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Phytochemicals, Antibacterial, Antioxidant and Cytotoxic Analyses of *Balanites Aegyptiaca* Stem Bark Extracts

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ABSTRACT

Powdered stem bark of *B. aegyptiaca* was extracted using ethanol, aqueous and n-hexane. Test for the secondary metabolites showed the presence of flavonoids, saponins, tannins, phenols and carbohydrates in aqueous and ethanol extracts, while ethanol extract has in addition cardiac glycoside and steroids. Anthraquinone was the only metabolite detected in n-hexane extract. The antibacterial activity of the plant extracts showed ethanol extract to have a broad spectrum of activity against the test isolates with varying zones of inhibition, as shown by the results of the minimum inhibitory concentration (MIC) which for hexane extract was obtained at 50 mg/ml for *S. aureus*, while that of ethanol extract was obtained at25mg/ml for S. *typhi*, 50 mg/ml *for E.coli and* 100 mg/ml for *S. aureus.* However, the test organisms were not susceptible to the aqueous extract. DPPH (1,1-diphenyl-2 picrylhydrazyl) free radical scavenging assay was used to determine the antioxidant property of the extracts. The result showed that DPPH% inhibition varied between extracts with ethanol extract having a maximum of 80% while aqueous extract had 32% inhibition. The crude extracts of the plant sample were also subjected to brine shrimp lethality bioassay. The results obtained were 0.46, 3.13, 9.70 and13.35µg/ml for vincristine sulphate, hexane, ethanol and aqueous extracts respectively. Comparing this result with the standard vincristine sulphate, hexane extract showed significant cytotoxicity having LC_{50} of 3.13µg/ml.

Keywords: Antibacterial, Antioxidant, *Balanites aegyptiaca,* Flavonoids, Phytochemicals

INTRODUCTION

Balanites aegyptiaca (L.) Del., belongs to the family Zygophyllaceae. It is widely grown in the Sudano-Sahielian region of Africa, the Middle East and South Asia [1]. It is a multibranched evergreen tree reaching between 6 to 10 m in height, and grows under wide range of habitat,

tolerating a wide variety of soil types and climatic moisture levels [2]. *B. aegyptiaca (L) Del* has a long history of usage in African folk medicine to treat different diseases. In Egyptian folk medicine, the fruits are used as an oral hypoglycaemic and as an antidiabetic [3], while in Sudanese folk medicine, an aqueous extract of the fruit mesocarp is used in the treatment of jaundice[4].In Senegal, Nigeria, Morocco, and Ethiopia, *B. aegyptiaca* is taken as a purgative for colic and stomach ache [5]. In Nigeria, it has been reported that a mixture of dried leaves powder of *B. aegyptiaca* with *Ricinus communis* in water has been used for the treatment of contraception while the seed kernel oil is used for wounds [6]. Similarly, in Somalia, the powdered root bark is also used for contraception [7]. In Chad, fresh twigs are put on the fire in order to keep insects away, and the dried fruits are mashed in millet porridge and eaten for intestinal worms. In Libya and Eritrea, the leaves are used for cleaning infected wounds [5]. In Kenya, a root infusion is used as an emetic while the powdered seed is taken for asthma [8].

The pharmacological activity of different parts of this plant extracts has been studied. The aqueous extracts of fruit kernel and stem bark were reported to be effective against the larvae of *Aedes arabiensis***,** *Culex quinquefasciatus* and *Aedes aegypti* [9]. The larvicidal activity of the plant extract may be due to the interaction of saponin molecules with cuticle membrane of the larvae, thereby disordering it [10].The methanolic and aqueous extracts of whole plant of *B. aegyptiaca* were reported to be weakly active against *Staphylococcus aureus* and *Staphylococcus epidermidis* [11]. Stem bark extracts was also reported to show high antifungal activity against *Candida albican* [12]. This activity may be due to the presence of several triterpene saponins and steroidal saponins in *B. aegyptiaca* stem [13]. Ojo and co-workers [14] screened the extracts of leaf, stem, stem bark and root of this plant samples for hepatoprotective activity in Wistar albino rats. The stem bark extract was reported to show significant hepatoprotective effects than other extracts as compared to control rats. The stem bark extracts of *B. aegyptiaca* has been reported to show moderate antidiabetic activity while the aqueous extract of mesocarps of the fruits exhibit prominent antidiabetic activity. It is believed that the antidiabetic activity was due to the presence of steroidal saponins in the extracts [15, 16].

The main aim of this study was to determine the antibacterial, antioxidant and cytotoxic activity of different solvent extracts of stem bark of *B. Aegyptiaca.*

MATERIALS AND METHODS

Sample collection and Preparation

The stem bark of *B. aegyptiaca* was collected from the mature tree found around boys hostel of Nasarawa State University, Keffi, Nigeria, and was authenticated at the Department of Forestry and Wildlife, Nasarawa State University, Keffi, Nigeria, with a Voucher No FWF/O132 which was deposited at the Departmental herbarium. The samples were washed in clean water to remove dirt, air dried at room temperature for four weeks, pulverized with the aid of pestle and mortar into fine particles. It was stored in air tight container and kept away from moisture until required for the experiment.

Extraction of the sample

The powdered dried stem bark of *B. aegyptiaca was* extracted using method described by Yinusa *et al.*, [17] with slight modification. Powdered stem bark (50 g) were each suspended in 200 ml of extracting solvents (aqueous, n-hexane or ethanol) for three days with occasional agitations. The extracts were then decanted and filtered out using Whatman No 1 filter paper. The filtrates were each concentrated using rotary evaporator, weighed and store in the refrigerator at 4 °C.

Phytochemical Screening

The stem bark extracts of *B. aegyptiaca* were screened for the presence of secondary metabolites such as tannins, saponins, alkaloids, steroids, terpenes, anthraquinone, flavonoids, cardiac glucosides, phenols, resins, carbohydrates and phlobatannins using standard procedures [18].

Test Organisms

The test isolates were *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*, collected from the Microbiology Department of Nasarawa State University Keffi, Nigeria, and were maintained on Mueller Hinton broth at 37 °C for 24 hours. The organisms' viability were checked by streaking each test culture on a sterile plate and incubated at 37 °C for 24 hours according to the method described by Cheesbrough [19].

Agar Well Diffusion Method

The antibacterial effect of the n-hexane, ethanol and aqueous leaves extracts of *B. aegyptiaca* was determined by the method of agar well diffusion method alongside the minimum inhibitory

concentration (MIC) [19]. An inoculum from the stock culture was picked and inoculates into a selective media (sterile nutrient broth) using sterilized wire loop and incubated for 24 h at 37 °C. The nutrient agar was put in each sterile petri dish and allowed to set and then labelled. Using a sterile 5 mm cork borer, 5 wells were bored in the inoculated agar and the agar was then removed. About 0.1 ml of different concentration of the prepared extract (200, 100, 50 and 25 mg/ml) were dispensed into four wells, while the 5th well contained gentamicin (10 µg) which was used as a control. These were then left on the bench for 40 min for adequate diffusion of the extract and incubated at 37 °C for 24 hours. After incubation, the diameter of the zones of inhibition around each well was measured to the nearest millimetre along two axes 90° to each other and the mean of the two readings were then calculated.

DPPH-Free Radical Scavenging Activity

The analysis of the DPPH radical scavenging activity of the plant extracts was performed according to the method described by Koleva *et al.* [20]. Stock solution was prepared by dissolving 100 mg of extract in 1 ml of methanol and five, two fold serial dilutions was made. 0.5 ml of each of the concentrations was measured into separate test tubes and 0.3 ml of 0.5 mM DPPH was added. The reaction mixtures were vigorously shaken for 30 s in a Vortex apparatus and allowed to stand in the dark at room temperature for 30 minutes. Ascorbic acid was used as a standard for the investigation of the antiradical activity and was prepared in a similar manner. The absorbance was read using spectrophotometer at 517 nm against the blank. The blank was prepared by mixing 0.5 ml of the extract or ascorbic acid with 3.3 ml of methanol. Similarly, the control solution was prepared by mixing 3.5 mL of methanol and 0.3 ml of DPPH radical solution. The percentage of scavenging activity (X %) was calculated according to the equation below:

$$\mathbf{X\%} = \frac{\text{Absorbance of sample} - \text{Absorbance of blank}}{\text{Absorbance of control}} \times 100$$

Cytotoxicity activities

The cytotocixity screening of the crude extracts against Artemia salina was carried out in an in vivo simplified assay as described by Meyer *et al.*, [21]. In this experiment, 500 µg of the

extracts were dissolved in 1 ml of DMSO and by serial dilution technique, solutions of varying concentrations such as 250, 125, 75, 37.5, 18.75, 9.375, 4.6875, 2.34375, 1.171875, 0.5859375 µg/ml were obtained. Then 0.5 ml each of these standard concentrations was added to test tubes containing 10 shrimps in simulated brine water. After 24 h, the median lethal concentration (LC_{50}) of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration. Vincristine sulphate was used as positive control in this assay to compare the cytotoxicity of the extracts.

RESULTS AND DISCUSSION

Table 1: Characteristics of different extracts

Extract	Percentage dry weight	Colour	Consistency
Aqueous	10.0±0.9	Dark brown	Sticky
Ethanol	8.6±0.4	Dark brown	Sticky
Hexane	3.1±0.1	Yellowish brown	Waxy

Values are presented as mean (SD of three replicates)

Table 2: Phytochemical screening of *B. aegytiaca* stem bark extracts,

Test	Hexane extract	Aqueous extract	Ethanol extract
Flavonoids	-	+	+
Saponins	-	+	+
Tannins	-	+	+
Anthraquinone	+	-	-
Phenols	-	+	+
Phlobatannins	-	-	-
Carbohydrates	-	-	+
Cardiac glycosides	-	-	+
Terpeniods	-	-	-
Resins	-	-	-
Alkaloids	-	-	-
Steroids	-	-	+

Key: + = Present — = Absent

Table 3: Minimum inhibition concentration (MIC) of *Balanites aegytiaca* stem bark extracts against the test microbes (mm)

	Hexane extract (mg/ml)					Aqueous extract (mg/ml)					Ethanol extract (mg/ml)					Gentamicin (µg/ml)
Organism	200.0	100.0	50.0	25.0	12.5	200.0	100.0	50.0	25.0	12.5	200.0	100.0	50.0	25.0	12.5	10
Escherichia coli	-	-	-	-	-	-	-	-	-	-	14	12	8	-	-	22
Staphylococcus auteus	6	5	-	-	-	-	-	-	-	-	15	13	-	-	-	23
Salmonella typhi	-	-	-	-	-	-	-	-	-	-	23	14	11	9	-	22

Table 4: Result of antioxidant activities of ethanol and aqueous extracts of *B. aegytiaca* stem bark extract and ascorbic acid standard.

Concentration (mg/ml)	Ethanol extract	Aqueous extract	Ascorbic acid
2.0	80.03±1.2	32.21±0.3	85.91±1.2
1.5	77.02±1.5	29.01±0.4	83.22±2.1
1.0	63.09±2.0	27.31±0.2	86.34±1.3
0.5	60.16±1.1	22.75±0.4	84.62±1.6
0.25	54.11±0.9	25.15±0.2	85.48±2.5

Values are presented as mean (SD of three replicates)

Table 5: Result of LC_{50} data of *B. aegytiaca* stem bark extracts and vincristine sulphate standard.

Sample extract	LC_{50}(µg/ml)
Vincrisine sulphate standard	0.46±0.01
Hexane extract	3.13±0.02
Ethanol extract	9.70±0.20
Aqueous extract	13.35±0.40

Values are presented as mean (SD of three replicates)

The stem bark of *B. aegyptiana* was extracted using ethanol, aqueous and n-hexane. The percentage yields of these extracts were as shown in Table 1. From the result, aqueous extract has the highest value because it a universal solvent which is used in traditional medicine setting to prepare plants decoction, while hexane has the least value because they are generally used for extracting non-polar compounds.

The phytochemical screening of the ethanol and aqueous extracts of the stem bark of *B. aegyptiaca* revealed the presence of flavonoids, saponins, tannins, phenols and carbohydrates

while ethanol extract has in addition cardiac glycoside and steroids. In the hexane extract, only anthraquinones was detected as presented in Table 2. Phytochemicals like phlobatannins, terpenoids, resins, and alkaloids were not detected in all the sample extracts. The result shows that the presence of phytochemicals in different extracts is dependent on the polarity of the solvents used for the extraction [22]. Studies has shown that the presence of secondary metabolites such as saponins, tannins and anthraquinones in the stem bark of *B. aegyptiaca* is responsible for the antibacterial activity of this plant part [11, 23, 24]. Similarly, the presence of triterpenes, saponins and steroidal saponins were reported to be responsible for the antifungal activity [12, 13, 25]. Other phytochemicals like saponins, terpenoids, flavonoids, tannins and steroids have been reported to have anti-inflammatory effects [26, 27, 28], while saponins was reported to be responsible for the insecticidal and hepatoprotective activities of the plant [9, 14, 29].

The results obtained from the antibacterial screening of the stem bark of this plant indicated that the ethanol extract showed remarkable activity against all the tested organisms with varying sizes of zones of inhibition. This could be due to the synergistic effect of the presence of metabolites such as saponins, tannins and anthraquinone in the crude extract. The aqueous extract could not inhibit the growth of all the organisms tested while hexane extract is only weakly sensitive to *S. aureus*as shown in Table 3. The test organisms differed however in their level of susceptibility to the extracts as shown by the results of the minimum inhibitory concentration (MIC) which for hexane extract was obtained at 50 mg/ml, while that of ethanol extract was obtained at 25mg/ml for S. *typhi,* 50 mg/ml *for E.coli and* 100 mg/ml for *S. aureus.* The result obtained in this study agrees with findings of Parekh and Chanda [11] that aqueous extract of the stem bark of *B. aegyptiaca* is weakly active against *Staphylococcus aureus.* The high activity of ethanolic extract against all the bacteria tested may provide scientific bases for local usage of this plant in the treatment of different diseases. This finding is important because of the possibility of developing therapeutic agents that will be active against multidrug resistant bacteria. Therefore, this study shows the importance of the extract of the stem bark of *B.aegyptiaca* in antibiotics to control resistant bacteria that are becoming a threat to human health.

The ethanolic, aqueous and n-hexane extracts of stem bark of *B.aegyptiaca* were subjected to 1,1,-diphenyl-2 picryl hydrazyl (DPPH) radical scavenging assay. The ethanol extract showed a substantial free radical scavenging activity which is concentration dependent as presented in

Table 4. On the contrary, the aqueous extract showed mild antioxidant activity while n-hexane extract shows no activity. This study concurred with earlier report [30 - 32]. The antioxidant activity of this plant extract is reported to be due to the presence of flavonoid and phenolic contents, and these metabolites have redox properties that allow them to act as hydrogen donors, single oxygen quenchers and reducing agents [33]. Anselmi et al., [34] also reported that the presence of flavonoid and phenolic in food prevents lipid oxidation and thus inhibiting many diseases as cancer and atherosclerosis.

The cytotoxic potentials of crude extracts of ethanol, aqueous and n-hexane of the stem bark of *B.aegyptiaca* were screened by brine shrimp lethality bioassay for probable cytotoxic activity. The LC_{50} obtained from the best fit line slope were found to be 0.46 µg/ml for vincristine sulphate (Positive control), 3.13 µg/ml for n-hexane, 9.70 µg/ml for ethanol and
13.35 µg/ml aqueous extract respectively as shown in Table 5. Comparing the results with the positive control (0.46 µg/ml), the cytotoxicity exhibited by ethanol extract was not significant. Agreeing with the report that ethanol and aqueous extracts are weekly cytotoxic [35].

CONCLUSION

The stem bark extracts of *B. aegyptiaca* is conventionally utilized in the management of different diseases such as diarrhoea and syphilis. This report provided scientific bases for local usage of this plant in the treatment of these diseases. This finding is important because of the possibility of developing therapeutic agents that will be active against multidrug resistant bacteria.

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