ANTIGIARDIAL, ANTIAMOEBIC, ANTIMICROBIAL, ANTIOXIDANT ACTIVITY, CYTOTOXICITY AND PHYTOCHEMICAL OF ETHANOLIC FRUITS EXTRACT OF *Balanites aegyptiaca* (L.) DEL. FROM SUDAN

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**ABSTRACT**

*Balanites aegyptiaca* (L.) Del. belongs to family balanitaceae. The plant has antidiabetic, antihelmintic, antibacterial and antiviral activities. The bark, unripe, fruits, and leaves of this plant are reported to have anthelminthic, antifertility, purgative and antidyserteric Properties. The present study was conducted to investigate the in-vitro antigiardial activities (*Giardia lamblia*), antiamoebic activites (*Entamoeba histolytica*), antimicrobial (bacteria and fungi), antioxidant (DPPH assay), cytotoxic (MTT assay) and phytocemical of ethanol extract of *B. aegyptiaca* (fruit). The ethanol extracts of *B. aegyptiaca* (fruit) was screened for its antigiardial activities (*Giardia lamblia*), antiamoebic activites (*Entamoeba histolytica*), antimicrobial activity against four standard bacteria, two Gram-positive bacterial strains (*Bacillus subtilis* and *Staphylococcus aureus*), two fungal strains (*Apergillus niger* and *Candida albicans*) and two fungal strains (*Apergillus niger* and *Candida albicans*) using the cup plate agar diffusion method. Antioxidant screening for their free radical scavenging properties using 2,2Di (4-tert-octylphenyl)-1-picryl-hydrazyl (DPPH), while propyl galate was used as standard antioxidant and screened for their cytotoxicity using 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT). Ethanol extracts of *B. aegyptiaca* (fruit). showed 100% inhibition at a concentration 500 µg/ml after 96 h; this was compared with Metronidazole which gave 96% inhibition at concentration 312.5 µg/ml at the same time against *Giardia lamblia* and *Entamoeba histolytica*. The ethanol extract of *B. aegyptiaca* (fruit). exhibited
inhibitory effects against most of the tested organisms with zone of inhibition ranging from (15-25 mm). The tested antioxidant activity gave (70 ± 0.07 RSA%) in comparison to the control of propylgalate levels (88 ± 0.07 RSA%), MTT assay verified the safety of the examined extract and The results of Phytochemical screening of B. aegyptiaca (fruit) positive results were recorded for terpenoids, alkaloids, saponins and coumarins. Negative results were flavonoids, anthraquinone and tannins. In conclusion: These studies conducted for both B. aegyptiaca in the treatment of several protozoal, bacterial and fungal.

**KEYWORDS:** In vitro, antigiardial, antiamoebic, antimicrobial activity, antioxidant activities, cytotoxicity and phytochemical, Balanites aegyptiaca (fruit), Sudan.

I. INTRODUCTION

Medicinal plants are still invaluable source of safe, less toxic, lower price, available and reliable natural resources of drugs all over the world. People in Sudan and in other developing countries have relied on traditional herbal preparations to treat themselves. Therefore, it is useful to investigate the potential of local plants against these disabling diseases.[1,2]

*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. This plant is an indigenous species in Sudan, popular and of great concern, with diverse uses in folk medicine and many other applications. 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. It is also distributed in other countries located in the tropical dry belt of North Africa and dry areas of India and South Asia. 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-inflammatory, anti-helminthic, insecticidal, anti-ralarial, molluscicidal, anti-fungal, anti-bacterial and even for snake bites.[3]

Giardiasis is the most common cause of parasitic gastro-intestinal disease and it is estimated that up to two hundred million people are chronically infected with *giardia lamblia* globally, and 500,000 new cases reported annually.[4]
Giardia lamblia is a major cause of diarrhoea in humans.[5] Giardia is a flagellate protozoan with worldwide distribution that causes significant gastrointestinal diseases in a wide variety of vertebrates including cats and human. Giardiasis is one of the intestinal protozoa that cause public health problems in most developing countries as well as some developed countries. Giardia lamblia is considered to be one of the leading causative agents of diarrhoea in both children[6, 7, 8] and adults.[9, 10]

The World Health Organization (WHO) estimates that the protozoan Entamoeba histolytica is a major cause of morbidity worldwide, causing approximately 50 million cases of dysentery and 100,000 deaths annually.[11, 12] Intestinal amoebiasis due to the infection of E. histolytica is ranked third on the list of parasitic protozoan infections leading to death behind malaria and schistosomiasis.[13]

Amoebiasis is the infection of human gastrointestinal tract by E. histolytica; a protozoan parasite capable of invading the intestinal mucosa and that may spread to other organs, mainly the liver which usually leads to amoebic liver abscess. This infection remains a significant cause of morbidity and mortality world-wide.[14] Amoebiasis is a rare occurrence in developed countries of the world, but only found in travelers, immigrants, homosexuals and institutionalized persons. E. histolytica-associated dysentery is a common occurrence in the less developed and developing countries of the world, but is more common in areas of low socio-economic status, poor sanitation and nutrition especially in the tropics.[12] Thus the majority of E. histolytica infections, morbidity and mortality occur in Africa, Central and South America and the Indian sub-continent.[15]

Metronidazole is the drug now widely used and recommended in the treatment of giardiasis.[16] But it is less effective in the tissue than in the gut lumen.[17] In addition, it can eradicate only up to 50% of laminae infections.[18] Metronidazole sometimes causes adverse effects, example, myoplasia, neuralgia, and allergic dermatitis.[19] The present study was conducted to investigate the antigiardial, antiamoebic, antimicrobial antioxidant activities cytotoxicity and phytochemical of B. aegyptiaca (fruit) in Sudan.

II. MATERIALS AND METHODS

Plant materials
The B. aegyptiaca (fruit) were collected from central Sudan between January 2008 and February 2008. The plant was identified and authenticated by the taxonomists of Medicinal
and Aromatic Plants Research Institute (MAPRI). All plant parts were air-dried, under the shadow with good ventilation and then ground finely in a mill until their uses for extracts preparation.

Tables (1) showed the yield % of ethanolic, anti-giardial activity, anti-amoebic activity, antimicrobial, antioxidant, cytotoxicity and phytochemical of *B. aegyptiaca* (fruit) investigated in this study.

**Table (1): B. aegyptiaca (fruit) to be investigated for their anti-giardial, anti-amoebic, antimicrobial, antioxidant, cytotoxicity and phytochemical:**

<table>
<thead>
<tr>
<th>Scientific Name of Plants</th>
<th>Family name</th>
<th>Part Used</th>
<th>Yield %</th>
<th>Traditional medicine</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Balanites aegyptiaca</em> (L.) Del.</td>
<td>Balanitaceae</td>
<td>fruit</td>
<td>7.18 %</td>
<td>Infections, antimicrobial, sore throat, meningitis, anti-malaria, anti-tuberculosis, and antidiarrheal.</td>
</tr>
</tbody>
</table>

This table indicates the scientific names, families, parts used, yield% of ethanol extract and traditional uses of to *B. aegyptiaca* (fruit).

**Preparation of crude extracts**

Extraction was carried out for the fruits of *B. aegyptiaca* by using overnight maceration techniques according to the method described by Harbone.[20] About 50 g were macerated in 250 ml of ethanol for 3 h at room temperature with occasional shaking for 24 h at room temperature, the supernatant was decanted and clarity field by filtration through a filter paper, after filtration, the solvent was then removed under reduced pressure by rotary evaporator at 55°C. Each residue was weighed and the yield percentage was calculated then stored at 4°C in tightly sealed glass vial ready for use. The remaining extracts which is not soluble by successively extracted by Ethanol using the previous technique. Extracts kept in deep freezer for 48 h, then induced in freeze dryer (Virtis, USA) until completely dried. The residue was weighed and the yield percentage was calculated. The extracts were kept in 4°C until the time of their use.
Antigiardial activity and antimoebic activity of *B. aegyptiaca* extract

Parasite isolate

*G. lamblia* and *E. histolytica* used in all experiments were taken from patients of Ibrahim Malik Hospital (Khartoum). All taken samples were examined by wet mount preparation; the positive samples were transported to the laboratory in nutrient broth medium. Trophozoites of *G. lamblia* were maintained in RPMI 1640 medium containing 5% bovine serum at 37 ± 1°C. The trophozoites were maintained for the assays and were employed in the log phase of growth.

Inoculum

*G. lamblia* and *E. histolytica* was inoculated in the RPMI 1640 medium and incubated at 37 ± 1°C for 48 h. parasites were counted under the microscope by haemocytometer chamber.

*In vitro* susceptibility assays

*In vitro* susceptibility assays used the sub-culture method Cedillo-Rivera et al.,[21] which is being described as a highly stringent and sensitive method for assessing the anti-protozoal effects (gold standard) particularly in *E. histolytica*, *Gairdia intestinalis* and *Trichomonas vaginalis*. [22] 5 mg from each extract and compound was dissolved in 50 μl of dimethyl sulfoxide (DMSO) at Eppendorf tube containing 950 μl D.W in order to reach concentration of 5 mg/ml (5000 ppm). The concentrates were stored at -20°C for further analysis. Sterile 96-well microtitre plate was used for different plant extracts, positive control and negative control. Three columns of a microtitre plate wells [8 columns (C) × 12 rows (R)] were chosen.
for each extract, 40 μl) of an extract solution (5 mg/ml) were added to the first column wells C-1: On the other hand, 20 μl of complete RPMI medium were added to the other wells of the second column and third column (C-2 and C-3). Serial dilutions of the extract were obtained by taking 20 μl of extract to the second column wells and taking 20 μl out of the complete solution in C-2 wells to C-3 wells and discarding 20 μl from the total solution of C-3 to the remaining 20 μl serial solutions in the successive columns. 80 μl of culture medium was complemented with parasite and added to all wells. The final volume in the wells was 100 μl. In each test, Metronidazole (a trichomonocide) pure compound [(1-(2-hydroxyethyl)-2-methyl-5 Nitroimidazole], a was used as positive control in concentration 312.5 μg/ml, whereas untreated cells were used as a negative controls (culture medium plus trophozoites). For counting, the samples were mixed with Trypan blue in equal volume. The final number of parasites was determined with haemocytometer three times for counting after 24, 48, 72 and 96 h. The mortality % of parasite for each extracts activity was carried out according to the following formula:

\[
\text{Mortality of parasite (\%) = (Control negative – tested sample with extract) \times 100\%}
\]

Control negative

Only 100% inhibition of the parasite was considered, when there was no motile parasite observed.

**Antimicrobial activity of B. aegyptiaca extract**

**Test microorganisms**

The antibacterial activity of fruits of *B. aegyptiaca* extract was assessed against four bacterial species: two Gram-positive bacterial strains, *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 *Apergillus niger* (ATCC 9763) and *Candida albicans* (ATCC 7596).

National Collection of Type Culture (NCTC), Colindale, England.

American Type Culture Collection (ATCC) Rockville, Maryland, USA.

**Preparation of bacterial suspensions**

One ml aliquots of a 24 h broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 h. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about $10^8$ to $10^9$ CFU/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.\textsuperscript{[23]} 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 micropipette 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 h. 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.

**In vitro testing of extracts for antimicrobial activity**

**Testing for antibacterial activity:** The cup-plate agar diffusion method\textsuperscript{[24]} was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension $10^8$ to $10^9$ CFU/ml were thoroughly mixed with 100 ml of molten sterile nutrient agar which was maintained at 45°C. 20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agar was left to set and all of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. The cups were filled with 0.1 ml sample of each extracts using automatic microlitre pipette, and allowed to diffuse a room temperature for two hours. The plates were then incubated in the upright position at 37°C for 18 h. Two replicates were carried out for each extract against each of the test organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

**Testing for antifungal activity:** The same method as for bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25°C for two days for the *Candida albicans* and three days for *Aspergillus niger*.

**Determination of minimum inhibitory concentration (MIC) by agar plate dilution method:** The principle of the agar plate dilution is the inhibition of growth on the surface of the agar by the plant extracts incorporated into the medium. Plates were prepared in the series of increasing concentrations of the plant extract. The bottom of each plate was marked off.
into 6 segments. The organisms tested were grown in broth over night to contain $10^8$ CFU/ml. A loop-full of diluted culture was spotted with a standard loop that delivers 0.001 ml on the surface of segment. The end point (MIC) is the least concentration of antimicrobial agent that completely inhibits the growth. Results were reported as the MIC in mg/ml.

**Antioxidant activity of plant extracts**

**DPPH radical scavenging assay:** The DPPH radical scavenging was determined according to the method of Shimada et al.\textsuperscript{[25]} with some modification. In 96-wells plate, the test samples were allowed to react with 2,2-Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as 300 μM. The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group and Propyl Gallate (PG). All tests and analysis were run in triplicate.

**Cytotoxicity Screening**

Microculture tetrazolium MTT assay was utilized to evaluate the cytotoxicity of *B. aegyptiaca* (fruit).

**Microculture Tetrazolium (MTT) Assay**

**Principle**

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, blue colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.\textsuperscript{[27]}

**Preparation of Extracts, Solutions**

Using a sensitive balance 5 mg of each extracts were weighed and put in eppendorf tubes. 50 μl of DMSO were added to the extract and the volume was completed to 1 ml with distilled water obtaining a concentration of 5 mg/ml. The mixture was vortexed and stirred by magnetic stirrer to obtain a homogenous solution.
**Cell Line and Culturing Medium**

Vero (Normal, African green monkey kidney) cells were cultured in a culturing flask containing a complete medium consisting of 10% fetal bovine serum and 90% minimal essential medium (MEM) and then incubated at 37°C. The cells were subcultured twice a week.

**Cell line used**

Vero cells (Normal, African green monkey kidney).

**Cell counting**

Cell counts were done using the improved Neubauer chamber. The cover slip and chamber were cleaned with detergent, rinsed thoroughly with distilled water and swapped with 70% ethanol, then dried. An aliquot of cell suspension was mixed with equal volume of 0.4% trypan blue in a small tube. The chamber was charged with cell suspension. After cells had settled, the chamber was placed under light microscope. Using 40 X objective, cells in the 4 large corner squares (each containing 16 small squares) were counted. The following formula was used for calculating cells:

\[
N = \frac{\text{Number of cells counted} \times \text{dilution factor} \times 10^4}{4}
\]

**Procedure**

The monolayer cell culture formed in the culturing flasks was trypsinized and the cells were put in centrifuging tube and centrifuged for 5 minutes separating the cells from the supernatant that flicked out. 1 ml complete medium was added to the cells and all the cell suspension was contained in a basin. In a 96- well microtitre plate, serial dilutions of each extracts were prepared. 3 duplicated concentrations for each extracts i.e. 6 wells for each of 8 extracts. All wells in rows A, B and C were used in addition to first 4 wells from each rows D, E and F. The first 2 wells of row G were used for the negative control and the first 2 wells of row H were used for the positive control Triton X. 20 μl complete medium pipetted in all wells in rows B, C and mentioned wells of rows E and F. Then 20 μl from each extracts were pipetted in rows A and B and first 4 wells of rows E and F. 20 μl taken from row B were pipetted and mixed well in row C from which 20 μl were taken and flicked out. The same was done from E to F. After that 80 μl complete medium were added to all used wells. Then adjusting the cell account to 3000 cell/well, 100 μl of cell suspension were added completing all wells to the volume 200 μl. Now, we have duplicated three concentrations 500, 250, 125...
μg/ml for each extract. Then the plate was covered and incubated at 37°C for 96 hours. On the fourth day, the supernatant was removed from each well without detaching the cells. MTT suspension stock (5 mg/ml) prepared earlier in 100 ml phosphate buffer solution (PBS) was diluted (1:3.5) in a culture medium. To each well of the 96-well plate, 50 μl of diluted MTT were added. The plate was incubated for further 4 hours at 37°C. MTT was removed carefully without detaching cells, and 100 μl of DMSO were added to each well. The plate was agitated at room temperature for 10 minutes then read at 540 nm using microplate reader. The percentage growth inhibition was calculated using the formula below:

$$\% \text{ cell inhibition} = 100 - \left\{ \frac{(A_c - A_t)}{A_c} \right\} \times 100$$

Where, $A_t$ = Absorbance value of test compound; $A_c$ = Absorbance value of control.

**Phytochemical Screening**

Phytochemical screening is of great importance in providing us with information about chemicals found in the plant in term of their nature and range of occurrence. This information would enable us to correlate between the nature and range of occurrence of these chemicals and biological assays held to investigate a certain bioactivity of the mentioned plant. In this study the preliminary phytochemical screening was conducted according to Haborne.\(^{[28]}\)

**Preparation of the Extracts**

10 mg of the powdered leaves of each plant were refluxed with 100 ml of ethanol 80% for 4 hours. The cool solution was filtered and enough ethanol 80% was passed through the volume of the filtrate 100 ml. This prepared extract (PE) was used for the various tests.

**Test for Unsaturated Sterols and Triterpenes**

10 ml of the prepared extract (PE) was evaporated to dryness on a water bath and the cooled residue was stirred several times with petroleum ether to remove most of the coloring materials. The residue was then extracted with 20 ml of chloroform. The chloroform solution was dehydrated over sodium sulphate anhydrous. 5 ml of chloroform solution was mixed with 0.5 ml acetic anhydride followed by two drops of conc. Sulphuric acid. The gradual appearance of green, blue pink to purple color was taken an evidence of the presence of sterol (green to blue) and or triterpenes (pink to purple) in the sample by Harborne.\(^{[28]}\)

**Test for Alkaloids**

7.5 ml of (PE) was evaporated to dryness on a water bath. 5 ml of HCl (2N) was added and stirred while heating on the water bath for 10 minutes, cooled filtered and divided into two
test tubes. To one test tube few drops of Mayer’s reagent were added. While to the other tube few drops of Valser’s reagent were added. A slight turbidity or heavy precipitate in either of the two test tubes was tanked as presumptive evidence for the presence of alkaloids by Harborne.[28]

**Test for Flavonoids**

17.5 ml of the (PE) was evaporated to dryness on a water bath, cooled and the residue was defatted with petroleum ether and the defatted residue was dissolved in 30 ml of ethanol (80%) and filtered. The filtrate was used for the following tests: (A) To 3 ml of the filtrate in a test tube 1 ml of 1% aluminum chloride solution was in methanol was added. Formation of yellow color indicated the presence of Flavonoids, (Flavones and / or chalcone). (B) To 3 ml of the filtrate in a test tube 1 ml of 1% potassium hydroxide solution was added. A dark yellow color indicated the presence of the Flavonoids compounds (flavones or flavanones) chalcone and/or flavonol. (C) To 2 ml of the filtrate 0.5 ml of magnesium turnings were added. Producing of defiant color to pink or red was taken as presumptive evidence that flavanones were present in the plant sample by Harborne.[28]

**Test for Tannins**

7 ml of the (PE) was evaporated to the dryness on water bath. The residue was extracted several times with n-hexane and filtered. The insoluble residue was stirred with 10 ml of saline solution. The mixture was cooled, filtered and the volume of the filtrate was adjusted to 10 ml with more saline solution. 5 ml of this solution was treated with few drops of gelatin salt reagent. Formation of immediately precipitate was taken as an evidence for the presence of tannin in plant sample. To another portion of this solution, few drops of ferric chloride test reagent were added. The formation of blue, black or green was taken as an evidence for the presence of tannins.

**Test for Saponins**

1 g of the original dried powdered plant material was placed in a clean test tube. 10 ml of distilled water was added and the tube was stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of (honeycomb). The appearance of honeycomb, which persisted for least an hour, was taken as an evidence for the presence of Saponins.
**Test for Anthraquinone Glycosides**

10 g of the powdered plant sample were boiled with 10 ml of 0.5N KOH containing 1 ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of benzene. 5 ml of the benzene solution was shaken with 3 ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of Anthraquinones was indicated if the alkaline was found to have assumed pink or red color.

**Test for Coumarins**

3 g of the original powdered plant sample was boiled with 20 ml of distilled water in a test tube and filter paper was 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 coumarins was indicated if the spot has found to be absorbed the UV light.

**Statistical analysis**

All data were presented as means ± S.D. Statistical analysis for all the assays results were done using Microsoft Excel program. Student t test was used to determine significant difference between control and plant extracts at level of P < 0.05.

**III. RESULTS**

**Antigiardial activity of B. aegyptiaca extract**

The antigiardial potential of the Ethanolic extract of the medicinal plant of *B. aegyptiaca* (fruit) were extracted by ethanol, with different concentrations (500, 250 and 125 ppm) and Mertronidazole (the reference control) with concentration (312.5 µg/ml) to be investigated against *giardia lamblia* trophozoites *invitro* (Figure 2).

![Figure (2): In vitro activity of B. aegyptiaca (fruit) ethanolic extract against Giardia lamblia.](image-url)
Antiamoebic activity of *B. aegyptiaca* extract

The antiamoebic potential of the Ethanolic extract of the medicinal plant of *B. aegyptiaca* (fruit) were extracted by ethanol, with different concentrations (500, 250 and 125 ppm) and Mertronidazole (the reference control) with concentration (312.5 µg/ml) to be investigated against *Entamoeba histolytica* trophozoites *in vitro* (Figure 3).

![Figure (3): In vitro activity of B. aegyptiaca (fruit) ethanolic extract against Entamoeba histolytica.](image)

Antimicrobial activity of *B. aegyptiaca* extract

The antimicrobial potential of the Ethanolic extract of *B. aegyptiaca* (fruit) were extracted by ethanol, with different concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml) and antibiotics (Antibacterial and antifungal the reference control) with different concentrations (40, 20, 10 and 5 µg/ml) to be investigated against four standard bacterial strains and two fungal species which include: *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 *Apergillus niger* (ATCC 9763) and *Candida albicans* (ATCC 7596). (Tables 2 and 3).
Table (2): The antimicrobial activity of *B. aegyptiaca* (fruit) against the standard bacterial and fungal

<table>
<thead>
<tr>
<th>Name of plant (part)</th>
<th>Concentration (mg/ml)</th>
<th>Tested Bacteria</th>
<th>Tested Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gram (+ve)</td>
<td>Gram (- ve)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B.s</td>
<td>S.a</td>
</tr>
<tr>
<td><em>B. aegyptiaca</em> (fruit)</td>
<td>100</td>
<td>23</td>
<td>22</td>
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<tr>
<td></td>
<td>50</td>
<td>19</td>
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<td>25</td>
<td>18</td>
<td>17</td>
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<td></td>
<td>12.5</td>
<td>16</td>
<td>15</td>
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</table>

Key: Standard bacteria used: (*E.c* = *Escherichia coli*, *Ps.a* = *Pseudomonas aeruginosa*, *B.s* = *Bacillus subtilis*, *S.a* = *Staphylococcus aureus*, *C.a* = *Candida albicans* and *Asp.n* = *Apergillus niger*). MDIZ (mm) = Mean diameter of growth inhibition zone in mm.

Interpretation of results: MDIZ (mm) : >18 mm: Sensitive, 14 to 18 mm: Intermediate: <14 mm: Resistant. (-): No inhibition.

Table (3): Antibacterial and antifungal activity of reference antibiotics against standard microorganisms.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Drugs</th>
<th>Concentrations (µg/ml)</th>
<th>Standard microorganisms used MDIZ* (mm)</th>
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<tr>
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<td>Tested bacteria used (M.D.I.Zmm)</td>
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<td>50</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.5</td>
<td>23</td>
</tr>
</tbody>
</table>

Key: Standard bacteria used: (*E.c* = *Escherichia coli*, *Ps.a* = *Pseudomonas aeruginosa*, *B.s* = *Bacillus subtilis*, *S.a* = *Staphylococcus aureus*, *C.a* = *Candida albicans* and *Asp.n* = *Apergillus niger*). MDIZ (mm) = Mean diameter of growth inhibition zone in mm.

Interpretation of results: MDIZ (mm) : >18 mm: Sensitive, 14 to 18 mm: Intermediate: <14 mm: Resistant. (-): No inhibition.
Antioxidant activity of *B. aegyptiaca* extract

As shown in Table (4), the results of antioxidant activity *B. aegyptiaca* (fruit) showed high antioxidant activity against the DPPH free radical (70 ± 0.07 RSA%).

**Table (4): Antioxidant activity of *B. aegyptiaca* (fruit)**

<table>
<thead>
<tr>
<th>No</th>
<th>Name of plant</th>
<th>Part</th>
<th>% RSA* ± SD (DPPH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>B. aegyptiaca</em></td>
<td>fruit</td>
<td>70 ± 0.07</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>PG</td>
<td>88 ± 0.07</td>
</tr>
</tbody>
</table>

**Key:** RSA* = Radicals scavenging activity

*Control = P.G = Propyl Gallate.*

This table indicate the anti DPPH of ethanol extract of *B. aegyptiaca* (fruit) the reading and propyl gallate was used as standard drug level. The tested antioxidant activity gave (70 ± 0.07 RSA %) in comparison to the control of propylgallate levels gave (88 ± 0.07 RSA %).

Cytotoxicity assay of *B. aegyptiaca* extract

**Table (5): Cytotoxicity of plants extracts on normal cell lines (Vero cell line) as measured by the MTT assay**

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of plant (part)</th>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
<th>Inhibition (%) ± SD</th>
<th>IC_{50} (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>B. aegyptiaca</em> (fruit)</td>
<td>500</td>
<td>2.22</td>
<td>35.5 ± 0.05</td>
<td>&gt; 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>2.82</td>
<td>18.0 ± 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>125</td>
<td>3.16</td>
<td>8.1 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>0.14</td>
<td>95.3 ± 0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Key:** Control = Triton-x100 was used as the control positive at 0.2 µg/mL.

The maximum concentration used was 500 µg/mL. When this concentration produced less than 50% inhibition, the IC_{50} cannot be calculated.

This table indicates the % inhibition of vero cell line growth in vitro by ethanolic extract of *B. aegyptiaca* (fruit). MTT colorimetric assay was used. Reading in triplicate for different concentrations 125-500 µg/mL.

**Table (6): Preliminary Phytochemical Screening *B. aegyptiaca* extract**

<table>
<thead>
<tr>
<th><strong>Scientific Name of Plants</strong></th>
<th><strong>Part Used</strong></th>
<th><strong>Unsaturated Sterol And/or Triterpenes</strong></th>
<th><strong>Alkaloids</strong></th>
<th><strong>Flavonoids</strong></th>
<th><strong>Tannins</strong></th>
<th><strong>Saponins</strong></th>
<th><strong>Anthraquinone glycoside</strong></th>
<th><strong>Coumarins</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. aegyptiaca</em></td>
<td>fruit</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Present  - = absent.
IV. DISCUSSION

Antigiardial activity of *B. aegyptiaca* extract

Despite the previous comprehensive screening of Sudanese medicinal plants for their antiprotozoal activity.\(^{29, 30, 31, 2}\) The extracts of ethanol of *B. aegyptiaca* (fruit) were screened for their antiparasitical activity against *Giardia lamblia* and *Entamoeba histolytica* one reference drugs Metronidazole (Flagyl\(^\circ\)) was used as suitable drug for treatment giardiasis and amoebaisis.

*Giardia lamblia* is one of the most common intestinal pathogenic protozoan parasite.\(^{32}\) It is becoming increasingly important among HIV/AIDS patients. There are reports that some cases of acute and chronic diarrhea in AIDS patients may be associated with giardial infection. However, Metronidazole, the common drug of choice, can cause mutagenicity in bacteria\(^{33}\) and is carcinogenic in rodents.\(^{34}\) It also possesses undesirable side effects and treatment failures have been reported.\(^{35}\) Ethanol extract of *B. aegyptiaca* (fruit) showed 100% inhibition at a concentration 500 µg/ml after 96 h; this was compared with Metronidazole which gave 96% inhibition at concentration 312.5 µg/ml at the same time against *Giardia lamblia*.

Antiamoebic activity of *B. aegyptiaca* extract

The World Health Organization (WHO) estimates that the protozoan *Entamoeba histolytica* is a major cause of morbidity worldwide, causing approximately 50 million cases of dysentery and 100,000 deaths annually.\(^{36, 12}\) Intestinal amoebiasis due to the infection of *E. histolytica* is ranked third on the list of parasitic protozoan infections leading to death behind malaria and schistosomiasis.\(^{13}\) Amoebiasis is the infection of human gastrointestinal tract by *Entamoeba histolytica* (*E.histolytica*), a protozoan parasite capable of invading the intestinal mucosa and that may spread to other organs, mainly the liver which usually leads to amoebic liver abscess. This infection remains a significant cause of morbidity and mortality worldwide.\(^{37}\) Ethanol extract of *B. aegyptiaca* (fruit) showed 100% inhibition at a concentration 500 µg/ml after 96 h; this was compared with Metronidazole which gave 96% inhibition at concentration 312.5 µg/ml at the same time against *Entamoeba histolytica*.

Antimicrobial activity of *B. aegyptiaca* extract

The mean diameter of inhibition zone produced by *B. aegyptiaca* (fruit) extract on the tested standard microorganism are presented. On the other hand, Table (2 and 3) showed antimicrobial activity of the reference chemotherapeutic drugs against the test
microorganisms. The results were interpreted as sensitive, intermediate and resistant. Based on the results of Table (2 and 3), plant extracts resulting in ≤15 mm mean diameter inhibition zone are considered to be active and those resulting in >15 mm are inactive. The minimum inhibitory concentration (MIC) of the ethanolic extract of *B. aegyptiaca* (fruit) against standard microorganism, are shown in Tables (2 and 3).

It is clear from Table (2) that the ethanolic extract *B. aegyptiaca* (fruit) showed high activity only against *Aspergillus niger, Bacillus* and *Staphylococcus aureus*, whereas it was an intermediately active against, *E. coli, Candida albicans* and, *Pseudomonas aeruginosa*.

It is clear from Table (2) that the ethanolic extract of *B. aegyptiaca* (fruit) showed high activity all bacteria and fungi.

**Antioxidant activity of *B. aegyptiaca* extract**

To explain the health benefits attributed to both plants focused in this present work, antioxidant activity tests was carried for ethanolic extract of *B. aegyptiaca* (fruit) through DPPH. The result of DPPH of ethanol extract of *B. aegyptiaca* (fruit) the reading and propyl gallate was used as standard drug level. The tested antioxidant activity gave (70 ± 0.07 RSA %) in comparison to the control of propylgalate levels gave (88 ± 0.07 RSA%).

**Cytotoxicity assay of *B. aegyptiaca* extract**

Interestingly, the cytotoxicity assays were conducted in this study to evaluate the ethanolic extract of *B. aegyptiaca* (fruit) their cytotoxicity effects by using MTT-assay include (vero cell line). The result of MTT assay verified the safety of the examined extract.

**Phytochemical Screening of *B. aegyptiaca* extract**

The Phytochemical analysis of crude ethanolic extract of *B. aegyptiaca* performed by the method described earlier and then and then analyzed for phytocompounds like steroids or terpenoids, alkaloids, flavonoids, coumarins, saponins, tannins and anthraquinone preliminary analyzed and present study.

The results of Phytochemical screening of *B. aegyptiaca* (fruit) positive results were recorded for terpenoids, alkaloids, saponins and coumarins. Negative results were flavonoids, anthraquinone and tannins.
V. CONCLUSION
This result enhances the ethno botanical uses of the plants as antidiarrheal in cases associated with giardiasis and amoebiasis in central Sudan. 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.

VI. ACKNOWLEDGEMENTS
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