

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL EFFECT OF THE AQUEOUS AND METHANOLIC EXTRACTS OF ROOTS OF *Balanites aegyptiaca* (Del.) ON SOME BACTERIA SPECIES.

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INTRODUCTION

Traditional medicine is the oldest method of curing diseases and infections and various plants have been used in different parts of the world to treat human diseases and infections (Caceres *et al.*, 1991 Nweze *et al.*, 2004, Vineela & Elizabeth, 2005). Different plant parts have also been used for the treatment of various form ailments. The *Balanites aegyptiaca* plants have been reported to be used in a variety of folk medicines in Africa and Asia. It has been used in the treatment of skin diseases and remedy for stomach ache and jaundice (Hammiche & Maiza, 2006), treatment of cough (John *et al.*, 1990), treatment of diarrhea and syphilis (Boulos, 1992) and Typhoid fever (Doughari *et al.*, 2007) The root was also reported to be used in treatment of inflammation (Kubmarawa *et al.*, 2007) antidote for snake bite (Inngerdigen, 2004). Earlier studies have shown that *B. aegyptiaca* contains steroidal saponins, with most of them reporting the presence of saponins as the main cause of these activities. Besides its medicinal uses, *Balanites* trees are widely used as fodder and for timber purposes (Arbonier, 2002). Medicinal plants are known to owe their curative potentials to certain biological active substances which exist in parts of the plants. The chemicals which are referred to as active principles or phytochemical substances include terpenes, flavonoid, bioflavonoid, benzophenones, xanthenes as well as some metabolites such as tannins, saponins, cyanates, oxalate and anthrax- quinones (Iwu, 1993; Asaolu, 2002). This study is therefore aimed at determining the antimicrobial efficacy of the aqueous and the methanolic extracts of *Balanites aegyptiaca*.

Collection of plant material: The leaves and roots of *Balanites aegyptiaca* were collected in the wild in Zaria (latitude 11.07N and longitude 4.28E) and taken to the herbarium of the Department of Biological Sciences, Ahmadu Bello University Zaria and identified with voucher number 900175.

Preparation of extracts: After shade drying the plant material was grounded into powder using pestle and mortar. Exposure to sunlight was avoided to prevent the loss of active components (Thakare, 2004).

Extraction of plant materials by maceration method: One liter of 80 % methanol extraction fluid was mixed with 200 g of powdered plant material. The mixtures were kept for 2 days in tightly sealed vessels at room temperature and stirred several times daily with a sterile glass rod. This mixture was filtered through muslin cloth. Further extraction of the residue was repeated 3-5 times until a clear colorless supernatant extraction liquid was obtained indicating that no more extraction from the plant material was possible. The extracted liquid was subjected to

water bath evaporation to remove the solvent. The water bath temperature was adjusted to 40^o C. The same procedure was used for the aqueous extract. The semi-solid extract produced was kept under a ceiling fan to dry. The extract was weighed and portion of it used for phytochemical screening while the rest was use for the susceptibility test.

Phytochemical screening: Standard phytochemical test was carried out on the plant samples using the method of Trease and Evans (1989) to determine presence of Alkaloids, flavonoids, glucosides, saponim, tannins, phlobatannins, anthroquinone, steroids and terpenoids.

Antimicrobial susceptibility studies: Inhibition of microbial growth was tested by using the well method (Kirby-Bauer Method as described by Drago *et al.*, 1999). Standard aseptic microbiological methods were followed throughout this antibacterial study.

Test Organisms: Clinical strains of *Pseudomonas auregenosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Candida albicans* were obtained from Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria.

McFarland Standard: 0.5 McFarland standard was prepared adding 0.5ml of 1% Barium chloride (BaCl₂) to 99.5ml of 1% sulphoric acid (H₂SO₄) solution. The turbidity (density) of the 0.5 McFarland standard was used for estimation of the amount of bacteria in broth culture (culture for 24 hours at 37^oC) to pour into 5ml of distilled water in order to obtain a standard bacterial suspension of 1x10⁸ bacterial cells m (Bauer *et al.*, 1986).

Serial Doubling Dilution: Stock solution was prepared using 1g of the solid plant extract dissolved in 10mls of normal saline making a stock of 100mg/ml. Serial doubling dilution was employed to obtain concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.25mg/ml, 6.125mg/ml and 3.125mg/ml respectively.

Well method for antibacterial activity: The well method was employed to assay the plant extracts for antimicrobial activity. Petri dishes were plated with Muller – Hinton agar and prepared according to the manufacturers manual was allowed for 30 minutes to solidify. The test organisms were then spread on the surface of the media using a sterile swap stick. Cork borer (3mm) was used to bore wells in the media, six/petri-dish. The different concentration of the plant extracts were dispensed into the wells using a sterile syringe and needle. These were then allowed a diffusion time of 1 hour after which it was incubated at 37^oC for 24 hour. Zones of inhibition were measured using a metre rule and the mean recorded in millimeter as described by Mukherjee *et al.*, (1995).

Minimum Inhibitory Concentration (MIC): The aqueous and methanolic extracts which showed significant zones of inhibition were chosen to assay for MIC. MIC was determined by the standard method of Wariso and Ebong (1996). Nutrient broth was prepared and sterilized using autoclave. One (1) ml of the prepared broth was dispensed in to the test tubes numbered 1-9

using sterile syringe and needle. A stock solution containing 25mg/ml of the extract was prepared. Then 1 ml of the solution was dispensed into the tubes numbered 1. Subsequently, from tube 1, serial dilution was carried out and 1 ml from tube 1 was transferred up to tube number 7 and 1 ml from the tube 7 was discarded. Tube 8 was control for sterility of the medium and tube 9 for viability of the organisms. An overnight culture (inoculums) of each of the test isolates was prepared in sterile nutrient broth. 1 ml of the inoculum was transferred into each tube from tube 1 to tube 9 with exception of tube 8, to which another sterile nutrient broth was added. The final concentration of the plant extract in each of the test tubes numbered 1-7 after dilution 25, 12.5, 6.25, 3.125, 1.563, 0.78 and 0.39mg/ml, were incubated at 37°C for 24 h and examined for growth. The last tube in which growth failed to occur was the MIC tube.

The preliminary phytochemical screening of the roots *B. aegyptiaca* indicated the presence of tannins, flavonoids, saponins, triterpenes and glycosides were identified in the plant extracts while alkaloids and steroids were found to be absent from the two extracts as shown in table 1 below. The antimicrobial activities of the root aqueous extract against *E. coli*, *S. aureus*, *P.*

aureginosa, *S. typhi* and *C. albicans* is presented in Fig. 1 where all the test organisms were inhibited at

TABLE 1. QUALITATIVE PHYTOCHEMICAL SCREENING OF AQUEOUS AND METHANOLIC EXTRACTS OF ROOTS OF *B. aegyptiaca*.

Phytochemical	Methanolic extract	Aqueous extract
Alkaloid	-	-
Tannins	+	+
Flavonoids	+	+
Saponins	+	+
Steroids	-	-
Triterpenes	+	+
Glycosides	+	+
Anthroquonones	+	-

+ = Present, - = Absent

different concentrations except *S. typhi* which was not inhibited even at the highest concentration of 100mg/ml. Similarly, methanolic extract showed significant zones of inhibition on all the test organisms. *C. albicans* had the highest zone of inhibition at 50mg/ml (Fig 2).

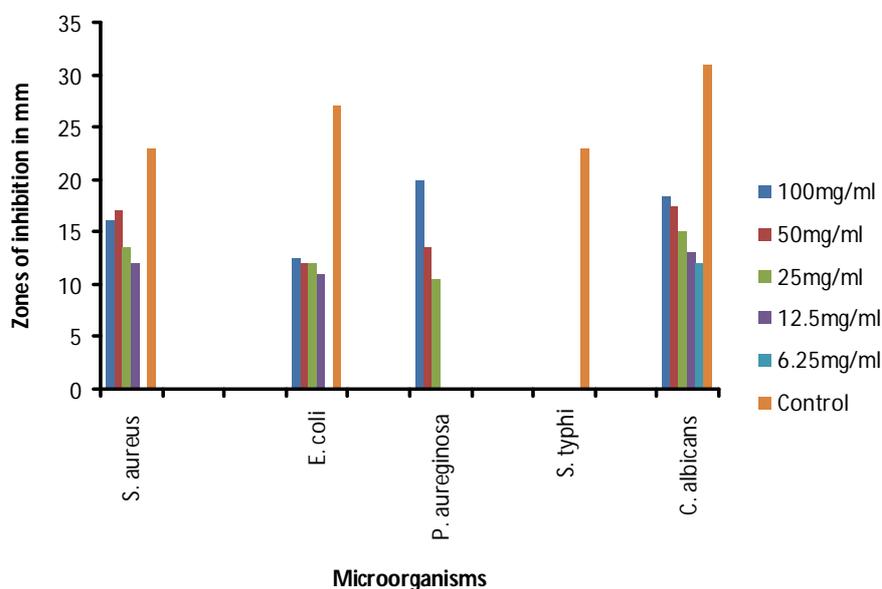


FIG. 1. SENSITIVITY OF DIFFERENT MICROORGANISMS TO DIFFERENT CONCENTRATION OF AQUEOUS EXTRACT OF *B. aegyptiaca*.

The methanolic root extract indicated the highest and least MIC of 50mg/ml and 1.563mg/ml against *S. typhi* and *C. albicans* respectively while that of the aqueous extract ranged between 3.125- 12.5mg/ml as shown in (Table 2).

The presence of phytochemicals in the root of *Balanites aegyptiaca* observed in this study confirmed the presence of flavonoid, saponins, tannins and glycoside Alkaloid, and steroids were absent in both extracts. Anthroquinone was present in the methanolic extract but absent in the aqueous extract. This difference can be attributed to the solubility of the active

component in different solvents (Ekpo & Etim, 2009). This was in agreement with the work of Kubmarawa *et al.*, (2007), who reported the presence of same in the root ethanolic extract of *Balanites aegyptiaca*. To a large extent, the chronological age of the plant, percentage humidity of the harvested material, situation and time of harvest, and the method of extraction were possible sources of variation for the chemical composition, toxicity and bioactivity of the extracts (Felix, 1982). The presence of these constituents has been reported to account for the exertion of antimicrobial activity by plants (Clark, 1981, Pretorius & Watt, 2001).

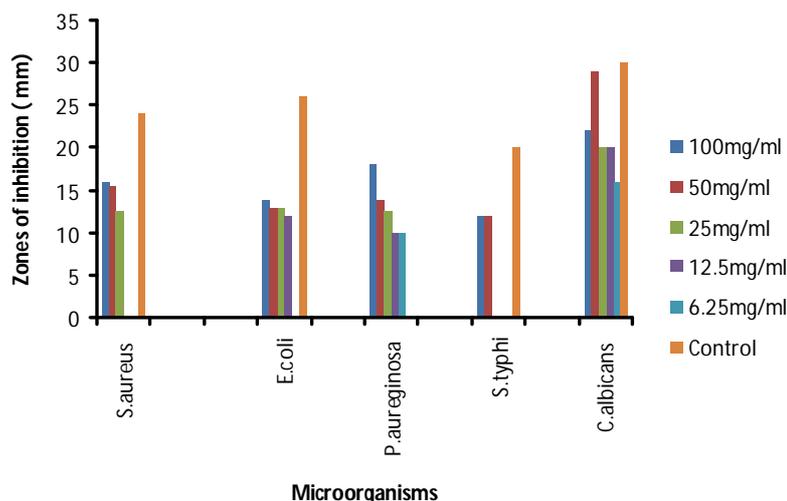


FIG. 2. SENSITIVITY OF DIFFERENT MICROORGANISMS TO DIFFERENT CONCENTRATION OF METHANOLIC EXTRACT OF *B. aegyptiaca*.

TABLE 2. MIC OF THE AQUEOUS AND METHANOLIC EXTRACTS OF *B. aegyptiaca* ON THE TEST ORGANISMS.

Microorganisms	Methanolic extract	Aqueous extract
<i>S. aureus</i>	6.25	3.125
<i>E. coli</i>	3.135	6.25
<i>P. aureginosa</i>	3.125	12.5
<i>S. typhi</i>	50	-
<i>C. albicans</i>	1.563	3.125

Methanolic extract of *Balanites aegyptiaca* showed significant zones of inhibition of the microorganisms used in the study. The aqueous extract was not able to inhibit *S. typhi*. This was in contrast with the result of Doughari *et al.*, (2007) who reported activity of aqueous root extract of *Balanite aegyptiaca* on *S. typhi* even though the methanolic and acetone extract performed significantly better. These disparities may be attributed to the change in environmental conditions which may affect the production of secondary metabolite in the plant (Waterman & Mole, 1989). There was no significant difference ($p > 0.05$) in the zone of inhibition between the two extract. The ability of *B. aegyptiaca* showing sensitivity to two different strains of bacteria (gram positive and gram negative bacteria) and fungi shows its application as a broad spectrum antimicrobial agent with the largest efficacy being the methanolic extract from this study. This justifies the use of this plant in traditional medicine practices. It is therefore recommended that more work be conducted to help optimally extract all the bioactive compounds in the plant and formulated into appropriate dosage for the treatment of infectious diseases.

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