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IN VITRO ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF BALANITES AEGYPTIACA(L.) DEL. OF AQUEOUS BARK EXTRACT

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ABSTRACT

aqueous extract of Balanites aegyptiaca. bark, which were collected from North Kordofan Province, Sudan was extracted in distilled water using soxhlet apparatus. Phytochemical analysis of the extract revealed the presence of tannins, saponins, alkaloids flavonoids, steroids and glycosides. Yield percentage of **a**queous. l was tested for its antimicrobial activity against five standard organism of bacteria and two standard organisms of fungi, it showed moderate activity against all organisms except E, coli, which was sensitive to the two concentrations used. hese findings support some of the traditional applications of the bark of B. aegyptiaca against microbial aliments. It is therefore recommended that further studies regarding fractionation, separation and purification of these active antimicrobial compounds are required.

KEYWORDS: Balanites aegyptiaca, aqueous extract, antimicrobial.

INTRODUCTION

Traditional medicines are widely utilized in our world today for the treatment of various ailments. About eighty percent of the world's population depend on traditional medicine for health sustenance. Many of these medicines have been scientifically validated to possess such biological activities and can be used to treat various ailments. Several studies have documented the toxic effects of some of these medicinal plants Balanites aegyptiaca (L.)

Del., known as Hegleig is a plant that belongs to the Balanitaceae family. It is an evergreen savanna tree, 4.5 to 6 m high, woody and with small spine scents (Koko et al., 2000). This plant is an indigenous species in Sudan, popular and of great concern, with diverse uses in folk medicine and many other applications (Elfeel and Warrag, 2011). It is widely distributed in arid and semi-arid regions of Sudan. It is estimated that up to one third of total trees population in central parts of Sudan is from this plant (NCR, 2008). It is also distributed in other countries located in the tropical dry belt of North Africa (Mohamed et al., 1999) and dry areas of India and South Asia (Chothani and Vaghasiya, 2011).

Almost all parts of this plant are used in traditional medicine. It is traditionally employed in treatment of jaundice, yellow fever, syphilis, diarrhea, epilepsy, cough and wound healing, in addition to its applications as anti-inflamatory, anti-helminthic, insecticidal, anti-ralarial, molluscicidal, anti-fungal, anti-bacterial and even for snake bites (Mohamed et al., 1999; Chothani and Vaghasiya, 2011; Koko et al., 2000; John et al., 1990; Inngerdingen et al., 2004; Kubmarawa et al., 2007; Maregesi et al., 2008). the bark of Balanites aegyptiaca is used in traditional medicine for various ailments including jaundice, malaria, and syphilis. It has demonstrated antimalarial activity in studies and also exhibits molluscicidal properties, making it effective against snails and other organisms. Additionally, the bark has been incorporated into soap in Sudan and has shown antimicrobial and antioxidant properties. The bark of the Balanites aegyptiaca tree treats hepatitis and reduces the risk of developing fibroids. It also effectively treats colon cancer. It expels gas and toxins and rids the body of any spleen problems.previous studies on the bark revealed the presence of tannins, saponins, alkaloids flavonoids, steroids and glycosides (Abdel-Rahim et al., 1986). This study deals with antimicrobial activity of the bark of Balanites aegyptiaca (L.) Del growing in Sudan (Sudanese variety).

MATERIAL AND METHODS

Collection of Plant Material

Preparation of plants extract The stem bark of Balanites aegyptiaca was collected were collected from North Kordofan State Province, identified by Dr. HayderAdbalgader and herbarium sheet was deposit at the herbarium of Medicinal and Aromatic Plants Research Institute. (MAPRI).

Preparation of the Aqueous Extract

The stem bark of the plant was air dried to a constant weight, pulverised to a dry powder and was extracted in distilled water using soxhlet apparatus extract 100 g of the stem bark sample with 500 ml of hot water for 4 hours the extract was evaporated to dryness in a hot air oven at 45oC then filtered with watt man filter paper. Extracts kept in deep freezer for 48 hours, then induced in freeze dryer till completely dried. The residue was weighed and the yield percentage was determined. The aqueous residue (2g) was dissolved in sterile was 20 ml (con 100 mg/ml), and Kept in refrigerator until used.

Phytochemical screening Test

General phytochemical screening for the active constituents was carried out for aqueous extract which showed the highest antimicrobial activity using the methods described by (Martinez & Valencia (1999), Sofowora (1993), and Harborne (1984))) with many few modifications.

Test of Tannins

0.2 g of the sample extract was dissolved in 10 ml of hot saline solution and divided in two tests tubes. To one tube 2-3 drops of ferric chloride added and to the other one 2 – 3 drops of gelatin salts reagent added. The occurrence of a blackish blue color in the first test tube and turbidity in the second one denotes the presence of tannins.

Test of Sterols and Triterpenes

0.2 g of the sample extract was dissolved in 10 of chloroform. To 5 ml of the solution 0.5 ml acetic anhydride was added and then 3 drop of conc. Sulphuric acid at the bottom of the test tube. At the contact zone of the two liquids a gradual appearance of green, blue pink to purple color was taken as an evidence of the presence of sterols (green to blue) and or triterpenses (pink to purple) in the sample.

Test for Alkaloids

0.5 g of the sample extract was dissolved in 10 ml of 2N HCL in water bath and stirred while heating for 10 minutes, cooled filtered and divided into two test tubes. To one test tube few drops of Mayer’s reagent was added while to the other tube few drops of Valser’s reagent was added. A slight turbidity or heavy precipitate in either of the tow test tubes was tanked as presumptive evidence for the presence of alkaloids.

Tests for Flavonoids

0.5 g of the sample extract was dissolved in 30 ml of 80% ethanol and filtered. The filtrate was used for following tests:

A/ to 3 ml of the filtrate in a test tube 1ml of 1% aluminum chloride solution was in methanol was added. Formation of a yellow color indicated the presence of Flavonoids. Flavones or and chalcone.

B/ to 3ml of the filtrate in a test tube 1ml of 1% potassium hydroxide solution was added. A dark yellow color indicated the presence of Flavonoids compounds (flavones or flavonenes) chalcone and or flavonols.

C/ to 2ml of the filtrate 0.5ml of magnesium turnings were added. Producing of defiant color to pink or red was taken as presumptive evidence that flavonenes were present in the plant sample.

Test for Saponins

0.3 g of the sample extract was placed in a clean test tube. 10 ml of distilled water were added, the tube stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of foam, which persisted for least an hour, was taken as evidence for presence of saponins.

Test for Cumarins

0.2 g of the aqueous extract dissolved in 10 ml distilled water in test tube and filter paper attached to the test tube to be saturated with the vapor after a spot of 0.5N KOH put on it. Then the filter paper was inspected under UV light, the presence of coumrins was indicated if the spot has found to be adsorbed the UV light.

Test for Anthraquinone Glycoside

0.2 g of the sample extract was boiled with 10 ml of 0.5N KOH containing 1ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of benzene. 5ml of the benzene solution was shacked with 3ml of 10% ammonium hydroxide solution and the two layers were allowed to separate.

The presence of anthraquinones wa+s indicated if the alkaline layer was found to have assumed pink or red color.

Test for Cyanogenic Glycoside

0.2 g of the sample extract was placed in Erlenmeyer flask and sufficient amount of water was added to moisten the sample, followed by 1ml of chloroform (to enhance every activity). A piece of freshly prepared sodium picrate paper was carefully inserted between a split crock which was used to stopper the flask, a change in color of the sodium picrate paper from yellow to various shades of red was taken as an indication of the presence of cyanogenic glycoside.

Antimicrobial activity of aqueous extract

Preliminary antimicrobial study was carried out using the method adopted by (Kavanagh 1972).

Tested organisms

Fungal micro-organisms

Aspergillus Niger AT cc 9763

Candida albicans AT cc 7596

Bacterial micro-organisms

Escherichia coli ATCC 25922

Klebsiella pneumonia ATCC 53651

Pseudomonas aeruginosa ATCC 27853

Staphylococcus ATCC 25923

Proteus vulgaris NCTC 8196

In vitro Testing of Antimicrobial Activity

Preparation of suspension

One ml aliquots of 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, washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about (108 – 109) colony forming units per ml. (Miles and Misra 1938).[12] The suspension was stored in the refrigerator at 4◦ C till use.

Screening the antibacterial activity of the extracts

3 ml of each of the 4 bacterial stock suspensions were thoroughly mixed with 300 ml of sterile melted nutrient agar which was maintained at 45◦ C. 20 ml of each of the inoculated nutrient agar were distributed into 6 sterile Petridishes. The agar was left to set and in each of

these plates, which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No. 40,). The agar discs were removed, alternate cups were filed with 0.1 ml of oil using adjustable volume automatic micro-titre pipette, and allowed to diffuse at room temperature for 2 hours. The plates were then incubated in the upright position at 37◦C for 18 hours. After incubation the diameters of the inhibition zones were measured.

3. RESULTS AND DISCUSSION

3.1. Preliminary Phytochemical Screening

The result showed the presence of alkaloid, tannins, saponins, glycosides, carbohydrates and steroids in varying degree These bioactive compounds contribute to a broad range of the plant's pharmacological profile, which includes antioxidant, wound healing, antimicrobial, anti-hepatitis, anticancer, anti-inflammatory, molluscicidal, anticonvulsant, antiplasmodial, antiparasitic effects, androgenic properties, and molecular docking. Toxicological studies have also confirmed the safety of B. aegyptiaca for medicinal purposes.

Table 1: Phytochemical Analysis of aqueous stem bark extract of Balanites aegyptiaca.

Constituents	Relative presence
Alkaloids	++
Steroids	++
Tritepenes	+
Flavonoids	++
Saponins	+++
Cumarins	-
Tannins	++
Anthraquenones	-
Cyanogenic	+

Key: +Trace, ++ Moderate, +++ High, - Negative

3.2. Antimicrobial activity of the aqueous extract

Antimicrobial activity of the aqueous extract using cup diffusion agar method and two concentrations (10 % and 20 %) against five standard organisms of bacteria and two fungi showed moderate activity against all used organisms except E, C which was sensitive to the two concentrations used. Inhibition zones ranged between 14 to 16 mm for all organisms, while for E. C was 19 to 20 mm. Obtained results of antimicrobial activity found to be on line with the findings of (Henna 2010)), who stated that the aqueous extract of Balanites

aegyptiaca showed antimicrobial activity ranged between 10 to 23 mm against different standard organisms of bacteria and fungi including the same species of this study.

Table: Inhibition zones (mm) of Balanites aegyptiaca of the Aqueous Extract against standard Organisms.

Organisms / Conc.	E. c	Ps.a	K. p	Pr. v	S. a	Ca.a	As. n
10 %	19	14	16	15	14	14	14
20 %	20	14	16	16	15	14	15

Isolated (Staphylococcus aureus: S.a, Escherichia coli: E.c, Pseudomonas aeruginosa: Ps.a, Candida albicans: Ca.a Aspergillus niger: As.n, Klebsiella pneumonia:k.p, and Proteus vulgaris: pr.v).

CONCLUSION

The current study revealed that the aqueous extract of the bark of B. aegyptiaca (desert dates) is a promising source of potential antimicrobial activity against wide-spectrum microorganisms, as many phytochemical investigation. The presence of alkaloids, saponins, terpenoids and tannins in the bark extracts of Balanite aegyptica has medicinal implications. These phytochemicals are known to be biologically active. Tannins were found to play a role in antifungal, antibacterial, astringent and antibiotic activities. Tannins were also found to form irreversible complexes with proline-rich proteins leading to the inhibition of the cell protein synthesis. In addition to antimicrobial activity exhibited by tannins, they also react and form complex with proteins to provide the typical tanning effect. This is important medicinally for the treatment of inflamed or ulcerated tissues. Tannins-containing herbs as their main component are astringent in nature and are used in the treatment of intestinal disorders such as diarrhoea and dysentery, thus exhibiting antimicrobial activity. One of the largest groups of chemical produced by plants is the alkaloids and their amazing effect on humans Accordingly, the bark of this ancient medicinal plant could play significant role in the search compounds of antimicrobial activity have been detected, in addition to the positive results of the antimicrobial for new antimicrobial drugs

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