

International Journal of Advanced Chemistry Research

ISSN Print: 2664-6781
 ISSN Online: 2664-679X
 IJACR 2023; 5(2): 46-51
www.chemistryjournals.net
 Received: 05-09-2023
 Accepted: 08-10-2023

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Antioxidant activity and LC-MS/MS analysis of phytochemicals in *Balanites aegyptiaca* L. leaves aqueous extract

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DOI: <https://doi.org/10.33545/26646781.2023.v5.i2a.158>

Abstract

The leaves of *Balanites aegyptiaca* L. plant are used in the form of juice in traditional folk medicines, especially in Africa and Southern Asia, in the form of juice to treat diarrhoea, curing anthrax, dysentery, cuts and clean malignant wounds; other parts of the plant have also shown to have their own medicinal activities. The purpose of this analysis study was to study the phytochemical composition and *in vitro* antioxidant activity of aqueous extract of *Balanites aegyptiaca* L. leaves using qualitative analysis methods and LC-MS/MS analyses. Using DPPH (2, 2-Diphenyl-1-picrylhydrazyl) free radical scavenging assay, the aqueous extract was found to have significant antioxidant activity with an IC₅₀ value 46.60±0.02 µg/mL. A qualitative analysis shows that the *Balanites aegyptiaca* L. plant leaves contain carbohydrate, protein, amino acids, glycoside, tannins, saponin, flavonoids and phenolic compounds. Bioactive phytochemicals in the extracts were detected by LC-MS/MS analysis and 8 phytochemicals responsible for antioxidant activity were reported. The results obtained in this analysis study indicate that the aqueous extract of *Balanites aegyptiaca* L. leaves has promising antioxidant properties for the scavenging of free radicals. For this reason, the plant leaves should contain bioactive components with antioxidant activity, and it is beneficial in the treatment of diseases related to free radicals and in reducing the problems associated with suppressed oxidative stress related complications.

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

Introduction

Balanites aegyptiaca L. 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 ^[1] 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 ^[2]. *Balanites aegyptiaca* L. is multipurpose plant used for food and fodder in different regions of Africa and South Asia ^[3]. Among the various parts of *Balanites aegyptiaca* L. plant, fleshy pulp of the fruit is eaten and also used as a food, beverage & medicines ^[4]. All part of *Balanites aegyptiaca* L. 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 ^[5]. 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 ^[6, 7]. The extract of root bark has been used in diarrhoea, haemorrhoid and also acts as a fish poison ^[8]. *Balanites aegyptiaca* L. fruits have various primary and secondary metabolites such as alkaloids, tannins, steroids, glycosides, flavonoids, furanocoumarin, saponins, fixed oil protein, fat, carbohydrates and vitamin C ^[9]. Along with this it has various electrolytes or minerals such as calcium ions, iron, magnesium, phosphorus, zinc, copper and potassium ions ^[10]. Though already some traditional information is available about the *Balanites aegyptiaca* L. plants and review has been intended on various aspects of ethnopharmacology and phytochemistry of *Balanites aegyptiaca* L. Almost all the parts of *Balanites aegyptiaca* L. plant are traditionally used in several folk medicines ^[11, 12].

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This evaluation study focusses on the antioxidant activity and phytochemicals present in leaf extract of *Balanites aegyptiaca* L. with beneficial health effects in reducing of oxidative stress related complications.

Materials and Methods

Collection of Plant material

Leaves of *Balanites aegyptiaca* L. 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 Extract

The *Balanites aegyptiaca* L. dried leaves were rushed and powdered with the help of grinder. 30 g of powdered plant material was macerated in 150 mL of aqueous solvent and kept on a magnetic stirrer for stirring and extracted using a soxhlet apparatus sequentially in aqueous 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 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 leaves extract, which confirms presence of free radical scavengers in extracts. Percentage of DPPH free radical scavenges by test solution were measured as

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

IC₅₀ values were determined by using graphical method.

Phytochemical analysis

The leaves aqueous 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 Discussions

In the present evaluation study, the antioxidant potential and phytochemical ingredients content in *Balanites aegyptiaca* L. leaves aqueous extract was carried out. The experimental data and results of leaves aqueous 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	43.05±0.03	12.15±0.03
2	10	44.91±0.04	19.41±0.04
3	20	53.21±0.04	33.93±0.02
4	30	54.78±0.02	42.87±0.03
5	40	58.64±0.01	47.48±0.02
6	50	62.11±0.04	51.53±0.01
IC ₅₀ value (µg/mL)		18.11±0.03	46.60±0.02

The above, result table-1 shows the percentage of DPPH free radical scavenged activity by leaves aqueous extract and ascorbic acid at different concentrations tested. Leaves extract of *Balanites aegyptiaca* L. exhibited potential scavenging activity [18] by IC₅₀ value 46.60±0.02 µg/mL and ascorbic acid as a standard 18.11±0.03 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 aqueous extract of *Balanites aegyptiaca* L. at all concentrations. From the current result it may be suggested that the leaves extract of *Balanites aegyptiaca* L. reduces the DPPH free radical and significantly noted antioxidant activity.

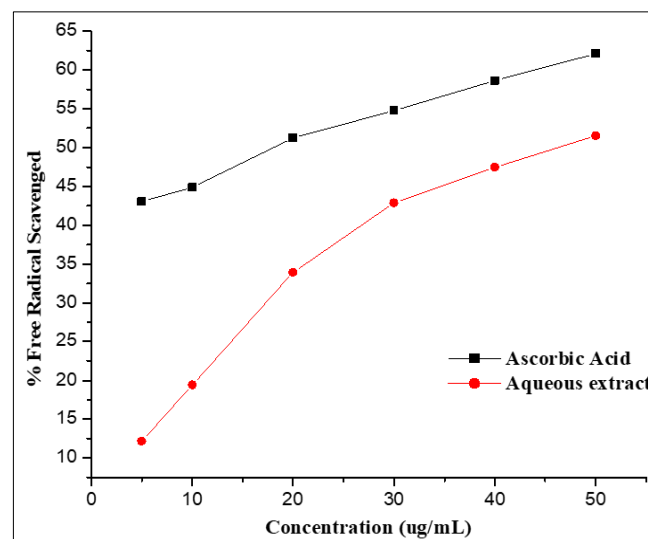


Fig 1: Antioxidant activity of *Balanites aegyptiaca* L. leaves aqueous extract

Phytochemical analysis

The qualitative various phytochemical test analysis of *Balanites aegyptiaca* L. leaves aqueous extract were showed the presence of carbohydrate, protein, amino acids, glycoside, tannins, saponin, flavonoids and phenolic compounds [19, 20] as shown in Table 2.

Table 2: Phytochemical analysis of leaves aqueous extract

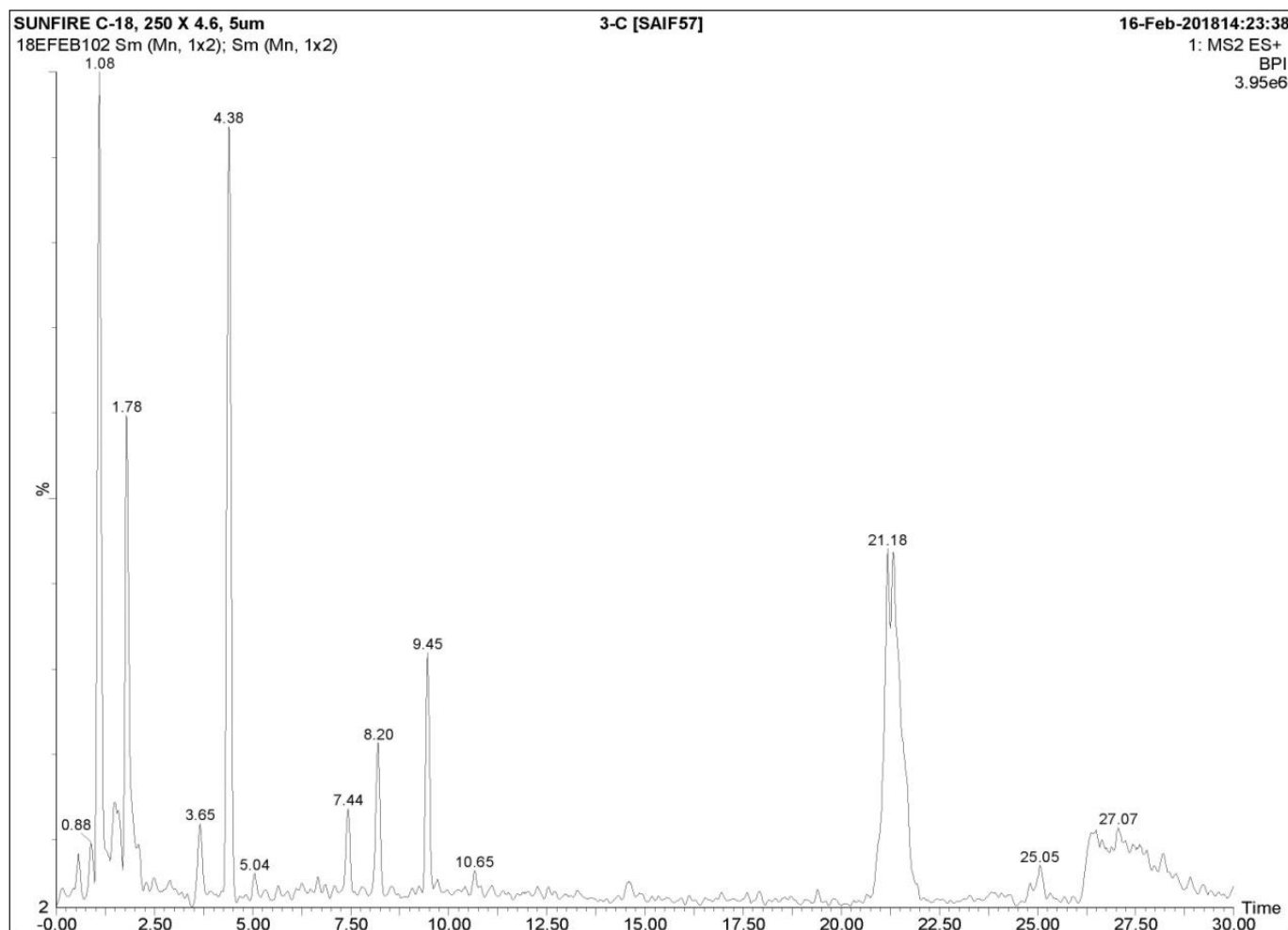
Sr. No.	Phytochemical Tests	Result
1	Alkaloid	-
2	Carbohydrate	+
3	Protein and amino acids	+
4	Glycoside	+
5	Tannin	+
6	Saponin	+
7	Flavonoids	+
8	Steroids	-
9	Triterpenoids	-
10	Phenolic compounds	+

(+) for present and (-) for absent

LC-MS/MS analysis of aqueous extract

The effective bioactive phytochemical ingredients in *Balanites aegyptiaca* L. 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* L. leaves aqueous extract was detected phytochemicals intensity peaks chromatogram (BPI and EIC) as shown in fig. 2.

**Table 3:** Phytochemical ingredients detected in leaves aqueous extract

Peak	R. Time	Name	Base m/z
1	1.08	L(-)-Verbenone	151.2
2	1.78	Na-Benzenol arginine ethylester	307.4
3	3.65	4,7-Phenanthroline	181.3
4	4.38	Na-Benzenol arginine ethylester	307.4
5	7.44	1-(11Z,14Z-eicosadienoyl)-glycero-3-phospho-(1'-myo-inositol)	625.3
6	8.20	Assamsaponin A	1193.7
7	9.45	1-[3-(4-tert-butylphenyl)-2-methyl-propyl]piperidine	274.5
8	21.18	Sphingosine (2-amino-4-octadecene-1, 3-diol)	300.4

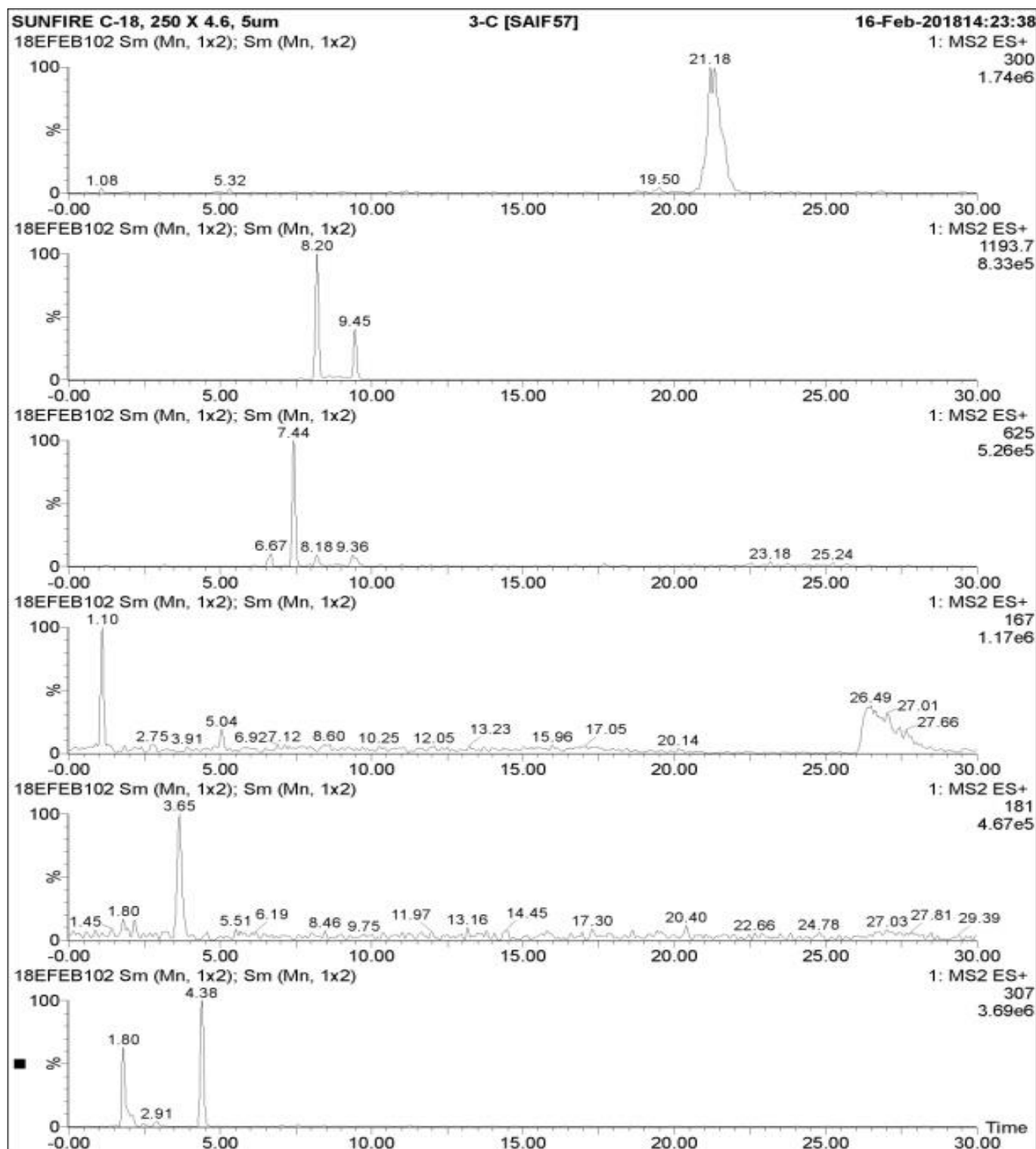


Fig 2: LC-MS/MS chromatogram (BPI and EIC) of aqueous extract

The eight phytochemical ingredients [21, 22] tentatively detected by LC-MS/MS analysis in leaves aqueous extract of *Balanites aegyptiaca* L. which contribute to antioxidant activity as shown in above table 3. Phytochemical analysis of *Balanites aegyptiaca* L. leaves aqueous extract showed different compounds as carbohydrate, protein, amino acids, glycoside, tannins, saponin, flavonoids and phenols, which could be help in the development of a suitable monograph for the plant. The phytochemical compositions investigation of stem bark, kernel and seeds extracts were analysed by gas chromatography-mass spectroscopy (GC-MS) and reported different bioactive contents [23-25] with significant

pharmacological activities [26-28]. The leaves of *Balanites aegyptiaca* L. is used in curing anthrax, antibacterial, antihelminthic, 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* L., 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 aqueous extract as L (-)-

Verbenone, Na-Benzenolarginine ethylester, 4, 7-Phenanthroline, Na-Benzenolarginine ethylester, 1-(11Z, 14Z-eicosadienyl)-glycero-3-phospho-(1'-myo-inositol), Assamsaponin-A, 1-[3-(4-tert-butylphenyl)-2-methyl-propyl] piperidine and Sphingosine (2-amino-4-octadecene-1, 3-diol ^[29]). Several research studies demonstrate that extracts and phytochemicals isolated from different parts of *Balanites aegyptiaca* L. are having rich bioactive contents, which responsible for various pharmacological activities ^[30] such as antiproliferative, antioxidant ^[31], antidiabetic ^[32], wound healing, antimicrobial, hepatoprotective, hypocholesterolemic, antiviral, diuretic and insecticidal activity ^[33]. Fruit pulp extract of *Balanites aegyptiaca* L. could thus confer neuroprotection through its antioxidant and anticholinesterase activity ^[34]. The natural health products contain 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 ^[35, 36]. Similarly, in aqueous extract of leaves also founds polyphenol and flavonoid contents, responsible for the DPPH free radical scavenged activity ^[37]. The dose dependent potential scavenging of free radicals reported by leaves aqueous extract *in vitro* study ^[38]. Therefore, *Balanites aegyptiaca* L. medicinal plant leaves has been used as potent antioxidant source to treat and prevent pathological conditions ^[39].

Conclusion

These analyses suggest that *Balanites aegyptiaca* L. leaves aqueous 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 leaf of *Balanites aegyptiaca* L. 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* L. leaves aqueous extract shows *in vitro* potential antioxidant activity in reducing role of oxidative stress related problems.

Acknowledgment

The authors are acknowledging sincere thanks to Research Institute, Maulana Azad College, Aurangabad, Director, CDRI, Lucknow, HOD and Principal of our college for provided facilities to do this research work.

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