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## Phytochemical screening and antioxidant activity of *Balanites aegyptiaca* linn. Leaves aqua-ethanol extract

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### Abstract

*Balanites aegyptiaca* Linn. Plant leaves has been reported to use as traditional folk medicines especially in Africa and southern Asia in the form of juice to treat diarrhea, curing anthrax, dysentery, cuts and clean malignant wounds; whereas other parts of this plant have also reported their own traditional medicinal activities. This screening study is intended to evaluate the phytochemical ingredients and antioxidant activity of *Balanites aegyptiaca* Linn. Leaves aqua-ethanol extract with qualitative analysis method according to standard protocol and LC-MS/MS analysis. Antioxidant activity of aqua-ethanol extract was found to be significant with IC<sub>50</sub> value 34.15±0.03 µg/mL using DPPH (2, 2-Diphenyl-1-picryl hydrazyl) free radical scavenging assay method. The qualitative analysis of *Balanites aegyptiaca* Linn. Leaves aqua-ethanol extract revealed that the plant contain carbohydrate, protein, amino acids, glycoside, tannins, saponin, flavonoids and phenolic compounds. The LC-MS/MS analysis was carried out for the detection of bioactive phytochemical ingredients in the extract and reported 8 phytochemical ingredients, which are responsible for antioxidant activity. The results obtained in the present screening study indicate that *Balanites aegyptiaca* Linn. Leaves aqua-ethanol extract content promising free radical scavenging bioactive ingredients. Hence plant content promising bioactive ingredients responsible to antioxidant activity and treating diseases related to free radical reactions in reducing of oxidative stress related complications.

**Keywords:** *Balanites aegyptiaca* Linn, phytochemical, LC-MS/MS, DPPH, antioxidant

### Introduction

*Balanites aegyptiaca* Linn. Is a drought tolerant perennial tropical ever green plant belongs to family Zygophyllaceae (Balanitaceae) and traditionally known as desert date. The *Balanites* name originally derived from the Greek word which means fruit resemble acorn <sup>[1]</sup> and also known by different vernacular names in various countries. It is native to arid and sub arid part of Africa and Middle East especially Arabian Peninsula, but most widely in various parts of Africa and South Asia <sup>[2]</sup>. *Balanites aegyptiaca* Linn. Is multipurpose plant used for food and fodder in different regions of Africa and South Asia <sup>[3]</sup>? Among the various parts of *Balanites aegyptiaca* Linn. Plant, fleshy pulp of the fruit is eaten and also used as a food, beverage & medicines <sup>[4]</sup>. All part of *Balanites aegyptiaca* Linn. Has several bioactive phytochemical contents, which possess miscellaneous medicinal properties by possessing an effect on causes of a reaction or trigger of a response in the living tissue <sup>[5]</sup>. The bark, fruit and oil of tree have been widely used to treat various disease or disorders such as cancer, tuberculosis, malaria, diabetes, sleeping sickness, wounds, colds, syphilis, liver and spleen disorders, jaundice, yellow fever, snake bite and aches <sup>[6,7]</sup>. The extract of root bark has been used in diarrhoea, haemorrhoid and also acts as a fish poison <sup>[8]</sup>. *Balanites aegyptiaca* Linn. Fruits have various primary and secondary metabolites such as alkaloids, tannins, steroids, glycosides, flavonoids, furanocoumarin, saponins, fixed oil protein, fat, carbohydrates and vitamin C <sup>[9]</sup>. Along with this it has various electrolytes or minerals such as calcium ions, iron, magnesium, phosphorus, zinc, and copper and potassium ions <sup>[10]</sup>. Though already some traditional information is available about the *Balanites aegyptiaca* Linn. Plants and review has been intended on various aspects of Ethnopharmacology and phytochemistry of *Balanites aegyptiaca* Linn. Almost all the parts of *Balanites aegyptiaca* Linn. Plant are traditionally used in several folk medicines <sup>[11, 12]</sup>. This evaluation study focus on the antioxidant activity and phytochemicals present in leaf extract of *Balanites aegyptiaca* Linn.

With beneficial health effects in reducing of oxidative stress related complications.

## Materials and Methods

### Collection of Plant material

Leaves of *Balanites aegyptiaca* Linn. Plant were collected from local area identified and authenticate with the help of our institute botanists. The collected leaves are cleaned with distilled water to remove dirt and air dried in shade.

### Preparation of Extraction

The *Balanites aegyptiaca* Linn. Dried leaves were washed and powdered with the help of grinder. 30 g of powdered plant material was macerated in 150 mL of aqua-ethanol and kept on a magnetic stirrer for stirring and extracted using a Soxhlet apparatus sequentially in aqua-ethanol solvent. The fraction of extract was collected and solvent was evaporated out to dryness. The extracted material was stored in airtight bottles for further investigation studies.

### In vitro antioxidant activity

The *in vitro* antioxidant activity was evaluated by using stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) with the help of UV-spectrophotometer [13-15]. 0.1 mM DPPH stock solution was prepared in ethanol solvent. 1.0 mL of this stock solution was added to 1.0 mL of extract solution in water at different concentrations (5-50 µg/mL) and final volumes were made to 3 mL by adding distilled water. After 20 minutes, the absorbance of each concentration of test solution was measured at 517 nm. Ascorbic acid was used as standard. The absorbance of the test solutions were decreases with increase in concentration of leaf extract, which confirms presence of free radical scavengers in extracts. Percentage of DPPH free radical scavenges by test solution were measured as

$\% \text{ Free Radical Scavenged} = (A_{\text{Control}} - A_{\text{Test}} / A_{\text{Control}}) \times 100$   
IC<sub>50</sub> values were determined by using graphical method.

### Phytochemical analysis

The leaves aqua-ethanol extract was qualitatively evaluated for the bioactive phytochemical contents reporting such as alkaloids, carbohydrate, protein, amino acids, glycoside, tannins, saponin, flavonoids, steroids, terpenoids and phenolic compounds etc. by the help of standard protocol [16, 17].

### LC-MS/MS analysis

LC-MS/MS analysis technique was used for identification of phytochemical ingredients separated by liquid chromatography. It provides separation of ingredients and detection by MS provides molecular weight of compounds. LC-MS/MS analysis of aqueous solvent extracted material was carried out on Waters UPLC-TQD Mass spectrometer. The ingredients were identified by comparison of mass spectra with the inbuilt Metlin, Lipid and Mass Bank databases.

### Statistical analysis

The tests were carried out in triplicate and its results expressed in mean ± SD. Values of  $p < 0.05$  were considered as statistically significant.

### Results and Discussion

In the present evaluation study, the antioxidant potential and phytochemical ingredients content in *Balanites aegyptiaca* Linn. Leaves aqua-ethanol extract was carried out. The experimental data and results of leaves aqua-ethanol extract and ascorbic acid as a standard were represents in Table 1.

**Table 1:** % Free radical scavenged activity of standard and leaves extract

Sr. No.	Concentration in (µg/mL)	% Free radical scavenged of standard	% Free radical scavenged of leaves extract
1	5	42.03±0.03	34.74±0.03
2	10	44.51±0.05	37.15±0.05
3	20	50.11±0.03	41.36±0.04
4	30	54.45±0.01	47.91±0.02
5	40	57.64±0.02	53.73±0.02
6	50	61.31±0.04	57.07±0.01
IC <sub>50</sub> value (µg/mL)		19.73±0.02	34.15±0.03

The above, result table-1 shows the percentage of DPPH free radical scavenged activity by leaves aqua-ethanol extract and ascorbic acid at different concentrations tested. Leaves extract of *Balanites aegyptiaca* Linn. Exhibited potential scavenging activity [18] by IC<sub>50</sub> value 34.15±0.03 µg/mL and ascorbic acid as a standard 19.73±0.02 µg/mL at concentrations ranging from 5µg/mL to 50µg/mL as shown in fig.1. In all concentrations of both samples shows DPPH radical scavenging activity. Ascorbic acid scavenging activity was found to be higher than leaves aqua-ethanol extract of *Balanites aegyptiaca* Linn. At all concentrations. From the current result it may be suggested that the leaves extract of *Balanites aegyptiaca* Linn. Reduces the DPPH free radical and significantly noted antioxidant activity.

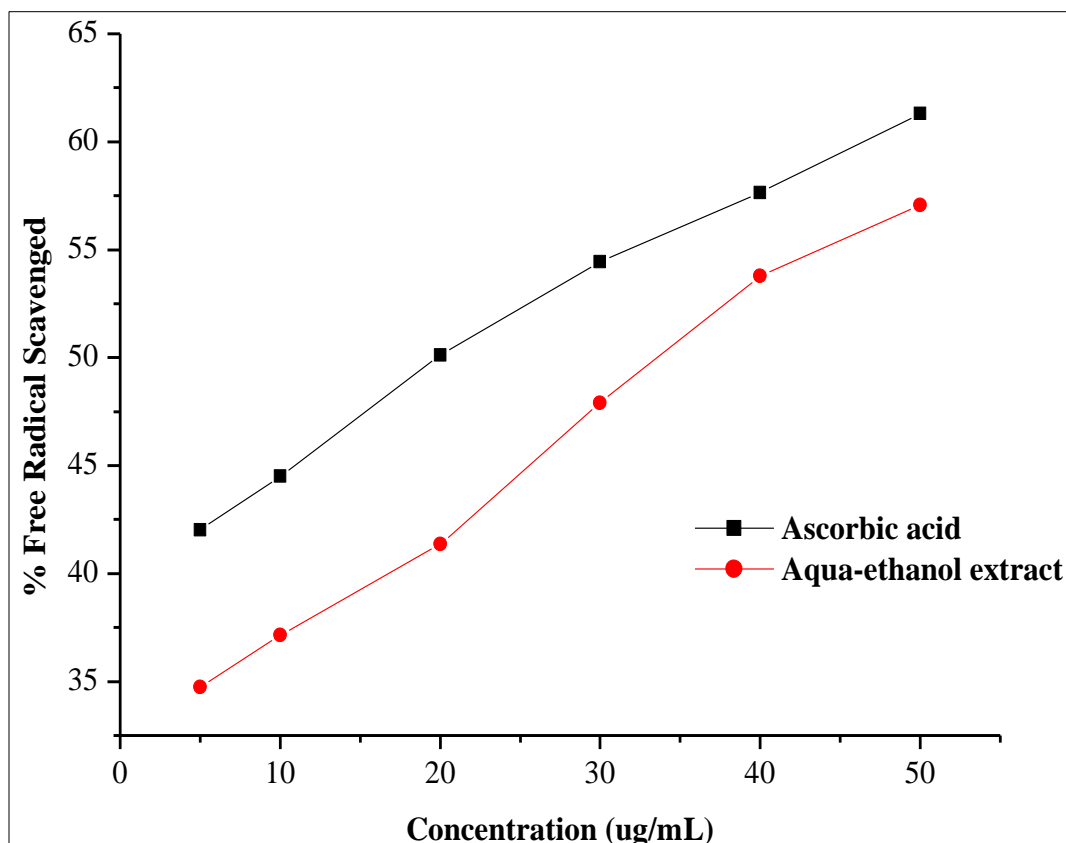
The qualitative various phytochemical test analysis of *Balanites aegyptiaca* Linn. Leaves aqua-ethanol extract were showed the presence of carbohydrate, protein, amino

acids, glycoside, tannins, saponin, flavonoids, triterpenoids and phenolic compounds [19, 20] as shown in Table 2.

**Table 2:** Phytochemical analysis of leaves aqua-ethanol extract

Sr. No.	Phytochemical Tests	Result
1	Alkaloid	-
2	Carbohydrate	-
3	Protein and amino acids	+
4	Glycoside	+
5	Tannin	+
6	Saponin	+
7	Flavonoids	+
8	Steroids	-
9	Triterpenoids	+
10	Phenolic compounds	+

(+) for present and (-) for absent

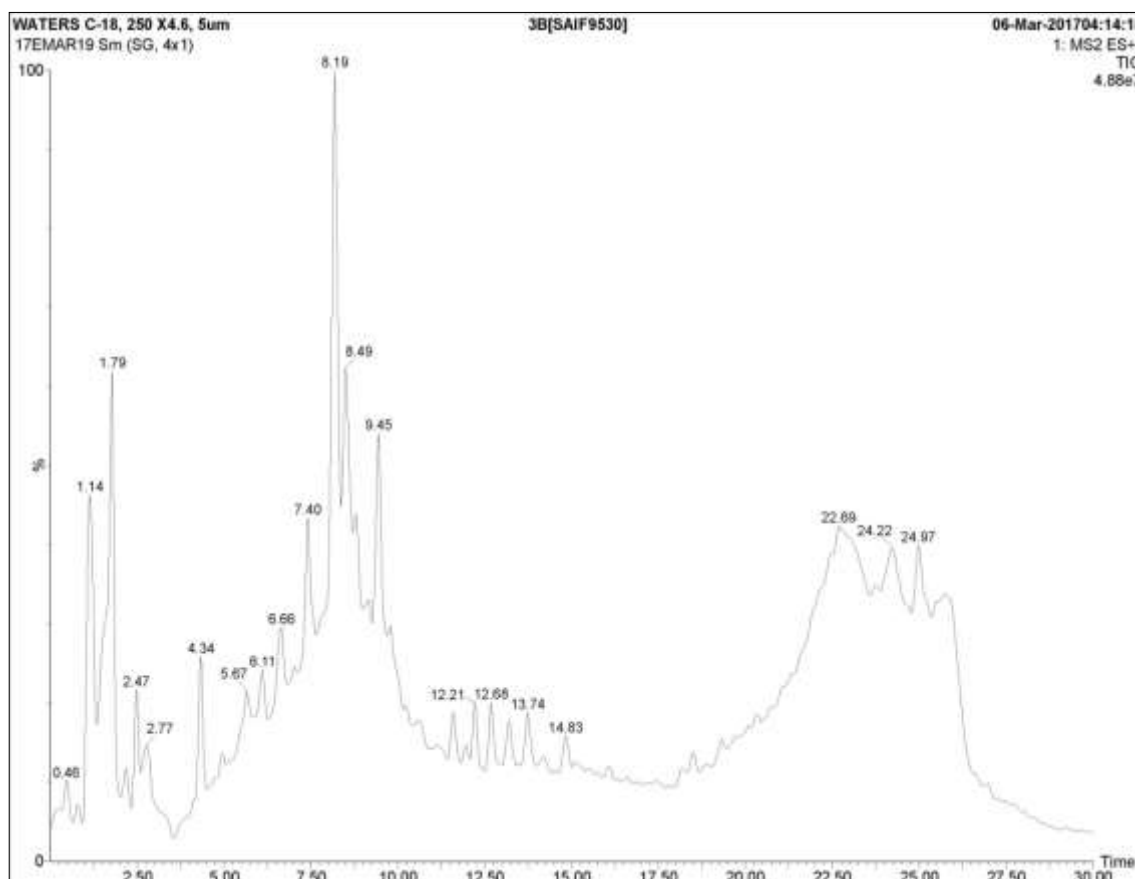


**Fig 1:** Antioxidant activity of *Balanites aegyptiaca* linn. Leaves aqua-ethanol extract  
Phytochemical analysis

#### LC-MS/MS analysis of aqua-ethanol extract

The effective bioactive phytochemical ingredients in *Balanites aegyptiaca* Linn. Leaves are responsible for potency of antioxidant activity were screened by LC-

MS/MS analysis spectral technique. The LC-MS/MS analysis of *Balanites aegyptiaca* Linn. Leaves aqua-ethanol extract was detected phytochemicals intensity peaks chromatogram (TIC and EIC) as shown in fig. 2.



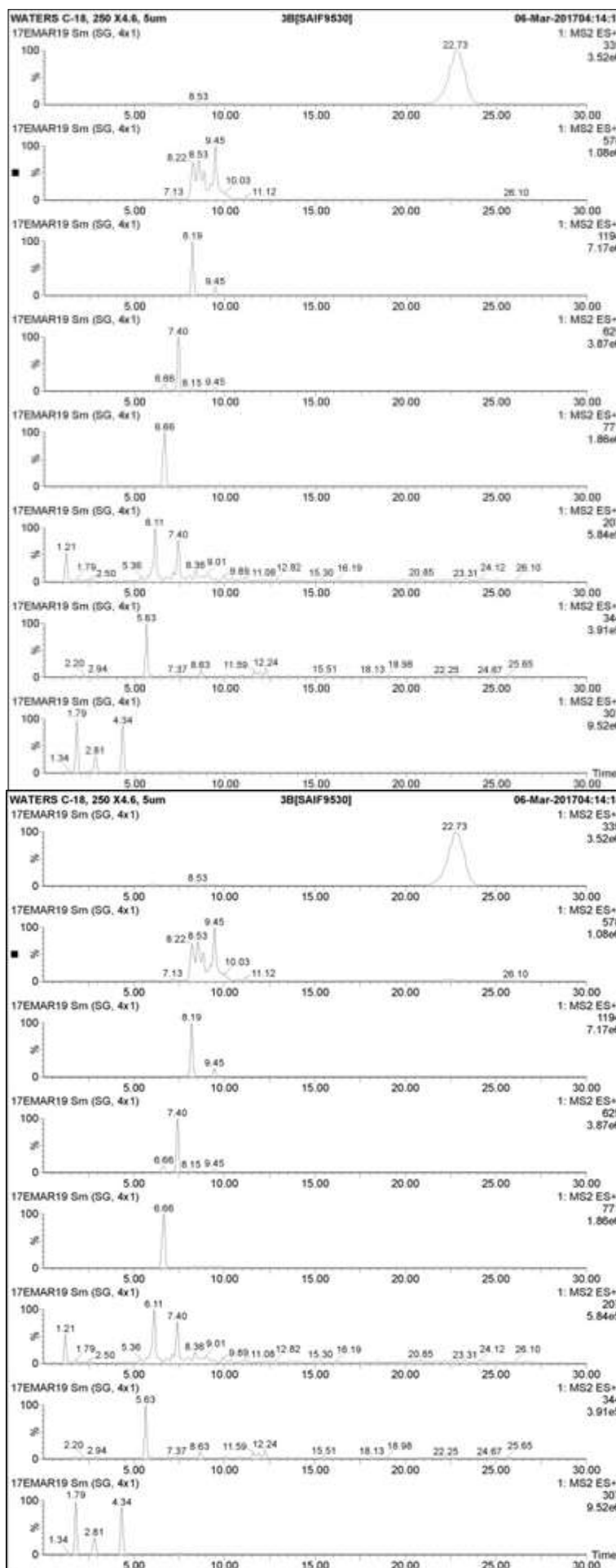


Fig 2: LC-MS/MS chromatogram (TIC and EIC) of aqua-ethanol extract

**Table 3:** Phytochemical ingredients detected in leaves aqua-ethanol extract

Peak	R. Time	Name	Base m/z
1	1.79	Matricin	307
2	5.67	4-((1R)-1-[(R)-hydroxy (phenoxy) phosphoryl] pentyl)amino)-4-oxobutanoic acid	344
3	6.11	(2R, 3S)-2-hydroxybutane-1, 2, 3-tricarboxylic acid; 3-C-carboxy-2, 4-dideoxy-2-methyl-D-threo-pentanic acid	207
4	6.66	7-Methylquercetin-3-Galactoside-6"-Rhamnoside-3"-Rhamnoside	771
5	7.40	Isorhamnetin-3-O-rutinoside	625
6	8.19	Assam Saponin A	1194
7	9.45	Rhoifolin	578
8	22.69	(4Z, 7R, 8E, 10E, 12Z, 14S)-7, 14-dihydroxyoctadeca-4, 8, 10, 12-tetraenedioic acid	339

The eight phytochemical ingredients <sup>[21, 22]</sup> tentatively detected by LC-MS/MS analysis in leaves aqua-ethanol extract of *Balanites aegyptiaca* Linn. Which contribute to antioxidant activity as shown in above table 3.

Phytochemical analysis of *Balanites aegyptiaca* Linn. Leaves aqua-ethanol extract showed different compounds, which could be help in the development of a suitable monograph for the plant. Phytochemicals investigation of stem bark and kernel extracts were reported different bioactive contents identified using GC-MS analysis <sup>[23, 24]</sup> with significant pharmacological activities <sup>[25-27]</sup>. The leaves of *Balanites aegyptiaca* Linn. Is used in curing anthrax, antibacterial, anthelmintic, contraception and to clean malignant wounds. LC-MS/MS analysis using feature-based molecular networks revealed diverse phytochemical ingredients classes viz. carbohydrate, glycoside, tannins, triterpenoids, steroidal, saponins, flavonoids, N-containing metabolites, phenolics, fatty acids and lipids as the constitutive metabolome in *Balanites aegyptiaca* Linn., whereas its vegetative parts such as leaves and stem were rich in amino acids and fatty acids. Principal phytochemical ingredients were reported in LC-MS/MS analysis of leaves aqua-ethanol extract as Matricin, 4-((1R)-1-[(R)-hydroxy (phenoxy) phosphoryl] pentyl)amino)-4-oxobutanoic acid, (2R, 3S)-2-hydroxybutane-1, 2, 3-tricarboxylic acid; 3-C-carboxy-2, 4-dideoxy-2-methyl-D-threo-pentanic acid, 7-Methylquercetin-3-Galactoside-6"-Rhamnoside-3"-Rhamnoside, Isorhamnetin-3-O-rutinoside, Assamsaponin A, Rhoifolin and (4Z, 7R, 8E, 10E, 12Z, 14S)-7, 14-dihydroxyoctadeca-4, 8, 10, 12-tetraenedioic acid <sup>[28]</sup>. Several research studies demonstrate that extracts and phytochemicals isolated from different parts of *Balanites aegyptiaca* Linn. Are having rich bioactive contents, which responsible for various pharmacological activities such as antiproliferative, antioxidant <sup>[29]</sup>, antidiabetic, wound healing, antimicrobial, hepatoprotective, hypocholesterolemic, antiviral, diuretic and insecticidal activity <sup>[30]</sup>. Fruit pulp extract of *Balanites aegyptiaca* Linn. Could thus confer neuroprotection through its antioxidant and anticholinesterase activity <sup>[31]</sup>. The natural health products contains bioactive metabolites and will be used in *in vitro* studies were clearly indicates as a promising avenue for the prevention of oxidative stress related disorders <sup>[32, 33]</sup>. Similarly in aqua-ethanol extract of leaves also founds polyphenol and flavonoid contents, responsible for the DPPH free radical scavenged activity <sup>[34]</sup>. The dose dependent potential scavenging of free radicals reported by leaves aqua-ethanol extract *in vitro* study. Therefore, *Balanites aegyptiaca* Linn. Medicinal plant leaves has been used as potent antioxidant source to treat and prevent pathological conditions <sup>[35]</sup>.

## Conclusion

These screenings suggest that *Balanites aegyptiaca* Linn. Leaves aqua-ethanol extract has acceptable antioxidant activity, but it has less efficiency than standard ascorbic acid. This indicates that this plant can have great scope of important bioactive antioxidant phytochemical ingredients, which can be formulated to make antioxidant dosage forms. The bioactive phytochemicals were content in the extract reveals important role in potent antioxidant activity. A leaves of *Balanites aegyptiaca* Linn. Plant could be become a source of natural antioxidant agents responsible for prevent and management of oxidative stress cause complications in humankind. Therefore, these screening studies concluded that *Balanites aegyptiaca* Linn. Leaves aqua-ethanol extract shows *in vitro* potential antioxidant activity in reducing role of oxidative stress related problems.

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