

**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 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

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 [1]. Traditional medicine has remained the most affordable and easily accessible source of treatment in the primary health care system of resource poor communities and local therapy is the only means of medical treatment for such communities [2]. According to [3] about 80% of Nigerian homes, maintain some sort of private family traditional medicine practitioner. Existing data and contemporary researchers seem to authenticate the assumption for general health improvement of the masses by traditional healers. Plants have broader uses than just food and genetic reservoirs. Medicinal plants

34 have been used for centuries to treat a wide variety of ailments [4]. The presence of secondary
35 metabolites in plants has been associated in most of their therapeutic activities [5]. Herbal medicines are
36 now considered a part of Complementary and Alternative medicine (CAM) and are gaining popularity due
37 to their potent antioxidant activity, minimal side effects and economic viability [6]. Many research efforts
38 have been directed towards the provision of empirical proofs to back up the use of plants species in trade
39 and medicinal practices in recent years [7].

40 **MATERIALS AND METHODS**

41 **Source and preparation of plant materials**

42 The plant materials were collected from Gombe State University botanical garden Biological science
43 department; Gombe State University, it located in Gombe between the latitude 10°00N to 10°20N and
44 longitude 11°0E to 11° E. These were brought and identified by a taxonomist with voucher number
45 900191 at the Herbarium unit of the Department of Biological Sciences, Gombe State University. The
46 plant parts were air-dried for two weeks at room temperature (25°C) in the laboratory and then ground to
47 powder.

48 **Extraction procedures**

49 The ground plant parts were extracted at the Department of Pharmacognosy, Faculty of
50 Pharmaceutical Sciences, Gombe State University, following the methods of [8].

51 **Preparation of Ethanol Extraction of *Balanites aegyptiaca***

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

56

57

58 **Phytochemical analysis**

59 The method of [8] was employed for the test of the presence of the phytochemical properties.

60 **Source and preparation of test microorganisms**

61 The stock cultures of the test microorganisms were obtained from the Department of Microbiology,
62 Gombe State University. Their validity was determined by sub culturing onto nutrient agar and confirmed
63 by standard cultural, morphological and biochemical techniques as described by [9]. The inocula of the
64 test organisms were standardized by the method of [10]. This was done by suspending each test
65 organism in 5ml of nutrient broth and the turbidity was compared with that of 0.5 McFarland standard.
66 McFarland standard was prepared by adding 0.6ml of 1% barium chloride (BaCl_2) to 99.4ml of 1%
67 sulphuric acid (H_2SO_4) solution. The turbidity of the 0.5 McFarland standards was used for estimation of
68 the number of bacteria in broth culture (culture for 24 hours at 37°C) to pour into 5ml of distilled water in
69 order to obtain a standard bacterial suspension of 1×10^5 cfu/ml [11].

70 **Preparation of concentration of extracts**

71 Approximately 1g of each extract was dissolved in 5mls of distilled water to yield 200mg/ml. 1ml of the
72 200mg/ml was taken and added to 1ml of distilled water to give a concentration of 100mg/ml. 1ml of the
73 100mg/ml extract concentration was also taken and added to 1ml of distilled water to get a concentration
74 of 50mg/ml. The procedure was repeated twice to give concentrations of 25mg/ml and 12.5mg/ml.

75 **Antibacterial susceptibility testing**

76 The antibacterial activity of the fractions of *B. aegyptiaca* was determined using the well method (Kirby-
77 Bauer Methods) as described by [12]. Standard aseptic Microbiological methods were followed
78 throughout this antibacterial study.

79

80 **Well method for antibacterial activity**

81 The well method was employed to assay the plant fractions for antibacterial activity. Petri dishes were
82 poured with nutrient agar and allowed for 30 minutes to solidify (This was done in duplicate for each

83 fraction and test organism). The test organisms were then inoculated by spreading on the inocula on the
84 surface of the medium using a sterile swab stick. A sterile Cork borer (size 3) was used to bore 4 wells in
85 the medium. The different concentration of the plant fractions were placed in the wells using a sterile
86 syringe and needle (different for each sample and test organism). These were then allowed a diffusion
87 time of 1 hour after which the plates were incubated at 37 °C for 24 hours. The positive control was
88 Ceftriaxone (100mg/ml). The potency of the extracts was determined by the clear zones of inhibition
89 around the wells and was respectively measured as the diameter zones of inhibition. MIC was determined
90 using the method of [13], while MBC was determined using the method of [14].

91 **Data analysis**

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

95 **Result and Discussion**

96 The result of preliminary phytochemical screening (table 1) revealed the presence of alkaloids,
97 flavonoids, tannins, saponins and steroids in the roots and stem bark, while anthraquinones were present
98 in the leaf only. This is similar to that of [15] who investigated the activity of root, stem-bark and leaves
99 extracts of *Terminalia glaucescens*' against some pathogenic organisms. Phytochemical screening of the
100 fractions of *Terminalia glaucescens*' also revealed the presence of alkaloids, tannins, saponins, steroids,
101 flavonoids, anthraquinones and phlobatannins (mostly in root and stem-bark). Also, [16] in the screening
102 of the methanolic and water extracts of the stem bark of *Jatropha curcas* revealed the presence of
103 saponin, steroids, tannin, glycosides, alkaloids and flavonoids in the extracts. [17] Found tannins and
104 anthraquinones (the largest group of quinones) to possess antibacterial effects by inhibiting nucleic acid
105 synthesis. Anthraquinones were absent in the root and stem-bark extracts. This is similar to the work of
106 [18] who investigated the ethanol extract of *Maranta arundinacea* rhizomes and found alkaloids to be
107 present in the ethanol extract, but absent in the ethyl-acetate fraction. The presence of these
108 phytochemicals has been reported to account for the exertion of antimicrobial activity by plants [19].

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

114
 115 It was shown from previous studies that the root extract of the plant has more and lethal effect on
 116 microorganisms, followed by the bark among the various part tested [20]. But based on these work and
 117 the statistical analysis, the leaf extract showed MIC value with low concentration of 12.5mg/ml for *S.*
 118 *aureus* sand 50mg/ml for *K. Pneumoniae*, than the root and stem bark extract on both the tested bacteria
 119 (Table 5). The root showed MIC value with concentration of 50mg/ml for both the tested organisms. The
 120 result of the MIC and MBC also showed that *Balanites aegyptiaca* is bactericidal (Table 6).

121

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

	Root	Stem Bark	Leaf
	Ethanol	Ethanol	Ethanol
Alkaloids	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Steroids	+	+	+
Anthraquinones	-	-	+

123 + = Present, - = Absent.

124

125

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

128 Zones of inhibition (mm)

129 Microorganisms	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.125mg/ml	control
130 <i>K. pneumoniae</i> ¹³	10	5	2	2	26	
131 <i>S. aureus</i>	16	13	9	7	4	24

132 Control: Ceftriaxone

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

137 Zones of inhibition (mm)

138 Microorganisms	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.125mg/ml	control
139 <i>K. pneumoniae</i>	14	11	9	5	3	24
140 <i>S. aureus</i>	8	12	9	7	5	25

141 Control: Ceftriaxone

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

145 Zones of inhibition (mm)

146 Microorganisms	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.125mg/ml	control
147 <i>K.pneumoniae</i>	16	12	9	7	5	26
148 <i>S. aureus</i>	20	16	13	10	6	24

149 Control: Ceftriaxone

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

153 Microorganisms	Root	Stem bark	Leaf
154 <i>K. pneumoniae</i>	25	25	12.5

155 *S. aureus* 50 25 25

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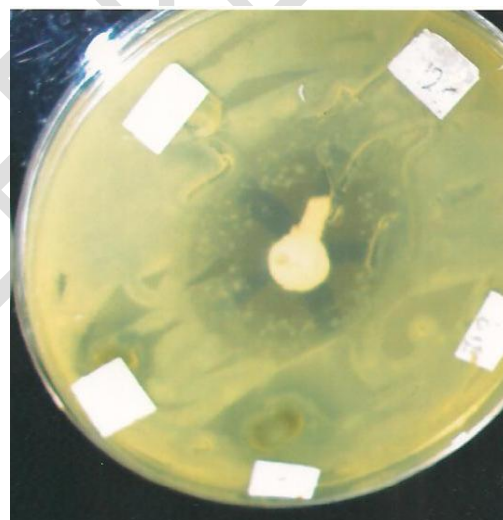
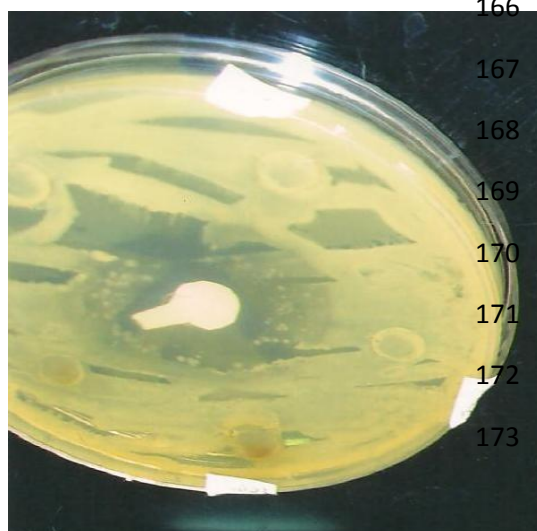
157 **Table 6:** Minimum Bactericidal Concentration (MBC) for microorganism of different extracts of *Balanites*
158 *aegyptiaca* in mg/ml

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

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



174 *Klebsiella pneumoniae*

Staphylococcus aureus

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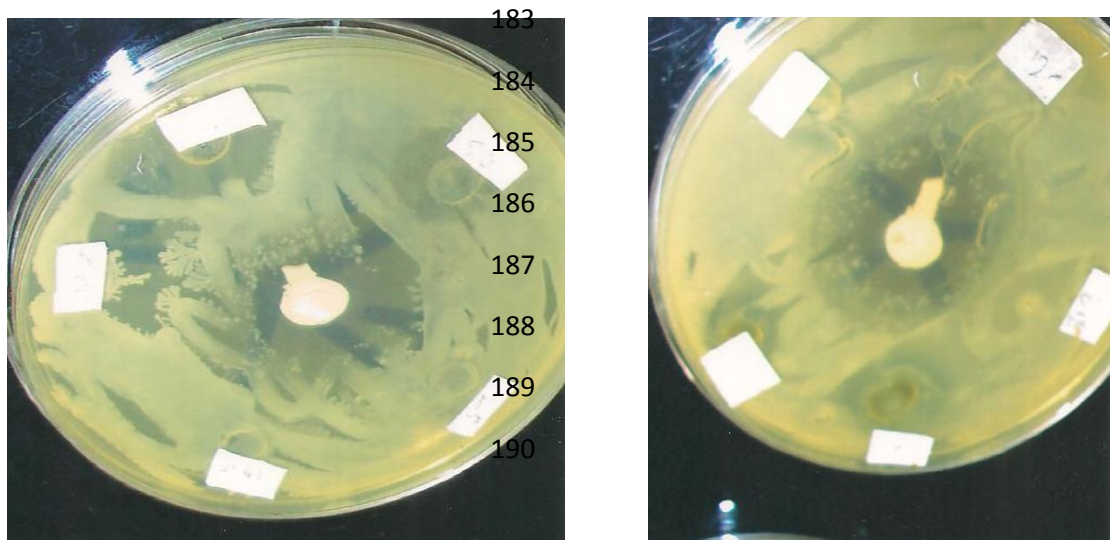
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181 **Plate 2:** inhibition zone of the stem bark extract at different concentrations against *Klebsiella pneumoniae*
182 and *Staphylococcus aureus*

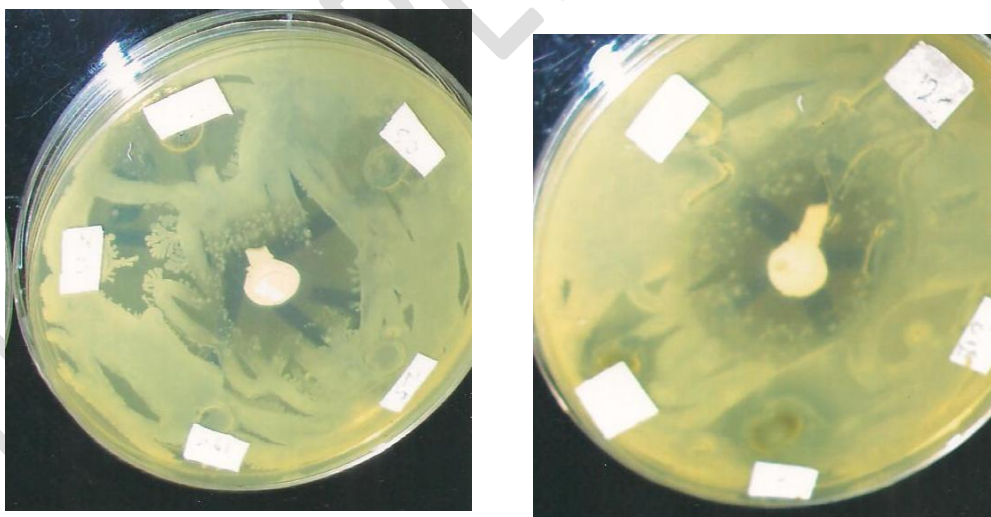


191 *Klebsiella pneumoniae*

Staphylococcus aureus

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193 **Plate 3:** Inhibition zone of the leaf extract at different concentrations against *Klebsiella pneumoniae* and
194 *Staphylococcus aureus*



204 *Klebsiella pneumoniae*

Staphylococcus aureus

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208 **Conclusion**

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

212
213 The result of the antibacterial activity of the ethanol extract of the root, bark and leaf of *Balanites*
214 *aegyptiaca* inhibited the growth of *Klebsiella pneumoniae* and *Staphylococcus aureus* with MIC value of
215 12.5mg/ml for *K. pneumoniae* and 25mg/ml for *S. aureus*.

216
217 The demonstration of antibacterial activity of the *Balanites aegyptiaca* is indeed a development that will
218 help to discover new antibiotics that can serve for treatment of infections that are caused by bacteria that
219 are becoming resistant to most of the antibiotics used for treatment of diseases caused by the
220 microorganisms. The fact that plants are very common makes it a cheaper alternative for drugs
221 development for human consumption and used.

223 **References**

- 224 1. Misrak, K., Amare, A. and Mohammed, Y. (2013). Efficacy of plant extracts, traditional materials and
225 antibacterial chemicals against *Xanthomonas campestris* pv. *vesicatoria* on tomato seed. *African*
226 *Journal of Microbiology Research*, 7(20): 2395-2400
227
- 228 2. Yinger, H. and Yewhalaw, D. (2007). Traditional medicinal plant knowledge and use by local healers in
229 Sekoru district, Jimmazon, Southwestern Ethiopia. *Journal of Ethnobiology and Ethnomedicine*. 3: 24-
230 30
- 231 3. Alaribe, S.I. (2008). A Survey of the Importance and Problems of Traditional Health care medicine, A
232 case study of Ezinihitte Mbaise L.G.A. Imo State. Unpublished B.Sc. project, A.I.F.C.E. Owerri, Imo
233 State.
234
- 235 4. Vaidya, A.D. and Devasagayam, T.P. (2007). Current status of herbal drugs in India: an overview.
236 *Journal of Clinical Biochemical Nutrition*, 41:1-11.
237
- 238 5. Ogunleye, D.S. and Ibitoye, S.F. (2003). Studies of antimicrobial activity and chemical constituents of
239 *Ximema Americana*. *Tropical Journal of Pharmacology* 2:239-241.
240
241
- 242 6. Auddy, B., Ferreira, M., Blasina, F., Lafon, L., Arredondo, F., Dajas, F., Tripathi, P.C., Seal, T. and
243 Mukherjee, B. (2003). Screening of antioxidant activity of three Indian medicinal plants, traditionally

244 used for the management of neurodegenerative diseases. *Journal of Ethno-Pharmacology*, 84:131-
245 138.
246

247 7. Parker, J. D. and Burkepile, D. E. (2017). Recent advances in plant-herbivore interactions. *Public*
248 *Medicine*, 1(6): 1-3

249 8. Sofowora, A. (2006). *Medical Plants and Traditional Medicine in Africa*. (2nd edn). Spectrum books Ltd,
250 Ibadan. Nigeria. PP. 150 –153.
251

252 9. Cowan, S. T., and Steel, K. J. (2004). *Manual for the identification of medical bacteria*. Cambridge
253 University Press, New York. PP. 45 – 63.
254

255 10. Barry, A. L. and Thornsberry, C. (1991). Susceptibility tests: diffusion test procedures. In Balows, A.
256 (ed.): *Manual of Clinical Microbiology*, 40: 213-219.
257

258 11. Bauer, A.W., Kirby, M.D.K., Sherras, J.C. and Trick, M. (2003). Antibiotic susceptibility testing by
259 standard single disc diffusion method. *American Journal of clinical Pathology* 45:490-496.
260

261 12. Abalaka, M.E., Mann, A. and Adeyemo, S.O. (2011). Studies on in-vitro antioxidant and free radical
262 scavenging potential and phytochemical screening of leaves of *Ziziphus mauritiana* L and *Ziziphus*
263 *spinachristi* L compared with Ascorbic acid. *Journal of Medical Genetics and Genomics*, 3(2):28-34.
264
265

266 13. Doughari, J. H., Pukuma, M. S. and De, N. (2007) Antibacterial effects of *Balanites aegyptiaca* L. Drel.
267 and *Moringa oleifera* Lam. on *Salmonella* Typhi. *African Journal of Biotechnology*. 6 (19), pp. 2212-
268 2215.

269 14. Rotimi, V. O., Laughon, B. E., Bartlett, J. G. and Mosadami, H. A. (1988). Activities of Nigerian chewing
270 stick extracts against *Bacterioides melaninogenius*. *Antimicrobial Agents of Chemotherapy*, 32: 598-
271 600.
272

273 15. Adebayo, E. A. and Ishola, O. R. (2009). Phytochemical and antimicrobial screening of crude extracts
274 from the root, stem bark and leaves of *Terminalia glaucescens*. *African Journal of Pharmacy and*
275 *Pharmacology* 3 (5): 217-222.
276
277

278 16. Igbinosa, O. O., Igbinosa, E. O. and. Aiyegoro, O. A (2009). Antimicrobial activity and phytochemical
279 screening of stem bark extracts from *Jatropha curcas* (Linn). *African Journal of Pharmacy and*
280 *Pharmacology*. 3 (2): 058-062

281 17. Harbone, J. B. (1999). *Phytochemical methods*. Chapman and Hall Limited, London. PP. 149 – 188
282

283 18. Nishaa, S., Vishnupriya, M., Sasikumar, J.M. And Gopalakrishnan, V.K. (2013). Phytochemical
284 Screening and GC-MS Analysis of Ethanolic Extract of Rhizomes of *Maranta arundinacea*. *Research*
285 *Journal of Pharmaceutical, Biological and Chemical Sciences*. 4 (2): 52
286
287

288 19. Lutterodt, G.D., Ismail, A., Basheer, R.H. and Baharudin, H.M. (1999). Antimicrobial effects of *Psidium*
289 *guajava* extracts as one mechanism of its antidiarrhoeal action. *Malaysia Journal of Medical Science*.
290 6(2): 17-20
291

292 20. Chapaga, B. and Wiesma, Z. (2005). Larvacidal effects of aqueous extracts of *Balanites aegyptiaca*
293 (desert date) against the larva of culexpiplens mosquitoes. *African Journal of Biotechnology*, 4:1351-
294 1354

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UNDER PEER REVIEW