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# **RESEARCH ARTICLE**

# EVALUATION OF ANTIOXIDANT POTENTIAL AND PHYTOCHEMICALS OF BALANITES AEGYPTIACA (LINN.) LEAF EXTRACT

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### ARTICLE INFO

## ABSTRACT

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Keywords Balanites aegyptiaca (Linn.), phytochemical, GC-MS, DPPH, antioxidant. Leaf of *Balanites aegyptiaca* (Linn.) plant have been used 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. So this study is intended to evaluate the antioxidant potential and phytochemical contents in *Balanites aegyptiaca* (Linn.) leaf ethanol extract with GC-MS analysis and qualitative method according to standard protocol. Antioxidant potential of ethanol extract was found to be significant with IC<sub>50</sub> value 37.61  $\pm$  0.64 µg/mL using DPPH (2, 2-Diphenyl-1-picryl hydrazyl) free radical scavenging assay method. The presence of various bioactive phytochemical contents in *Balanites aegyptiaca* (Linn.) leaf ethanol extract like carbohydrate, protein, amino acids, glycoside, tannins, saponin, flavonoids and phenolic compounds were found to be positive. The GC-MS analysis was carried out for the identification of active compounds in the extract and reported 12 phytochemicals, which are responsible for antioxidant activity. This evaluation revealed the antioxidant potency of leaf extract and its content active compounds role in reducing of oxidative stress related complications.

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# **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 (Gupta S.C. et al., 2012) 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 (Al-Thobaiti & Abu Zeid, 2018). Balanites aegyptiaca (Linn.) is multipurpose plant used for food and fodder in different regions of Africa and South Asia (Elseed et al., 2002). Among the various parts of Balanites aegyptiaca (Linn.) plant, fleshy pulp of the fruit is eaten and also used as a food, beverage, & medicines (National Research Council, 2008). 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 (Abdelkarim et al., 2014).

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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 (Al-Thobaiti S. A. and Abu Zeid I. M., 2018: Mohamed Hussain S.A. et al., 2019). The extract of root bark has been used in diarrhoea, haemorrhoid and also acts as a fish poison (Bukar A. et al., 2004). 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 (Datti Y. et al., 2020). Along with this it has various electrolytes or minerals such as calcium ions, iron, magnesium, phosphorus, zinc, copper and potassium ions (Stadlmayr B. et al., 2013). 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 plant are traditionally used in several folk medicines (Yadav J. P. and Panghal M., 2010; Chothani D. L. and Vaghasiya H. U., 2011; and Saboo et al., 2014). This evaluation study focus on the antioxidant potential 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**: Leafs of *Balanites aegyptiaca* (Linn.) plant were collected from local area identified and authenticate with the help of our institute botanists. The collected leafs are cleaned with distilled water to remove dirt and air dried in shade.

**Preparation of Extraction:** The *Balanites aegyptiaca* (Linn.) dried leafs were rushed and powdered with the help of grinder. 30 g of powdered plant material was macerated in 150 mL of ethanol and kept on a magnetic stirrer for stirring and extracted using a soxhlet apparatus sequentially in 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 potential: The *in vitro* antioxidant potential was evaluated by using stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) with the help of UV-spectrophotometer (Blois M. S., 1958; Shendge P. *et al.*, 2011 and Gawade B. *et al.*, 2020). 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  $\mu$ g/mL) and final volumes were made to 3 mL by adding distilled water. After 20 minutes, the absorbance of each concentrations 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

% Free Radical Scavenged Potential =  $(A_{Control} - A_{Test} / A_{Control}) \times 100$ 

IC<sub>50</sub> values were determined by using graphical method.

**Phytochemical analysis:** The leaf 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 (Tiwari *et al.*, 2011; Gawade B. and Farooqui M, 2018).

**GC-MS analysis:** GC-MS analysis study was carried out with the help of on Shimadzu GC-MS model number QP 2010S. Its column Rxi-5Sil MS, 30 meter length, 0.25 mm ID and 0.25 µm thickness was used. The detected bioactive phytochemical contents were identified by comparing of mass spectra with NIST-11 and WILEY-8 inbuilt libraries.

**Statistical analysis:** The tests were carried out triplicate in 3 sets and its results expressed in mean  $\pm$  SD. Values of P < 0.05 were considered as statistically significant.

## **RESULTS AND DISCUSSION**

In the present evaluation study, the antioxidant potential and phytochemical contents in *Balanites aegyptiaca* (Linn.) leaf

ethanol extract was carried out. The experimental data and results of leaf ethanol extract and ascorbic acid as a standard were represents in Table-1. The above, result table-1 shows the percentage of DPPH free radical scavenged potential by leaf ethanol extract and ascorbic acid at different concentrations tested. Leaf of Balanites aegyptiaca (Linn.) exhibited potential scavenging activity (Kahsay T. et al., 2014) by IC<sub>50</sub> values of ethanol extract 37.61  $\pm$  0.64 µg/mL and ascorbic acid as a standard 15.90  $\pm$  0.45 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 DPPH free radical scavenging potential was found to be greater than leaf ethanol extract of Balanites aegyptiaca (Linn.) at all concentrations. From the current result it may be suggested that the leaf extract of Balanites aegyptiaca (Linn.) reduces the DPPH free radical and significantly noted antioxidant potential.

**Phytochemical analysis:** The qualitative various phytochemical test evaluation of *Balanites aegyptiaca* (Linn.) leaf ethanol extract were showed the presence of carbohydrate, protein, amino acids, glycoside, tannins, saponin, flavonoids and phenolic compounds (Kumawat B. K. *et al.*, 2012; Farooqui M. & Gawade B. 2018) as shown in Table-2.

GC-MS analysis: The effective bioactive phytochemical compounds in Balanites aegyptiaca (Linn.) leaf are responsible for potency of antioxidant activity were studied by GC-MS analysis technique as shown in Fig.-2. These contribute to antioxidant potential. The chromatogram is run upto 44.0 minutes. The first peak appears at 16.54 minutes, overall there are twelve peaks reported. The probable compounds as per peak library search are given in the Table-3. The glycopyranoside is found to be the major content of the leaf ethanol extract. Phytochemical analysis of Balanites aegyptiaca (Linn.) leaf ethanol extract showed different compounds, which could be help in the development of a suitable monograph for the plant (Kumawat B. K. et al., 2012). Phytochemicals investigation of stem bark and kernel extracts were reported different bioactive contents identified using GC-MS analysis (Abu Zeid I. M. et al., 2019) with significant pharmacological activities (Mutwali I. E. F. A. and Abdelgadir S., 2016; Shafik N. H. et al., 2016). The leaf of Balanites aegyptiaca (Linn.) is used in curing anthrax, antibacterial, antihelminthic, contraception and to clean malignant wounds. Principal phytochemical constituents contains saponin, furanocoumarin, flavonoids namely quercetin 3-glucoside, quercetin-3-rutinoside, 3-glucoside, 3-rutinoside, 3-7diglucoside and 3-rhamnogalactoside of isorhamnetin, six diosgenin glucosides including di-, tri- and tetraglucosides. The different parts of Balanites aegyptiaca (Linn.) are having rich bioactive contents, which responsible for various pharmacological activities such as antiproliferative, antioxidant (Amadou I. et al., 2019), antidiabetic, wound healing, antimicrobial, hepatoprotective, hypocholesterolemic, antiviral, diuretic and insecticidal activity (Yadav J. P. and Panghal M., 2010; Chothani D. L. and Vaghasiya H. U., 2011; and Saboo et al., 2014). The natural health products contains bioactive phytoconstituents were clearly indicates as a promising avenue for the prevention of oxidative stress related disorders. Similarly in ethanol extract of leaf also founds polyphenol and flavonoid contents, responsible for the DPPH free radical scavenged potential (Abdelaziz S. M. et al., 2020; Usman A. et al., 2020).

Sr. No.	Concentration in (µg/ml)	% Free Radical Scavenged of Standard	% Free Radical Scavenged of leaf extract
1	5	$43.06 \pm 0.08$	$27.44 \pm 0.63$
2	10	$45.71 \pm 0.16$	$33.10 \pm 0.55$
3	20	$53.09 \pm 0.89$	$39.66 \pm 0.70$
4	30	$55.65 \pm 0.35$	$44.91 \pm 0.12$
5	40	$59.75 \pm 1.25$	$51.70 \pm 0.32$
6	50	$62.31 \pm 0.91$	$56.03 \pm 0.41$
IC <sub>50</sub> Val	ue (µg/mL)	$15.90 \pm 0.45$	$37.61 \pm 0.64$

Table-1. % Free Radical Scavenged Potential of standard and leaf extract

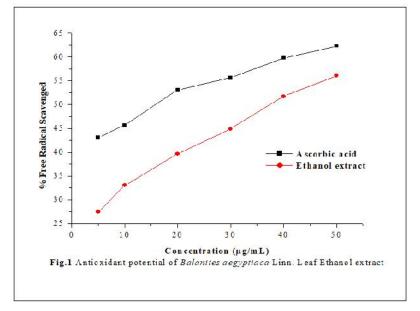
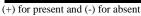


Table-2. Phytochemical analysis of leaf ethanol extract

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	+



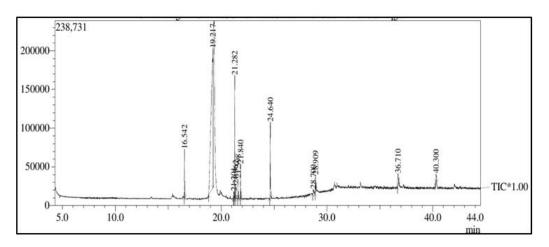


Fig. 2. GC-MS Chromatogram (TIC) of Balanites aegyptiaca (Linn.) leaf ethanol extract

Peak #	R. Time	Phytochemicals detected	Base m/z
1	16.542	Phenol, 2,4-Bis(1,1-Dimethylethyl)-	191.05
2	19.217	AlphaD-Glucopyranoside, methyl	53.90
3	21.201	1-HexenE, 3,5,5-Trimethyl-	70.05
4	21.282	Neophytadiene	68.00
5	21.362	1-Hexanol, 4-methyl-	70.05
6	21.598	6-Octen-1-ol, 3,7-Dimethyl-, Propanoate	82.05
7	21.840	16-Heptadecenal	82.05
8	24.640	2-Hexadecen-1-ol, 3,7,11,15-Tetramethyl-, [R-[R*,R*-(E)]]- (T-Phytol)	71.00
9	28.700	1H-Indene, 1-hexadecyl-2,3-dihydro-	117.00
10	28.909	1-Tridecanol	57.00
11	36.710	Carbonic acid, neopentyl cyclohexylmethyl ester	97.05
12	40.300	Cyclopentanemethanol, .alphacyclohexyl-2-nitro-	95.05

Table 3. Phytochemical contents detected in GC-MS analysis of leaf ethanol extract

The dose dependent potential scavenging of free radicals reported by leaf ethanol extract in vitro study. Therefore, *Balanites aegyptiaca* (Linn.) medicinal plant leaf has been used as potent antioxidant source to treat and prevent pathological conditions (Meshram R. L. and Umbarkar S. N., 2011).

#### Conclusion

These evaluations suggest that *Balanites aegyptiaca* (Linn.) leaf ethanol extract has good antioxidant potential, but it has less efficiency than standard ascorbic acid. This indicates that this plant can have great scope of important bioactive antioxidant molecules, which can be formulated to make antioxidant dosage forms. The bioactive phytochemicals were content in the extract reveals important role in potential antioxidant activity. A leaf of *Balanites aegyptiaca* (Linn.) plant could be become a source of natural antioxidant agents responsible for prevent and management of oxidative stress cause complications. Therefore, these evaluations concluded that *Balanites aegyptiaca* (Linn.) leaf ethanol extract shows *in vitro* potential antioxidant activity in reducing role of oxidative stress related problems.

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**Conflicts of Interest:** The authors have no conflict of interest to declare.

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