Original Research Article

THE ETHANOLIC 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 ethanolic 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, steroids, and anthraquinones, although anthraquinones were absent in ethanolic extracts of root and stem bark of *Balanites aegyptiaca* but present in the ethanolic 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 ethanolic 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 ethanolic 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 aegpytiaca* Delile) has long history of traditional uses for wide ranges of diseases [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 aegpytiaca* Delile and *Ricinus communis* in water is used for contraception [3]

[4]. 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 [5]. According to Alaribe [6] 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 have been used for centuries to treat a wide variety of ailments [7]. The presence of secondary metabolites in plants has been associated in most of their therapeutic activities [8]. Herbal medicines are now considered a part of Complementary and Alternative Medicine (CAM) and are gaining popularity due to their potent antioxidant activity, minimal side effects and economic viability [9]. Many research efforts have been directed towards the provision of empirical proofs to back up the use of plants species in trade and medicinal practices in recent years [10]. This work intends to study specific microorganisms' response to extracts of *B. aegyptiaca*

2. MATERIALS AND METHODS

Source and preparation of plant materials

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

2.1 Extraction procedures

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

2.2 Preparation of Ethanolic Extraction of *Balanites aegyptiaca*

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

2.3 Phytochemical analysis

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

2.4 Source and preparation of test microorganisms

The stock cultures of the test microorganisms were obtained from the Department of Microbiology, Gombe State University. Their validity was determined by sub culturing onto nutrient agar and confirmed by standard cultural, morphological and biochemical techniques as described by [13]. The inocula of the test organisms were standardized by the method of Bauer and Thornsberry [14]. This was done by suspending each test organism in 5ml of nutrient broth and the turbidity was compared with that of 0.5 McFarland standard. McFarland standard was prepared by adding 0.6ml of 1% barium chloride ($BaCl_2$) to 99.4ml of 1% sulphuric acid (H_2S0_4) solution. The turbidity of the 0.5 McFarland standards was used for estimation the number of bacteria in broth culture (culture for 24 hours at 37^{0}C) to pour into 5ml of distilled water in order to obtain a standard bacterial suspension of 1 x 10^{5} cfu/ml [15].

2.4 Preparation of concentration of extracts

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

2.6 Antibacterial susceptibility testing

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

2.7 **Well method for antibacterial activity**

The well method was employed to assay the plant fractions for antibacterial activity. Petri dishes were poured with nutrient agar and allowed for 30 minutes to solidify (This was done in duplicate for each fraction and tested organism). The tested organisms were then inoculated by spreading on the inocula on the surface of the medium using a sterile swab stick. A sterile Cork borer (size 3) was used to bore 4 wells in the medium. The different concentration of the plant fractions were placed in the wells using a sterile syringe and needle (different for each sample and tested organism). These were then allowed a diffusion time of 1 hour after which the plates were incubated at 37 ^{0}C for 24 hours. The positive control was Ceftriaxone (100mg/ml). The potency of the extracts was determined by the clear zones of inhibition around the wells and was respectively measured as the diameter zones of inhibition. The MIC was determined using the method of Doughari [17], while MBC was determined using the method of Rotimi [18].

2.8 Data analysis

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

2.9 Result and Discussion

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

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

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

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

Table 1: Qualitative phytochemical screening of *Balanites aegyptiaca*

	Root Ethanol	Stem Bark Ethanol	Leaf Ethanol
Alkaloids	+	+	+

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

+ = Present, - = Absent.

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

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

Control: Ceftriaxone

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

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

Control: Ceftriaxone

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

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

Control: Ceftriaxone

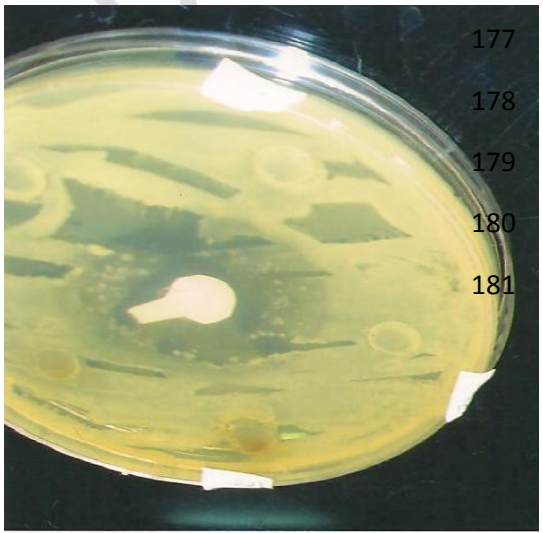
Table 5: Minimum inhibitory concentration (MIC) for microorganism of different extracts of *Balanites aegyptiaca* in mg/ml

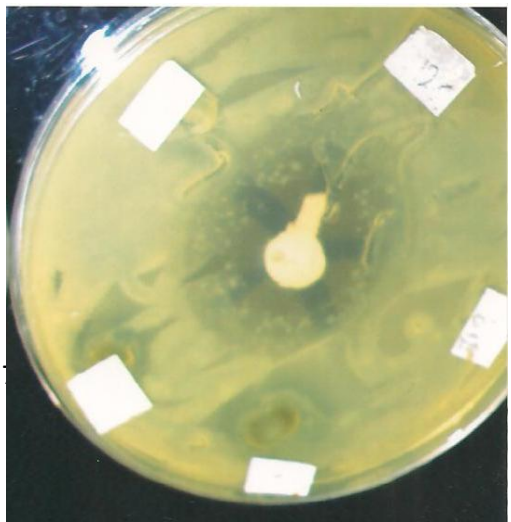
Microorganisms	Root	Stem bark	Leaf
K. pneumoniae	25	25	12.5
S. aureus	50	25	25

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

Microorganisms	Root	Stem bark	Leaf
K. pneumoniae	50	25	12.5
S. aureus	50	25	50

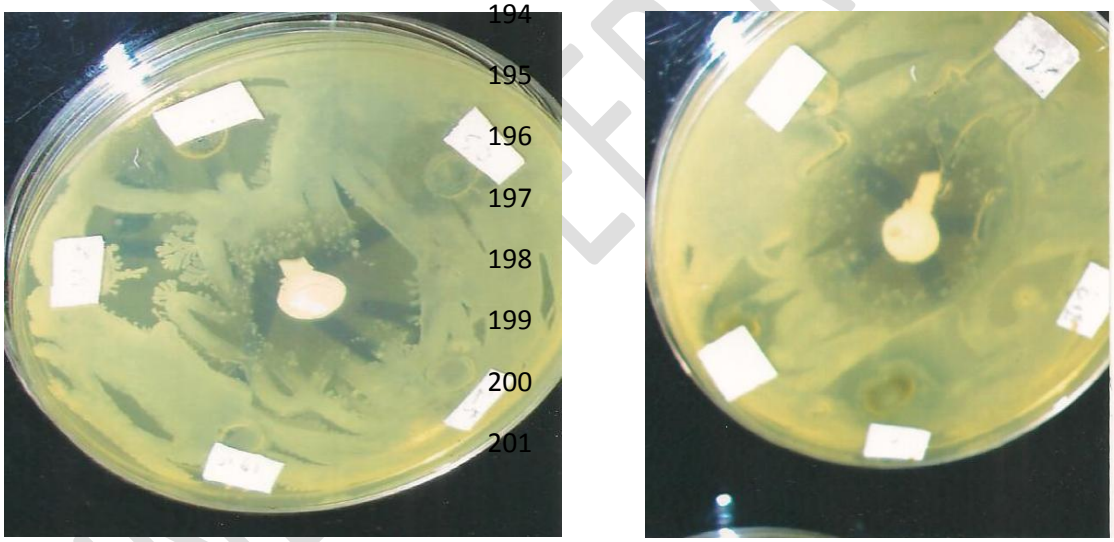
Plate 1: Inhibition zone of the root extract at different concentrations against *Klebsiella pneumoniae* and *Staphylococcus aureus*





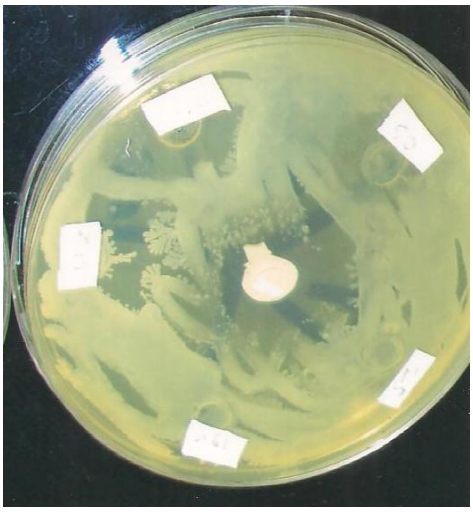
Klebsiella pneumoniae *Staphylococcus aureus*

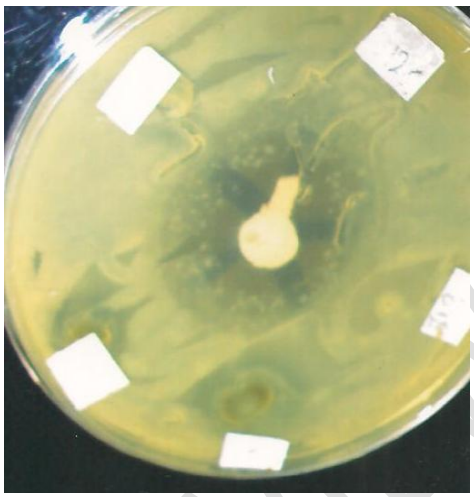
Plate 2: inhibition zone of the stem bark extract at different concentrations against *Klebsiella pneumoniae* and *Staphylococcus aureus*



Klebsiella pneumoniae *Staphylococcus aureus*

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





Klebsiella pneumoniae *Staphylococcus aureus*

3.0 Conclusion

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

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

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

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