

ANTIMICROBIAL ACTIVITY OF *BALANITES AEGYPTIACA* LEAFSShams Eldien Koko¹, Talib M. A.², Suliman I. Suliman¹, Muddathir S. Alhassan¹ and Ahmed S. Kabbashi*¹¹Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), P.O. Box 2404, National Center for Research, Khartoum, Sudan.²Africa City of Technology, Khartoum, Sudan.

*Corresponding Author: Ahmed S. Kabbashi

Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), P.O. Box 2404, National Center for Research, Khartoum, Sudan.

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ABSTRACT

Balanites aegyptiaca has been reported to be an anti-helminthic, a purgative, febrifuge, emetic and can also cure other types of ailments like skin boils, malaria, wounds, colds, syphilis, liver and spleen disorders. Various parts of this plant have their own traditional medicinal properties. The seed is used as a febrifuge, and its oil is used to treat tumors and wounds. An aqueous extract of the bark is used in Sudanese folk medicine in the treatment of jaundice. The kernel oil exhibited anticancer activity against lung, liver, and brain carcinoma cell lines. It also has anti-mutagenic activity against *Fasciola gigantica*-induced mutagenicity besides anthelmintic activity against hepatic worms (*Schistosoma mansoni*), the aqueous leaf extract and saponins isolated from its kernel cakes have antibacterial activity. The branches are used as tooth brush. The root extracts have proved to be slightly effective against experimental malaria. The purpose of the paper was to investigate the *in-vitro* antimicrobial activity of ethanol, Petroleum ether and chloroform extracts of *B. aegyptiaca* (Leafs). The ethanol, Petroleum ether and chloroform extracts of *B. aegyptiaca* was tested against four standard bacteria i.e.: two Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*), two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and against two standard fungi species i.e. *Aspergillus niger* and *Candida albicans* using the disc diffusion method. The microbial activities were provide that most of the extracts ethanol, petroleum ether, and chloroform extract of *B. aegyptiaca* (Leafs). The ethanol, petroleum ether, and chloroform extract exhibited inhibitory effects against most of the tested organisms with the zone of inhibition ranging from (11 to 14 mm), (15-16 mm) and (13-15mm) respectively. In conclusion: This study conducted for *B. aegyptiaca* (Leafs) proved to have potent activities against antibacterial as well as antifungal activity *in vitro*.

KEYWORDS: *In vitro*, antimicrobial, *Balanites aegyptiaca*, (leafs).

INTRODUCTION

Balanites aegyptiaca is a widely grown desert plant with multi-use potential. It is mainly found in arid and semi-arid areas throughout Africa, the Middle East, and South Asia. It is believed that the plant is indigenous to all dry lands south of the Sahara, extending southward to Malawi in the Rift Valley, and to the Arabian Peninsula. It has wide ecological distribution, but it is mainly found on level alluvial sites with deep sandy loam and free access to water. It is a lowland species, growing up to 1000 m altitude in areas with mean annual temperature of 20 to 30°C and mean annual rainfall of 250 to 400 mm.^[2] In Sudan it is widely spread in the northern arid and the central semi-arid regions.^[3] In many African countries (e.g. Senegal, Nigeria, Ethiopia and Sudan) *B. aegyptiaca* has been reported to be an anti-helminthic, a purgative, febrifuge, emetic and can also cure other types of ailments like skin boils, malaria, wounds, colds, syphilis, liver and spleen disorders.^[4] Various parts of this plant have their own traditional medicinal properties.

The seed is used as a febrifuge,^[5] and its oil is used to treat tumors and wounds.^[6] An aqueous extract of the bark is used in Sudanese folk medicine in the treatment of jaundice,^[7,8] The kernel oil exhibited anticancer activity against lung, liver, and braincarcinoma cell lines. It also has anti-mutagenic activity against *Fasciola gigantica*-induced mutagenicity besides anthelmintic activity against hepatic worms (*Schistosoma mansoni*), the aqueous leaf extract and saponins isolated from its kernel cakes have antibacterial activity.^[9,10] The branches are used as tooth brush.^[11] The root extracts have proved to be slightly effective against experimental malaria.^[12]

Infectious diseases account for high proportion of health problems and are the leading cause of death worldwide.^[13] Even though pharmaceutical industries have produced a number of new antimicrobial drugs in the last years, resistance to these drugs by micro-organisms has increased. This is due to indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious diseases.^[14] In general,

bacteria have the ability to acquire and transmit resistance to drugs used as therapeutic agents,^[15] Incidents of epidemics due to such drug resistant micro-organisms are now a common global problem posing enormous public health concerns. The global emergence of multidrug resistant bacterial strains is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure of infections.^[16,17] The present study was conducted to investigate the antimicrobial activity of *B. aegyptiaca* (leaf) in Sudan.

MATERIALS AND METHODS

Plant Material

The *B. aegyptiaca* (Leaf) was collected from Central Sudan between January and February 2017. The plant was identified and authenticated by the taxonomists of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan.

Preparation of crude extracts

Processing and Extraction of Plant Samples.

The dried powdered leaf (50 g) was extracted successively by ethanol, Petroleum ether and chloroform in a Soxhlet extractor by continuous hot percolation. Each time before extracting with the next solvent of higher polarity the powdered material was dried in a hot air oven below 50 °C for 10 minutes and dried in hot air oven.^[18]

Test microorganisms

The ethanol, Petroleum and chloroform extracts of *B. aegyptiaca* leaf were tested against four bacterial species: two Gram positive bacteria viz., *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923), two Gram-negative bacterial strains *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and two fungal strains viz., *Aspergillus niger* (ATCC 9763) and *Candida albicans* (ATCC 7596). The bacterial and fungal strains used in the study were obtained from the Department of Microbiology, of the Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI) and National Health Laboratory of Khartoum in Sudan. The bacterial cultures were maintained on nutrient agar and incubated at 37°C for 18 h and then used for the antimicrobial test.

Preparation of the test organisms

Preparation of bacterial suspensions

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37° C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10⁸- 10⁹ C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used.

The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique.^[19] Serial dilutions of

the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

Preparation of fungal suspension

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

Testing of antibacterial susceptibility

Disc diffusion method

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines.^[20] Bacterial suspension was diluted with sterile physiological solution to 10⁸cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

RESULTS AND DISCUSSION

The leaf of ethanol, Petroleum and chloroform extracts of *B. aegyptiaca* family (Balanitaceae) was screened for antimicrobial activity against two Gram positive bacteria (*B. subtilis*, *S. aureus*), two Gram negative bacteria (*E. coli*, *P. aeruginosa*) as well as two fungi namely (*A. niger* and *C. albicans*) using the disc diffusion method.

The microbial activities were provided that most of the extracts ethanol, petroleum ether, and chloroform extract of *B. aegyptiaca* (Leaf). The ethanol, petroleum ether, and chloroform extract exhibited inhibitory effects against most of the tested organisms with the zone of inhibition ranging from (11 to 14 mm), (15-16 mm) and (13-15mm) respectively.

The ethanol extract of *B. aegyptiaca* (Leafs) dissolved in methanol (1:10) showed high activity (14, 12 and 11 mm) against (*C. albicans*, *S. aureus* and *B. subtilis*) respectively, Petroleum ether extract of *B. aegyptiaca* (Leafs) dissolved in methanol (1:10) showed high activity (16, 15, 15 and 13 mm) against (*A. niger*, *B.*

subtilis, *S. aureus* and *C. albicans*) respectively, and The chloroform extract of *B. aegyptiaca* (Leafs) dissolved in methanol (1:10) showed high activity (16, 15, 15, 13 and 13 mm) against (*C. albicans*, *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis*) respectively.

Table 1: Antimicrobial activity of leafs of *B. aegyptiaca* against the standard bacteria and fungi.

Standard microorganisms	Mean Diameter of Growth Inhibition Zone (mm)		
	Ethanol	Petroleum ether	Chloroform
Tested Bacteria used			
Bacillus subtilis	11	15	13
Escherichia coli	-	-	15
Staphyococcus aureus	12	15	13
Pseudomonas aeruginosa	-	-	15
Tested fungi used			
Apergillus niger	-	16	-
Candida albicans	14	13	16

Key: The results were expressed in terms of the diameter of the inhibition zone: < 9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active; >18 mm, very active.

CONCLUSION

The leaf extracts of *B. aegyptiaca* showed the various degree of inhibitory activity against the microorganisms tested. The obtained results may justify the use of the Sudanese leafs of *B. aegyptiaca* as antimicrobial therapy in traditional medicine in Sudan and the neighboring countries. Further investigations regarding the mode of action and other related pharmacological studies such as in vivo investigation, drug formulation and clinical trials are highly recommended.

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