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Free radical scavenging effect of various extracts of leaves of *Balanites aegyptiaca*(L.) Delile by DPPH method

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ABSTRACT

The study of free radicals and antioxidants in biology is producing medical revolution that promises a new age of health and disease management. The present study was performed to evaluate the in vitro antioxidant effect of the petroleum ether, alcoholic and aqueous extracts of Balanites aegyptiaca (L.) Delile (Zygophyllaceae) by using DPPH method. The alcoholic extract exhibited significant inhibition in DPPH free radical formation with IC_{50} values of 10.7474. The alcoholic extract showed potent activity on DPPH, which is compared to that of ascorbic acid (IC_{50} = 21.2253) taken as standards. The results of the present comprehensive analysis demonstrated that alcoholic extract of Balanites aegyptiaca (L.) Delile is a viable source of natural antioxidants and might be exploited for functional foods and nutraceutical applications.

Key words: *Balanites aegyptiaca* (L.) Delile, Successive solvent extracts, DPPH free radical scavenging.

INTRODUCTION

Balanites aegyptiaca (L.) Delile, also known as 'desert date' in English, belongs to family *Zygophyllaceae* . This tree is native to much of Africa and parts of the Middle East. In India, It is particularly found in drier parts of Rajasthan, Madhya Pradesh, Gujarat and Deccan. This is one of the most common but neglected wild plant species of the dry land areas of Africa and South Asia [1]. The tree can grow up to 10 meters in height with spiny branches, compound leaves and greenish yellow flowers, double root system and pale brown date-like fruits. [2].

It is highly resistant to stresses such as sandstorms and heat waves, and grows with minimal available moisture [3]. Literature has revealed antifeedent, molluscicide, antidiabetic, contraceptive activities and antihelminthic in various *Balanites aegyptiaca* (L.) Delile extracts [4-8]. The bark, unripe fruits, and leaves of this plant are reported to have anthelmintic, antifertility, purgative and antidysentric properties [9,10.11].

Antioxidants are reducing agents and limit oxidative damage to biological structures by passivating free radicals. These are compounds, when added to lipids and lipid containing foods increases their shelf-life by retarding the process of lipid peroxidation. Also, these have been widely used as food additives to avoid food degradation, and they play an important role in preventing many lifestyle-related diseases and aging, being closely related to the formation of ROS and to lipid peroxidation [12]. Antioxidant compounds are widely used compounds to counter the free radicals mediate oxidative stress in the cell. These antioxidant compounds can be derived from natural and

chemical sources. Natural sources are much safer to use due to less toxicity and side effects, so the production of antioxidant compound from the natural sources such as plants and algae is in great demand [13].

Fig. 1: *Balanites aegyptiaca* (L.) Delile

DPPH assay has been extensively used for screening antioxidant activity because it can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentration. When DPPH radicals encounter a proton donating substance such as an antioxidant, it would be scavenged and the absorbance is reduced. Thus, the DPPH radicals were widely used to investigate the scavenging activity of some natural compounds [14].

Free radicals are implicated for more than 80 diseases including Diabetes mellitus, arthritis, cancer, ageing etc. In treatment of these diseases, antioxidant therapy has gained an utmost importance. Current research is now directed towards finding naturally occurring antioxidant of plant origin. Therefore we investigated free radical scavenging effect of various successive extracts of leaves of Balanites Aegyptiaca(L.) Delile by DPPH method.

MATERIALS AND METHODS

Collection and authentication

The plant leaves of Balanites Aegyptiaca (L.) Delile was collected from uncultivated fields in and around the Village Maroth of Nagaur District, Rajasthan, India during 2011. The Plant was identified from "Department of Botany, University of Rajasthan, Jaipur and confirmed by compared with the help of herbarium maintained at the Department of Botany, University of Rajasthan, Jaipur. A voucher specimen (No. RVBL21073) was deposited and preserved in Herbarium Department of Botany, University of Rajasthan, Jaipur for further reference.

Preparation of plant extract

The leaves after collection were shade-dried, powdered (40 mesh size) to get a coarse powder and stored in a well closed container. The dried coarse powder (450 g) was subjected to soxhlet extraction successively with petroleum ether (60-80°), ethanol (95%) and distilled water to get the crude extracts. Each time before extracting with next solvent, the powdered material was dried in hot air oven below 50^0C. The extracts were concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature (50-60°). All the extracts were stored in refrigerator for further study [15].

Chemicals

2,2'- diphenylpicryl-1-hydrazyl (DPPH) was purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA). Ascorbic acid was obtained from Merck Ltd., Mumbai, India. Methanol was analytical grade and obtained from SD fine chemicals Ltd., Mumbai, India. All reagents used for the experiments were of analytical grade (AR).

DPPH free radical scavenging activity

The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as [16].

DPPH + (H - A) → DPPH - H + A
(Purple) (Yellow)

The hydrogen atom- or electron-donation ability of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-colored methanol solution of 2,2'- diphenylpicryl-1-hydrazyl (DPPH). This spectrophotometric assay used stable radical DPPH as a reagent [17,18]. 50 ml of various concentrations of the successive extracts in methanol were added to 5 ml of a 4 mg/100 ml methanol solution of DPPH, it was protected from light by covering the test tubes with aluminum foil. The mixture was shaken vigorously and left to stand for 30 min incubation period at room temperature, and the absorbance was measured at 517 nm using methanol as blank. Extract concentration providing 50% inhibition (IC50) was calculated using the graph by plotting inhibition percentage against extract concentration. Ascorbic acid (AA) was used as positive controls and all tests were carried on triplicates. [19]

The free radical scavenging activity (FRSA) (% antiradical activity) was calculated using the following equation:
% Antiradical activity = [(Control Absorbance - Sample absorbance) / Control Absorbance] x100

RESULTS AND DISCUSSION

Free radical scavenging effects of petroleum ether, alcoholic and aqueous extracts of leaves of *Balanites aegyptiaca* (L.) Delile at different concentrations were measured with ascorbic acid as standard compound by using DPPH method. The results are tabulated in table 1-4. Free radical scavenging capacity increased with increasing extracts concentration (Fig 6).

Table-5 shows the DPPH radical scavenging activity of different successive extracts which is expressed in terms of IC_{50} value with respect to ascorbic acid as standard. Lower IC_{50} value shows more antioxidant potential. The IC_{50} value for alcoholic extract was 10.7474 µg/ml which was comparatively lower than the IC_{50} **(**21.2253 µg/ml**)** of ascorbic acid, showed that alcoholic extract of *Balanites aegyptiaca* (L.) Delile are more effective as antioxidant compared to ascorbic acid.

Table1. Free radical scavenging effects of petroleum ether extract of *Balanites aegyptiaca* (L.) Delile leaves.

Conc.	Absorbance of sample	Control	Ctrl.-sample/ctrl	% Inhibition
10	0.212	0.256	0.171875	17.1875
20	0.201	0.256	0.214844	21.48438
30	0.189	0.256	0.261719	26.17188
40	0.175	0.256	0.316406	31.64063
50	0.153	0.256	0.402344	40.23438
60	0.127	0.256	0.503906	50.39063
70	0.102	0.256	0.601563	60.15625
80	0.079	0.256	0.691406	69.14063
90	0.063	0.256	0.753906	75.39063
100	0.048	0.256	0.8125	81.25

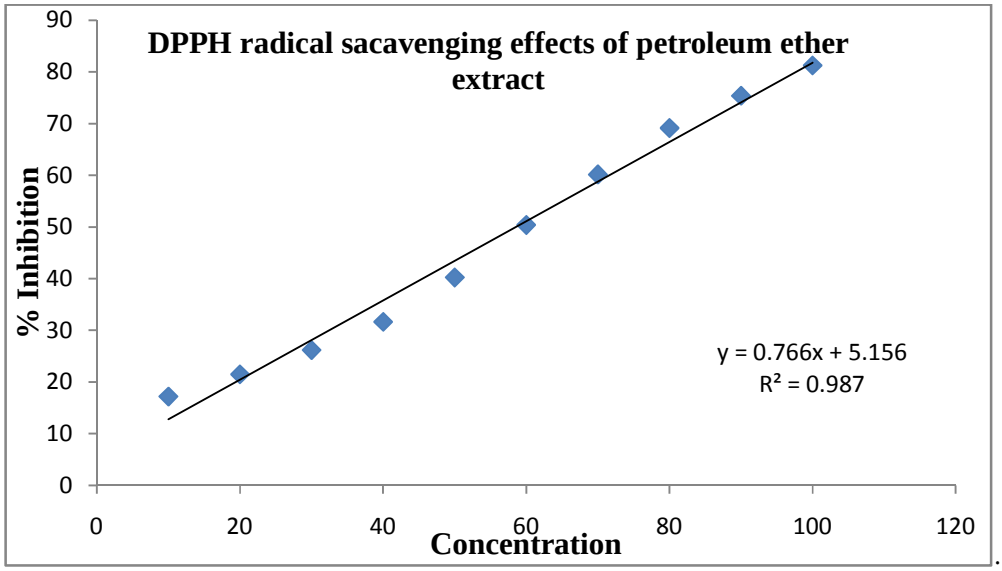

Fig. 2: Free radical scavenging effects of petroleum ether extract of *Balanites aegyptiaca* (L.) Delile leaves.

Table2. Free radical scavenging effects on alcoholic extract of *Balanites aegyptiaca* (L.) Delile leaves.

Conc.	Absorbance of sample	Control	Ctrl.-sample/ctrl	% Inhibition
10	0.129	0.256	0.496094	49.60938
20	0.12	0.256	0.53125	53.125
30	0.109	0.256	0.574219	57.42188
40	0.097	0.256	0.621094	62.10938
50	0.084	0.256	0.671875	67.1875
60	0.078	0.256	0.695313	69.53125
70	0.073	0.256	0.714844	71.48438
80	0.067	0.256	0.738281	73.82813
90	0.049	0.256	0.808594	80.85938
100	0.034	0.256	0.867188	86.71875

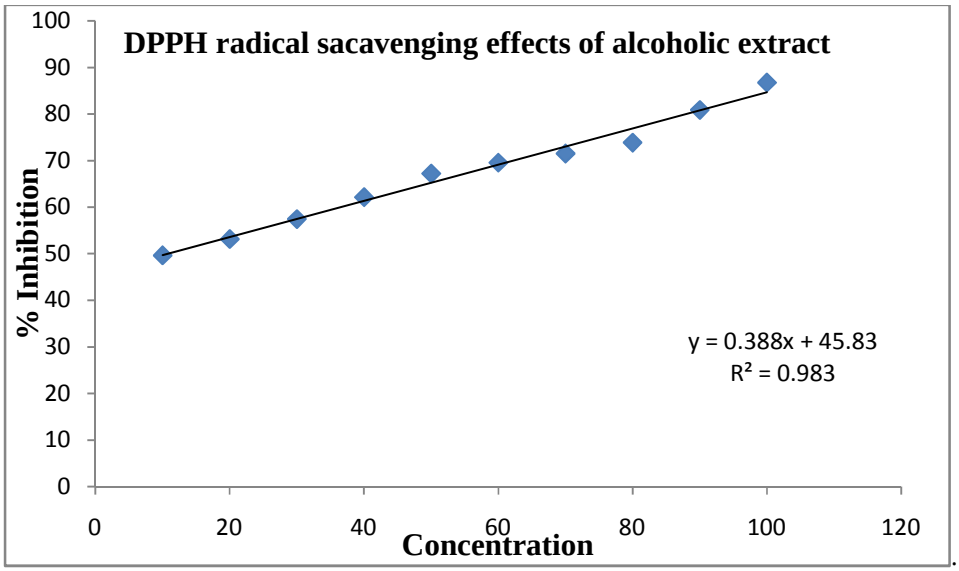

Fig. 3: Free radical scavenging effects on alcoholic extract of *Balanites aegyptiaca* (L.) Delile leaves.

Table3. Free radical scavenging effects on aqueous extract of *Balanites aegyptiaca* (L.) Delile leaves.

Conc.	Absorbance of sample	Control	Ctrl.-sample/ctrl	% Inhibition
10	0.164	0.256	0.359375	35.9375
20	0.155	0.256	0.394531	39.45313
30	0.146	0.256	0.429688	42.96875
40	0.134	0.256	0.476563	47.65625
50	0.126	0.256	0.507813	50.78125
60	0.115	0.256	0.550781	55.07813
70	0.102	0.256	0.601563	60.15625
80	0.075	0.256	0.707031	70.70313
90	0.068	0.256	0.734375	73.4375
100	0.044	0.256	0.828125	82.8125

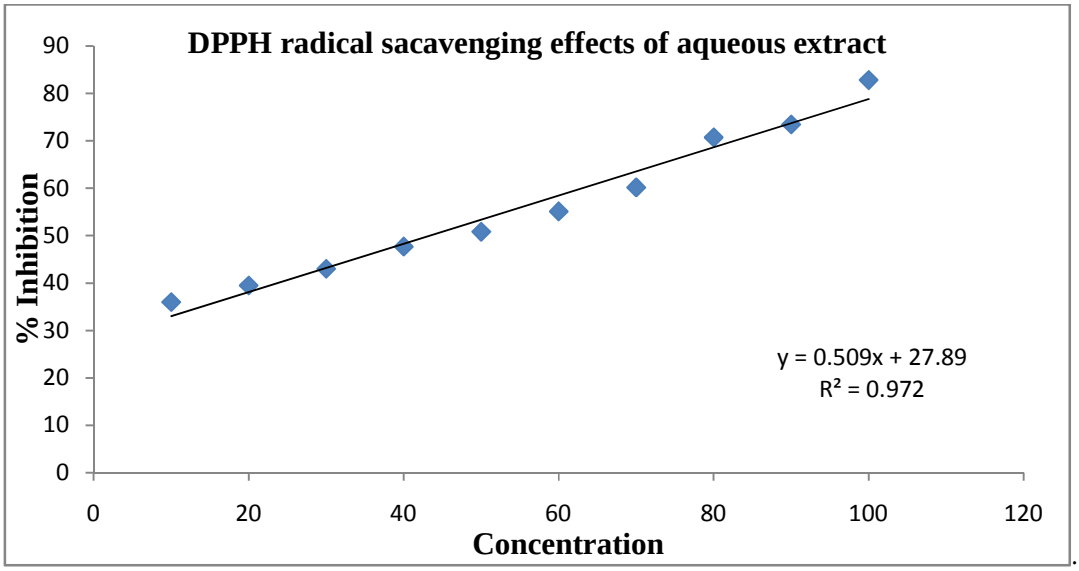

Fig. 4: Free radical scavenging effects on aqueous extract of *Balanites aegyptiaca* (L.) Delile leaves.

Table4. Free radical scavenging effects of Ascorbic acid

Conc.	Absorbance of sample	Control	Ctrl.-sample/ctrl	% Inhibition
10	0.136	0.256	0.46875	46.875
20	0.129	0.256	0.496094	49.60938
30	0.122	0.256	0.523438	52.34375
40	0.114	0.256	0.554688	55.46875
50	0.11	0.256	0.570313	57.03125
60	0.103	0.256	0.597656	59.76563
70	0.097	0.256	0.621094	62.10938
80	0.089	0.256	0.652344	65.23438
90	0.084	0.256	0.671875	67.1875
100	0.077	0.256	0.699219	69.92188

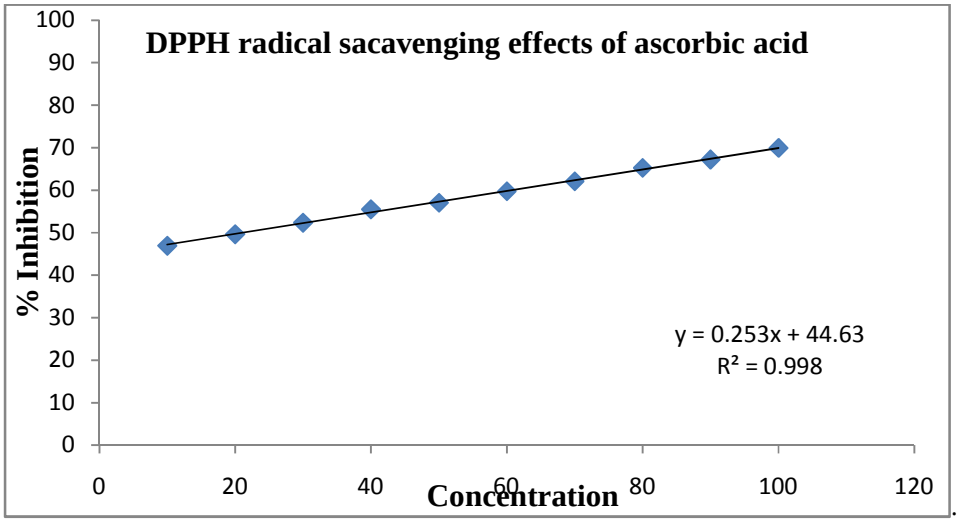

Fig. 5: DPPH radical scavenging activity of Ascorbic acid

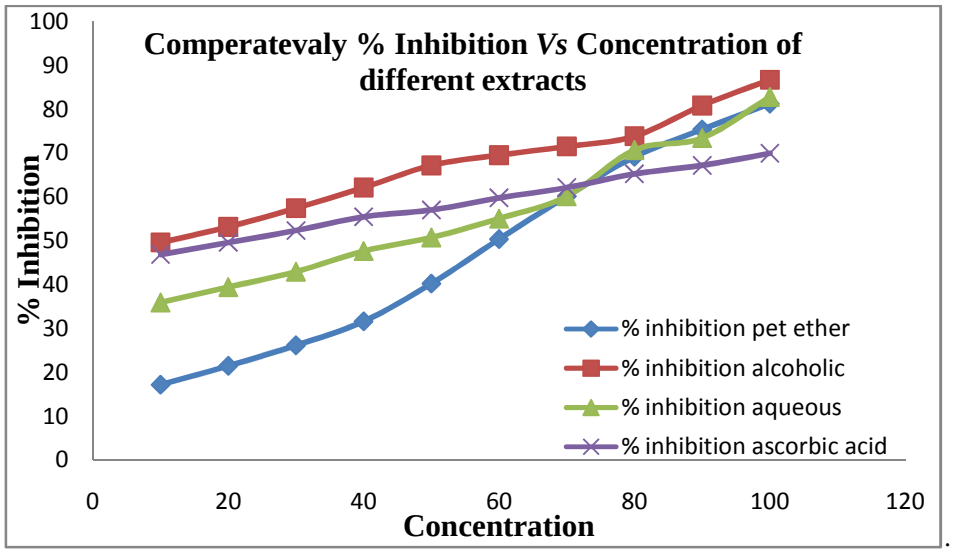

Fig. 6: Free radical scavenging effects of various extract of leaves of *Balanites aegyptiaca* (L.) Delile compared with standard ascorbic acid

Table 5 DPPH radical scavenging IC_{50} values of all extracts and ascorbic acid.

Compound	IC_{50} Value (µg/ml)
Pet ether extract	58.5431
Alcoholic extract	10.7474
Aqueous extract	43.4381
Ascorbic acid	21.2253

CONCLUSION

Based on the results of the present study, we conclude that the plant extract possesses antioxidant potential. The findings of the present study also suggested that alcoholic extract of Balanites *Aegyptiaca* (L.) Delile could be a potential natural source of antioxidants and could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases. However, further studies are necessary to examine underlying

mechanisms of antioxidant effect and to isolate the active compound(s) responsible for these pharmacological activities.

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