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## Preliminary Phytochemical Investigation on Leaves of *Balanites Aegyptiaca* (L.) Delile

Bhupendra Kumar Kumawat<sup>1\*</sup>, Mahesh Gupta<sup>2</sup>, Tara Chand<sup>3</sup>, Yogendra Singh<sup>4</sup>

<sup>1</sup> NIMS University, jaipur-303001, Rajasthan, India.

<sup>2</sup> Kota College of Pharmacy, Kota-, Rajasthan, India.

<sup>3</sup> Regional College of Pharmacy, Jaipur-302022, Rajasthan, India.

<sup>4</sup> Shanti Niketan College of Pharmacy, Mandi-175008, Himachal Pradesh, India.

### ABSTRACT

*Balanites Aegyptiaca* (L.) Delile, 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, Gujarat, Madhya Pradesh and deccan. The tree can grow up to 10 meters in height with spiny branches, compound leaves and greenish yellow flowers and pale brown date-like fruits. It is highly resistant to stresses such as sandstorms and heat waves, and grows with minimal available moisture. The bark, unripe fruits, and leaves of this plant are reported to have anthelmintic, antifertility, purgative and antidysentric properties. The usefulness of this plant is described in many folk books including Ayurveda and different biologically active phytoconstituents were isolated from plant. But no reports are available on phytochemical studies, hence present work was undertaken to investigate the preliminary phytochemical and Physico-chemical studies on the leaves of *Balanites Aegyptiaca* (L.) Delile. The study reveals the Physicochemical parameters like ash value, extractive value and phytochemical screening with different reagents showed the presence of fluorescence compounds, carbohydrates, protein & aminoacid, glycosides, saponin, tannins and flavonoids. The various Physico-chemical and phytochemical parameter were obtained which could help in the development of a suitable monograph for the plant.

**Keywords:** *Balanites Aegyptiaca* (L.) Delile, Successive solvent extracts, Physico- chemical analysis, Preliminary phytochemical screening, Foaming index and Fluorescence analysis.

\*Corresponding author

Email: bhupsa137@yahoo.com



## 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, Gujarat, Madhya Pradesh 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. Plant parts are used as soap substitutes because of high saponin contents; thorny branches used for fencing [2].

It is highly resistant to stresses such as sandstorms and heat waves, and grows with minimal available moisture [3]. Literature has revealed antifeedent, antidiabetic, molluscicide, antihelminthic, and contraceptive activities 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 antidyentric properties [9-11].

The local people (Warangal) use the leaves as a traditional medicine for the treatment of jaundice. Hepatoprotective activity of the bark as well as fruit pulp of this plant has been reported.

## 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 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°), benzene, chloroform, ethanol (95%), and finally it was macerated with distilled water and kept for 7 days to get the crude extracts. Each time before extracting with next solvent, the powdered material was dried in hot air oven below 50°C. 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 qualitative analysis [12].

### **Physico- chemical analysis**

Ash value represents the inorganic salts naturally occurring in the drug and adhering to it. Total ash is the residue remaining after incineration. The acid insoluble ash is the part of total ash which is insoluble in dilute hydrochloric acid. Mixing of sulphuric acid with powdered crude drug before ashing and this sulphated ash is normally less fusible than ordinary ash [12, 13].

The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign organic matter such as metallic salts and/or silica. The total ash value of plant material indicated the amount of minerals and earthy materials attached to the plant material. Air dried plant material was used for the quantitative determination of ash and extractive values according to standard procedure of Indian Pharmacopoeia, 1996 [14] and WHO/QCMMPM, 1992 [15].

Foreign organic matter was determined from the weight of the drug taken and moisture content was determined by loss on drying method in terms of percent w/w as per standard procedure mentioned in Indian Pharmacopoeia.

### **Extractive values**

The extraction of any crude drug with a particular solvent yields a solution containing different phyto-constituents. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. Extractive value is also useful for evaluation of crude drug, which gives an idea about the nature of the chemical constituents present in a crude drug and is useful for the estimation of specific constituents, soluble in that particular solvent used for extraction [16].

### **Preliminary phytochemical screening**

Powdered dried leaves were extracted successively with petroleum ether, benzene, chloroform, ethanol and distilled water. The extracts obtained from successive solvent extraction were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, carbohydrates, proteins/amino acids, glycosides, fixed oils & fats, phenolics, tannins, phytosterols, flavonoids and saponins. [16, 17]

### **Fluorescence characters**

Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Some constituents show fluorescence in the visible range in day light. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which do not visibly fluoresce in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different

reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation [18].

Organic molecules absorb light usually over a specific range of wave length; many of them reemit such radiations. So if the powder is treated with different chemical reagents and seen in the UV cabinet, different colours will be produced. Therefore it can be used for the identification of the drug. The fluorescence characteristic of the drug powder with different chemical reagent was studied by observing under UV Light at 254 & 366 nm. The fluorescence analysis is a tool for the determination of constituents in the plant that gives a definite idea of the chemical nature.

## RESULTS AND DISCUSSION

### Physicochemical analysis

Air dried powdered leaves were used for quantitative determination of various physicochemical parameters i.e. total ash, acid insoluble ash, water soluble ash, sulphated ash and loss on drying as per Indian Pharmacopoeia. The results are tabulated in the Table 1.

Table 1: Physico-Chemical parameters of powdered leaves of *Balanites Aegyptiaca* (L.) Delile

| Sr. No. | Parameters             | % W/W  |
|---------|------------------------|--------|
| 1.      | <b>Ash values</b>      |        |
|         | (a) Total ash          | 12.174 |
|         | (b) Acid insoluble ash | 0.650  |
|         | (c) Water soluble ash  | 8.508  |
|         | (d) Sulphated ash      | 12.202 |
| 2.      | <b>Loss on drying</b>  | 7.564  |

### Foaming Index

The foaming index of powdered leaves *Balanites Aegyptiaca* (L.) Delile was calculated by using this formula and was tabulated in Table 2

$$\text{Foaming index} = 1000/a = 1000/2 = 500$$

Thus, the foaming index of the powdered leaves of *Balanites Aegyptiaca* (L.) Delile was found to be 500. This result indicated that high saponin contents may be present.

### Fluorescence analysis

The fluorescence analysis of powdered leaves with different chemical reagent was observed under day light and U.V. light (254 & 366 nm) and data were tabulated in Table-3 and dried leaves powder extracts of various solvent were also observed and result are tabulated in the Table 4.

**Table 2: Foaming index of the powdered leaves of *Balanites Aegyptiaca* (L.) Delile**

| Sr. No. | Test volumetric flask no. (10ml) | Height of foam (cm.) |
|---------|----------------------------------|----------------------|
| 1.      | 1                                | 0.9                  |
| 2.      | 2                                | 1.4                  |
| 3.      | 3                                | 1.8                  |
| 4.      | 4                                | 2.0                  |
| 5.      | 5                                | 2.4                  |
| 6.      | 6                                | 2.7                  |
| 7.      | 7                                | 3.1                  |
| 8.      | 8                                | 3.4                  |
| 9.      | 9                                | 3.8                  |
| 10.     | 10                               | 4.4                  |

**Table 3: Fluorescence analysis of powdered leaves of *Balanites Aegyptiaca* (L.) Delile**

| Sr. No. | Chemical Treatment                          | Day light       | UV Light        |                 |
|---------|---|-----------------|-----------------|-----------------|
|         |   |                 | 254 nm          | 366 nm          |
| 1.      | Powder as such                              | Green           | Brown           | Greenish brown  |
| 2.      | Powder + Water                              | Green           | Dark green      | Light green     |
| 3.      | Powder + 1 N HCl                            | Light yellow    | Light green     | Light green     |
| 4.      | Powder + 5% NaOH                            | Greenish yellow | Greenish yellow | Greenish yellow |
| 5.      | Powder + 1 N NaOH (Alc.)                    | Green           | Yellowish green | Yellowish green |
| 6.      | Powder + 50% HNO <sub>3</sub>               | Orange          | Yellowish green | Yellowish green |
| 7.      | Powder + 50% H <sub>2</sub> SO <sub>4</sub> | Blackish brown  | Dark brown      | Green           |
| 8.      | Powder +Ammonia                             | Yellowish green | Yellowish green | Yellowish green |
| 9.      | Powder +Acetic acid                         | Greenish yellow | Greenish yellow | Tan yellow      |
| 10.     | Powder + I <sub>2</sub> sol <sup>n</sup> .  | Red             | Red             | Reddish brown   |
| 11.     | Powder + FeCl <sub>3</sub>                  | Yellowish Brown | Dark Green      | Greenish yellow |

**Table 4: Fluorescence Analysis of Successive Solvent Extraction of Leaves of *Balanites Aegyptiaca* (L.) Delile.**

| Sr. No. | Chemical Treatment             | Day light      | UV Light   |                |
|---------|--------------------------------|----------------|------------|----------------|
|         |                                |                | 254 nm     | 366 nm         |
| 1.      | Petroleum ether(60-80) extract | Dark green     | Dark green | Greenish black |
| 2.      | Benzene extract                | Dark green     | Black      | Black          |
| 3.      | Chloroform extract             | Dark green     | Black      | Black          |
| 4.      | Alcoholic extract              | Dark green     | Dark red   | Dark brown     |
| 5.      | Aqueous extract                | Greenish brown | Black      | Dark brown     |

### Extractive values

The extractive values of different extracts obtained from the successive solvent extraction of the leaves of *Balanites Aegyptiaca* (L.) Delile were carried out and results are tabulated in Table 5.

The petroleum ether, benzene, chloroform and ethanol extracts gave dark green colored residues, while water extract resulted in greenish brown colored amorphous solid.

Water soluble extractive value was found to be very high when compared to other extractable matter in the drug.

### Preliminary phytochemical screening

Different extracts obtained from the successive solvent extraction were analyzed for different phytoconstituents present in these by the method of qualitative phytochemical analysis. The results pertaining to this investigation were presented in Table 6.

They show the presence of the carbohydrates, protein & amino acid, glycosides, saponin, tannins, flavonoids and fixed oil.

**Table 5: Extractive values of successive solvent extracts of powdered leaves of *Balanites Aegyptiaca* (L.) Delile**

| Sr. No. | Solvent extracts               | Colour         | Consistency | Extractive values (% W/W) |
|---------|--------------------------------|----------------|-------------|---------------------------|
| 1.      | Petroleum ether(60-80) Extract | Dark green     | Sticky      | 1.62%                     |
| 2.      | Benzene Extracts               | Dark green     | Powder      | 1.33%                     |
| 3.      | Chloroform Extract             | Dark green     | Powder      | 1.05%                     |
| 4.      | Alcohol Extract                | Dark green     | Sticky      | 8.79%                     |
| 5.      | Aqueous extract                | Greenish brown | Powder      | 14.75%                    |

**Table 6: Preliminary phytochemical analysis of successive solvent extracts of powdered leaves of *Balanites Aegyptiaca* (L.) Delile.**

| Sr. No. | Plant constituents     | Test                     | PE | BE | CE | Alc. E | Aq. E |
|---------|------------------------|--------------------------|----|----|----|--------|-------|
| 1.      | Alkaloids              | Dragendorff's test       | -  | -  | -  | -      | -     |
|         |                        | Mayer's test             | -  | -  | -  | -      | -     |
|         |                        | Hager's test             | -  | -  | -  | -      | -     |
| 2.      | Carbohydrates          | Molisch's test           | +  | +  | +  | +      | +     |
|         |                        | Fehling's test           | +  | +  | +  | +      | +     |
| 3.      | Proteins & Amino acids | Biuret test              | +  | +  | +  | -      | -     |
|         |                        | Xanthoprotein test       | +  | +  | +  | +      | -     |
|         |                        | Millon's reagent test    | +  | +  | -  | -      | -     |
| 4.      | Glycosides             | Legal's test.            | -  | +  | +  | +      | +     |
|         |                        | Borntragers test.        | -  | -  | +  | -      | -     |
|         |                        | Cardiac Glycosides test  | -  | +  | +  | +      | -     |
|         |                        | Coumarin Glycosides      | -  | -  | -  | -      | -     |
| 5.      | Saponins               | Foam test                | -  | +  | +  | +      | +     |
| 6.      | Flavonoids             | Shinoda's Test           | +  | +  | +  | -      | +     |
|         |                        | Lead acetate test        | +  | +  | +  | +      | +     |
| 7.      | Fixed oils & Fats      | Spot test                | ++ | +  | +  | +      | -     |
| 8.      | Phenolics/ Tannins     | FeCl <sub>3</sub> test   | -  | +  | +  | +      | -     |
|         |                        | lead acetate test        | +  | +  | +  | +      | +     |
|         |                        | Pot. dichromate test     | -  | +  | +  | +      | +     |
| 9.      | Steroids               | Liebermann-Burchard test | -  | -  | -  | -      | -     |

**Key:** + = Present, - = Absent, PE = Petroleum ether Extract BE = Benzene Extract CE = Chloroform Extract, Alc. E = Alcoholic Extract Aq. E = Aqueous Extract

## CONCLUSION

In the present physicochemical investigation and preliminary phytochemical screening of powdered leaves of *Balanites Aegyptiaca* (L.) Delile provide useful information in regard to its correct identity and evaluation, and help to differentiate from the closely related other species of *Balanites Aegyptiaca* (L.) Delile. The other study viz. foaming index, and Fluorescence analysis add to its quality control and quality assurance for proper future identification of the plant, and serves as a standard monograph for identification and evaluation of plant. Further research on this species may help in the isolation of therapeutically potent compounds which can be finally be subjected to pharmacological activities, thus leading to opening up new avenues in the use of natural products for therapeutic purpose.

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