

**ANTI-OXIDANT ACTIVITY AND PHYTOCHEMICAL SCREENING OF BALANITES  
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Article Received on 09/09/2017

Article Revised on 30/09/2017

Article Accepted on 20/10/2017

**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 present study was conducted to investigate the in-vitro antioxidant (DPPH assay) and phytochemical screening of ethanol, Petroleum ether and chloroform extracts of *B. aegyptiaca* (Fruits). The ethanol, Petroleum ether and chloroform extracts of *B. aegyptiaca* (Fruits) were tested for antioxidant screening for their free radical scavenging properties using 2,2-Di (4-tert-octylphenyl)-1-picryl-hydrazyl (DPPH-assay), while propyl galate was used as standard antioxidant and phytochemical screening. The ethanol, Petroleum ether and chloroform extracts of *B. aegyptiaca* (Fruits) antioxidant activity were ( $14.4 \pm 0.04$ ,  $13.3 \pm 0.03$  and  $14.2 \pm 0.13$  RSA %) respectively in comparison to the control of propyl galate levels ( $88 \pm 0.07$  RSA %) and Preliminary phytochemical screening of the ethanol of *B. aegyptiaca* (Fruits) revealed that the plant contain triterpenes, tannins, coumarins, saponins and flavonoids. Negative results were recorded for alkaloids, Sterols and glycosides, and petroleum ether of *B. aegyptiaca* (Fruits) revealed that the plant contain coumarins, and Sterols. Negative results were recorded for alkaloids, glycosides, flavonoids triterpenes, tannins and saponins, Hence, the results obtained in the present study indicate that *B. aegyptiaca* have promising antioxidant indicates that the plant could be promising agent in scavenging free radicals and treating diseases related to free radical reactions.

**KEYWORDS:** Antioxidant (DPPH-assay), Phytochemical screening, *B. aegyptiaca* (Fruits).**INTRODUCTION**

Recently in many African countries comprehensive research was conducted on medicinal plants for the treatment of different diseases and conditions, such as diabetes, malaria, anemia and cancer. The availability and relatively cheaper cost of medicinal plants in sub-Saharan Africa, makes them more attractive as therapeutic agents when compared to 'modern' medicines (Agbor et al., 2005). 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 (Amaral et al., 2006; Koko et al., 2008). The medicinal

properties of plants have been investigated, in the light of recent scientific developments, throughout the world due to their potent pharmacological activities and economic viability. A great number of aromatic, spicy, medicinal and other plants contain chemical compounds, exhibiting antioxidant properties. Source of natural antioxidants are primarily, plant phenolics that may occur in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks (Pratt, 1990). Many of these antioxidant compounds possess anti-inflammatory, ant atherosclerotic, antitumor, ant carcinogenic, antibacterial or antiviral activities to a greater or lesser extent (Sala et al, 2002). Crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolic are increasingly of interest in the food industry, because they retard oxidative degradation of lipids and thereby

improve the quality and nutritive value of food (Kahkonen *et al.*, 1999; Rice *et al.*, 1995).

*Balanites aegyptiaca* (Balanitaceae) is a widely distributed African plant of medicinal interest (Speroni *et al.*, 2005). The fruits are edible and known as desert dates. It is a small evergreen savanna tree with a dark brown stem which usually attains a height of 4.5-6 m (Koko *et al.*, 2005a). In Egyptian folk medicine, the fruits are used as an oral hypoglycemic and an anti-diabetic (Sarker *et al.*, 2005). An aqueous extract of the fruit mesocarp is used in Sudanese folk medicine in the treatment of jaundice (Sarker *et al.*, 2005). Indeed the plant is used as a purge to remove intestinal parasites with the root, branches, bark, fruit and kernel extracts shown to be lethal to the miracidia and cercariae of *Shistosoma mansoni* and to *Fasciola gigantica* (Koko *et al.*, 2005a). Additionally extracts of the tree display abortive and antiseptic properties (Speroni *et al.*, 2005). The roots and bark of *Balanites aegyptiaca* contain numerous steroidal saponins and yamogenin or diosgenin glycosides (Speroni *et al.*, 2005). The fruit mesocarp contains a large variety of chemicals amongst which are the pregnane glycosides, coumarins, flavonoids, 6-methyldiosgenin and saponins (Koko *et al.*, 2005b). The saponins are a structurally and biologically diverse class of glycosides of both steroids and triterpenes that are widely distributed in terrestrial plants and in some marine organisms (Deng *et al.*, 1999). The present study was conducted to investigate the antioxidant activity and phytochemical screening of *B. aegyptiaca* (fruits) in Sudan.

## MATERIALS AND METHODS

### Plant Material

The *B. aegyptiaca* (fruits) 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 fruit (50g) 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 (Harborne, 1984).

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

#### DPPH radical scavenging assay

The DPPH radical scavenging was determined according to the method by (Shimada *et al.*, 1992) with some modification. In 96-wells plate, the test samples were allowed to react with 2,2-Di (4-tert-octylphenyl)-1-picrylhydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as 300 µM. The extract was dissolved in DMSO (500 µg/ml.

concentration), 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.

### 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 (Harborne, 1984).

### Preparation of the Extracts

10 mg of the powdered leaves of 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 as evidence of the presence of sterol (green to blue) and or triterpenes (pink to purple) in the sample (Harborne, 1984).

### 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 (Harborne, 1984).

### 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 (Harborne, 1984).

#### 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 coumarone was indicated if the spot has found to be absorbed the UV light.

#### Statistical analysis

All data were presented as means  $\pm$  S.D. Statistical analysis for all the assays results were done using Microsoft Excel program 2007.

## RESULTS AND DISCUSSION

The fruits of *B. aegyptiaca* family (Balanitaceae) were screened for antioxidant screening for their free radical scavenging properties using 2,2-Di (4-tert-octylphenyl)-1-picryl-hydrazyl (DPPH), while propyl galate was used as standard antioxidant and phytochemical screening.

#### Antioxidant activity of *B. aegyptiaca* (Fruits) extract

This table indicates the antioxidant DPPH of ethanol, Petroleum ether and chloroform extracts of *B. aegyptiaca* (fruits), propyl gallate was used as standard drug level. The ethanol, Petroleum ether and chloroform extracts of *B. aegyptiaca* (Fruits) antioxidant activity was ( $14.4 \pm 0.04$ ,  $13.3 \pm 0.03$  and  $14.2 \pm 0.13$  RSA %) respectively in comparison to the control of propyl galate levels ( $88 \pm 0.07$  RSA %).

#### Phytochemical analysis of *B. aegyptiaca* (Fruits) extract

Preliminary phytochemical screening of the ethanol of *B. aegyptiaca* (Fruits) revealed that the plant contain triterpenes, tannins, coumarins, saponins and flavonoids. Negative results were recorded for alkaloids, Sterols and glycosides, and petroleum ether of *B. aegyptiaca* (Fruits) revealed that the plant contain coumarins, and Sterols. Negative results were recorded for alkaloids, glycosides, flavonoids triterpenes, tannins and saponins, (Table 2).

**Table 1: Antioxidant activity of *B. aegyptiaca* (Fruits).**

No.	Name of Extract	% RSA* $\pm$ SD (DPPH)
1	Fruit extract (Ethanol)	$14.4 \pm 0.04$
2	Fruit extract (Petroleum Ether)	$13.3 \pm 0.03$
3	Fruit extract (Chloroform)	$14.2 \pm 0.13$
4	*Control (PG)	$88 \pm 0.07$

**Key:** RSA\* = Radicals scavenging activity \*Control = P.G = Propyl gallate.

**Table 2: Preliminary Phytochemical Screening analysis of *B. aegyptiaca* (Fruits) extract.**

No.	Tested	Fruit extract	
		Ethanol	Petroleum Ether
1	Saponins	+	-
2	Cummrins	+	+
3	Tannins	-	-
4	Alkaloids	-	-
5	Sterols	-	+
6	Triterpens	+	-
7	Flavonoids	++	-
8	Anthraquine	-	-
9	Glycosides	-	-

+ = Present

- = Absent.

## CONCLUSION

From complete investigation about antioxidant and pharmaceutical screening studies of *B. aegyptiaca* (Fruits) it can be recommended that extracts could be used as a easily available foundation of natural antioxidants, which can be used as supplement to aid the therapy of free radical mediated diseases such as cancer, diabetes, inflammation, etc., diabetes swelling. Further studies are needed on the isolation and elucidation of their chemical structures of antioxidant components.

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