). The test organisms were then inoculated by spreading on the inocula on the
92 surface of the medium using a sterile swab stick. A sterile Cork borer (size 3) was used to bore 4 wells in
93 the medium. The different concentration of the plant fractions were placed in the wells using a sterile
94 syringe and needle (different for each sample and test organism). These were then allowed a diffusion
95 time of 1 hour after which the plates were incubated at 37 °C for 24 hours. The positive control was
96 Ceftriaxone (100mg/ml). The potency of the extracts was determined by the clear zones of inhibition
97 around the wells and was respectively measured as the diameter zones of inhibition. MIC was determined
98 using the method of Doughari [17], while MBC was determined using the method of Rotimi [18].

99 2.8 Data analysis

100 One way Analysis of Variance (ANOVA) of was used to assess the efficacy of the plant parts in terms
101 of the activity as was shown by the zones of inhibition. Since there was no significant difference,
102 Duncan's multiple range test (DMRT) and Student t-test was not carried.

103 2.9 Result and Discussion

104 The result of preliminary phytochemical screening (table 1) revealed the presence of alkaloids,
105 flavonoids, tannins, saponins and steroids in the roots and stem bark, while anthraquinones were present
106 in the leaf only. This is similar to that of Adebayo and Ishola [19] who investigated the activity of root,
107 stem-bark and leaves extracts of *Terminalia glaucescens*' against some pathogenic organisms.
108 Phytochemical screening of the fractions of *Terminalia glaucescens*' also revealed the presence of
109 alkaloids, tannins, saponins, steroids, flavonoids, anthraquinones and phlobatannins (mostly in root and

110 stem-bark). Also, Igbiosa [20] in the screening of the methanolic and water extracts of the stem bark of
 111 *Jatropha curcas* revealed the presence of saponin, steroids, tannin, glycosides, alkaloids and flavonoids
 112 in the extracts. [21] Found tannins and anthraquinones (the largest group of quinones) to possess
 113 antibacterial effects by inhibiting nucleic acid synthesis. Anthraquinones were absent in the root and
 114 stem-bark extracts. This is similar to the work of Nisha [22] who investigated the ethanol extract of
 115 *Maranta arundinacea* rhizomes and found alkaloids to be present in the ethanol extract, but absent in the
 116 ethyl-acetate fraction. The presence of these phytochemicals has been reported to account for the
 117 exertion of antimicrobial activity by plants [23].

118 The result of antibacterial activity (Table 2, 3 and 4) of the ethanol extracts of root, stem bark and leaf of
 119 *Balanites aegyptiaca* revealed the potentiality of the plant in treating and curing diseases cause by the
 120 tested bacteria and other microorganisms. The ethanol extract of the root, stem bark, and leaves has
 121 inhibited the growth of *Klebsiella pneumoniae* and *Staphylococcus aureus*, with MIC values with low
 122 concentration of 25mg/ml for *S. aureus* and 12mg/ml for *K. pneumoniae* respectively.

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 124 It was shown from previous studies that the root extract of the plant has more and lethal effect on
 125 microorganisms, followed by the bark among the various part tested . But based on these work and the
 126 statistical analysis, the leaf extract showed MIC value with low concentration of 12.5mg/ml for *S. aureus*
 127 sand 50mg/ml for *K. Pneumoniae*, than the root and stem bark extract on both the tested bacteria (Table
 128 5). The root showed MIC value with concentration of 50mg/ml for both the tested organisms. The result of
 129 the MIC and MBC also showed that *Balanites aegyptiaca* is bactericidal (Table 6).

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 131 **Table 1:** Qualitative phytochemical screening of *Balanites aegyptiaca*

	Root	Stem Bark	Leaf
	Ethanol	Ethanol	Ethanol
Alkaloids	+	+	+

Tannins	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Steroids	+	+	+
Anthraquinones	-	-	+

+ = Present, - = Absent.

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135 **Table2:** The mean of the sensitivity test of the microorganisms to the ethanol root extract of *Balanites*
136 *aegyptiaca*

Microorganisms	Zones of inhibition (mm)					
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.125mg/ml	control
<i>K. pneumoniae</i> ¹³	10	5	2	2	26	
<i>S. aureus</i>	16	13	9	7	4	24
Control: Ceftriaxone						

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144 **Table 3:** The mean of the sensitivity test of the microorganisms to the ethanol stem bark extract of
145 *Balanites aegyptiaca*

Microorganisms	Zones of inhibition (mm)					
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.125mg/ml	control
<i>K. pneumoniae</i>	14	11	9	5	3	24
<i>S. aureus</i>	8	12	9	7	5	25

150 Control: Ceftriaxone

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152 **Table 4:** The mean of the sensitivity test of the microorganisms to the ethanol leaf extract of *Balanites*
153 *aegyptiaca*

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Microorganisms	Zones of inhibition (mm)					
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.125mg/ml	control
<i>K.pneumoniae</i>	16	12	9	7	5	26
<i>S. aureus</i>	20	16	13	10	6	24

Control: Ceftriaxone

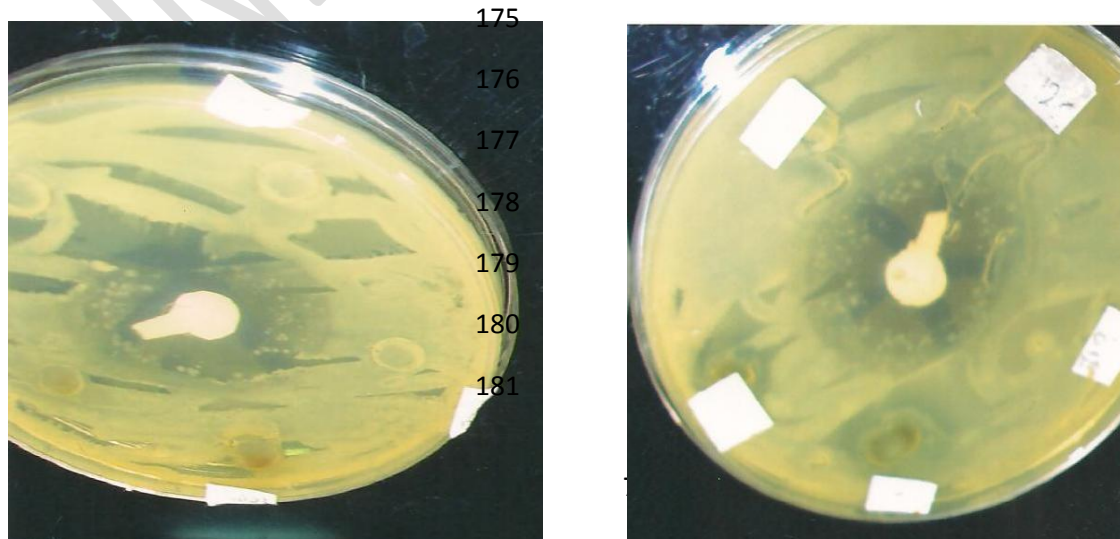
Table 5: Minimum inhibitory concentration (MIC) for microorganism of different extracts of *Balanites aegyptiaca* in mg/ml

Microorganisms	Root	Stem bark	Leaf
<i>K. pneumoniae</i>	25	25	12.5
<i>S. aureus</i>	50	25	25

Table 6: Minimum Bactericidal Concentration (MBC) for microorganism of different extracts of *Balanites aegyptiaca* in mg/ml

Microorganisms	Root	Stem bark	Leaf
<i>K. pneumoniae</i>	50	25	12.5
<i>S. aureus</i>	50	25	50

Plate 1: Inhibition zone of the root extract at different concentrations against *Klebsiella pneumoniae* and *Staphylococcus aureus*



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183 *Klebsiella pneumoniae*

Staphylococcus aureus

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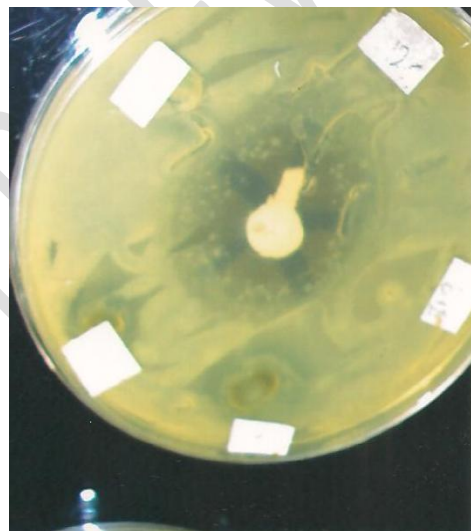
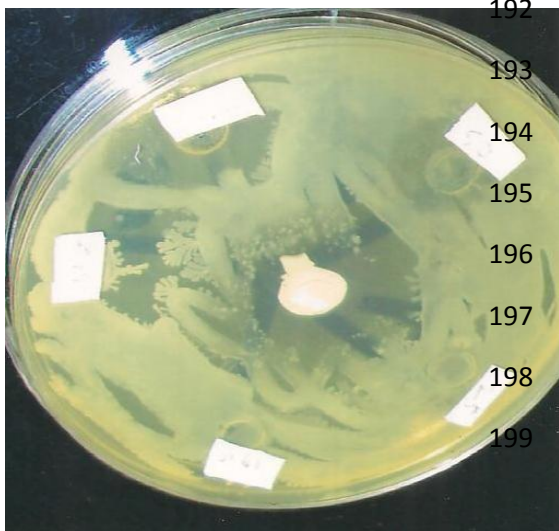
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190 **Plate 2:** inhibition zone of the stem bark extract at different concentrations against *Klebsiella pneumoniae*

191 and *Staphylococcus aureus*



200 *Klebsiella pneumoniae*

Staphylococcus aureus

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202 **Plate 3:** Inhibition zone of the leaf extract at different concentrations against *Klebsiella pneumoniae* and

203 *Staphylococcus aureus*

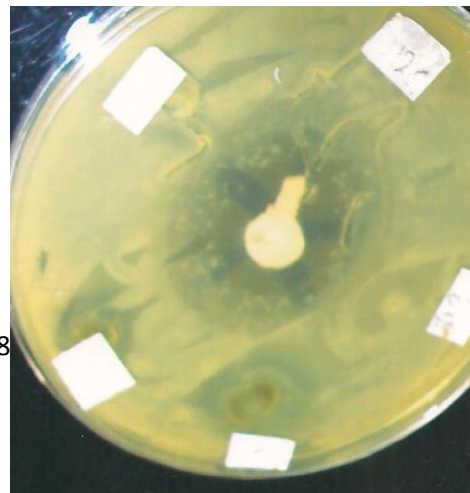
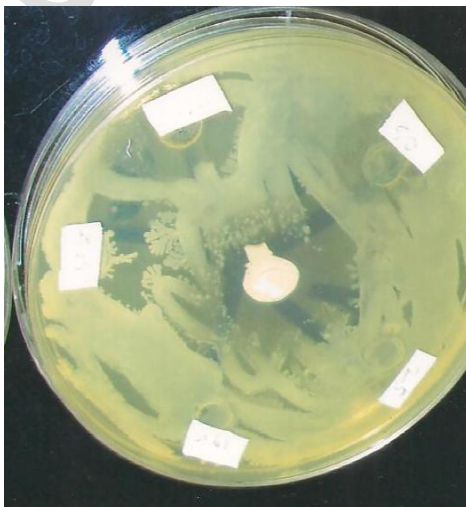
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213 *Klebsiella pneumoniae*

Staphylococcus aureus

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217 **3.0 Conclusion**

218 *Balanites aegyptiaca* is traditionally used in treatment of various ailments. The phytochemical screening
219 of the ethanol extract of root, stem bark, and leaves revealed the presence of; alkaloids, flavonoids,
220 saponins, tannins, and steroids, in both and anthraquinones in the leaf only.

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222 The result of the antibacterial activity of the ethanol extract of the root, bark and leaf of *Balanites*
223 *aegyptiaca* inhibited the growth of *Klebsiella pneumoniae* and *Staphylococcus aureus* with MIC value of
224 12.5mg/ml for *K. pneumoniae* and 25mg/ml for *S. aureus*.

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226 The demonstration of antibacterial activity of the *Balanites aegyptiaca* is indeed a development that will
227 help to discover new antibiotics that can serve for treatment of infections that are caused by bacteria that
228 are becoming resistant to most of the antibiotics used for treatment of diseases caused by the
229 microorganisms. The fact that plants are very common makes it a cheaper alternative for drugs
230 development for human consumption and used.

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