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Evaluation of pharmacognostical parameters of the stem of *Balanites roxburghii* planch

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Abstract
Balanites roxburghii Planch commonly called as hingota belonging to Zygophyllaceae family found in arid regions of India. This herb has been used traditionally for vomiting, vermifuge, antimycotic, laxatives, expectorant, treating pertussis and dog bites. *Balanites roxburghii* Planch stem is crucial due to its great phytochemical profile, which need careful Pharmacognostical standardization. Numerous pharmacological studies anti-inflammatories, anti-bacterial, antidiabetic, nutritional, anticonvulsant, antifertility, hepatoprotective properties, and antioxidant qualities, are well-known for *Balanites roxburghii*. Pharmacognostical standardization of the stem of *Balanites roxburghii* is the aim of this investigation. The plant's physiochemical analyses, ash value, moisture content, and pH, were in line with pharmacopoeial standards & reports. Proteins, alkaloids, amino acids, saponins, flavonoids, & other phenolic compounds were found in the plant extracts after phytochemical screening; glycosides, sterols, carbohydrates & volatile oils were not found. EEBR had a total flavonoids (195.09±1.132) and a low content of total phenolics (45.13±1.104).

Keywords: *Balanites roxburghii* planch, pharmacognostical standardization, ash value, extractive value, moisture contents

1. Introduction
Balanites roxburghii Planch in local language called as hingota, thorn tree, desert date, soap berry tree (due to presence of saponin and used for washing cloths locally), belonging to *Zygophyllaceae* family is an important genus of thorny shrubs or trees for medicinal value found in arid regions of India, including western Rajasthan, Punjab, West Bengal, Sikkim, and Myanmar. It is known for its emetics (vomiting), vermifuge (anthelmintic), antimycotics (anti-fungal), laxative, purgative, cathartic, colic, expectorant, and treating pertussis (whooping cough), skin problems, and dog bites [1, 2]. Ayurveda considers the bark to have anthelmintic and spasmolytic properties, making it useful for cough and skin problems. The leaf has anthelmintic properties, while the root has emetic properties. Fruits are used for treating whooping cough and dermatological conditions, and a bark paste is created and applied topically [2]. The fruit is known for its analgesic properties, anticonvulsant, antifertility, and hepatoprotective properties [3]. Phytochemical analyses of *Balanites roxburghii* reveal various components like saponin glycosides, flavonoids, tannins, alkaloids and phenols present in plant [4]. The seeds are used as expectorants for cough and colic, while the kernel is used for dermatological conditions and thermal injuries [3]. Diosgenin, a steroidal compound used in contraceptives, is found in roots and fruits. Traditional healers use the bark of plants to alleviate pain, reduce swelling and also exhibits anti-fertility efficacy and anti-inflammatory action [6].

In our present study, our main aim is to conduct a Pharmacognostical standardization of *Balanites roxburghii* Planch stem. The stem of *Balanites roxburghii* is crucial for its rich phytochemical profile, necessitating a thorough evaluation. Morphological and anatomical studies reveal key features like vessel arrangement, fiber types, and cellular structures. Organoleptic evaluations provide insights into sensory attributes influencing therapeutic applications. Pharmacognostical standardization promotes safe use in traditional healing practices and modern therapeutics by combining morphological assessments with phytochemical profiling, ensuring efficacy and safety while preserving traditional knowledge systems.

Fig 1: An overview of plant *Balanites roxburghii* Planch

2. Materials and Methods

2.1 Collection authentication of the Plant Material

The collection of stems of *Balanites roxburghii* Planch was done from JNV University, New Campus, Bhagat Ki Kothi, Jodhpur, Rajasthan (India) and its herbarium was prepared on herbarium sheet (11.5×16.5-inch size). Identification and authentication of *Balanites roxburghii* Planch was done by Dr. Sriman Lal Meena, Scientist-E & Head of Office, Botanical Survey of India (BSI), situated at Jodhpur, Rajasthan (India). The voucher number of certificate issued for plant authentication is {No.: BSI / AZRC / 1. 12012 / Tech. / 2023-24 (Pl. Id.)/489} on dated 31.10.2023 [7].

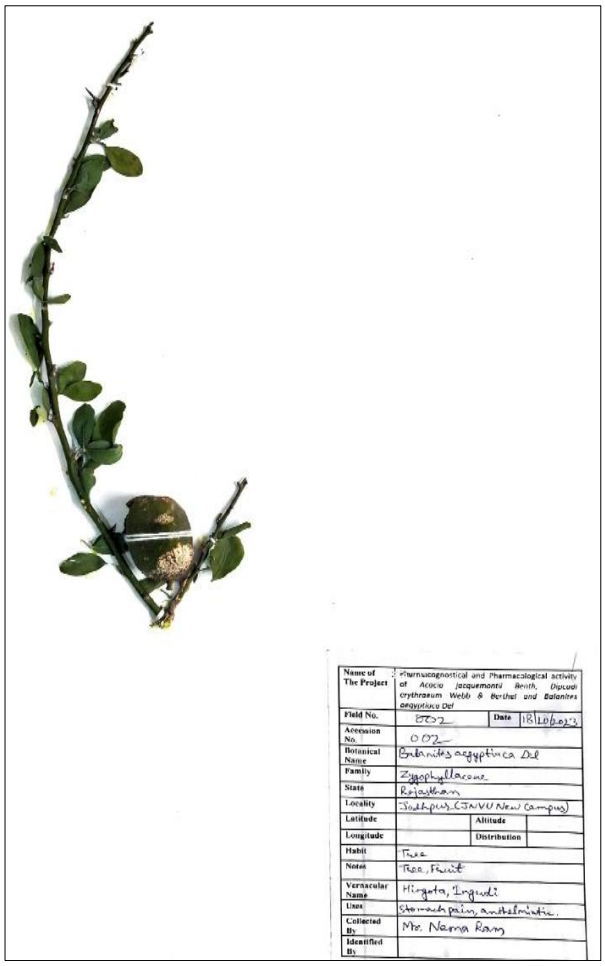

Fig 2: Herbarium sheet of *Balanites roxburghii* Planch

2.2 Pharmacogenetic Standardization of Crude Drug

- **Organoleptic Characters**
  The stem of *Balanites roxburghii* was examined as per standard procedure for organoleptic or macroscopic study [8].
- **Microscopic Characters**
  The transverse section of stem of *Balanites roxburghii* were taken with the help of sharp blade, stained with safranin as per standard procedure followed by mounting with glycerin, detected with the help of compound microscope and digital QUASMO microscope [8, 9].

Determination of Physico-Chemical Parameters

Determination of Total Ash Value

Ash values are used to detect inferior class products, exhausted drugs (fully or partially removal of content), and sandy or earthy matter. They are also used to identify chemical compounds by acid insoluble ash and total ash. To determine total ash values, 5gm air dried powdered drug was weighed on weighing balance, ignited in the muffle furnace at 600 ºC until white and then it was cooled in a desiccator. The residue of total ash (carbon-free) revealed, were weighed, and again ignited until constant weight was not obtained [10]. The percentage (%) of total ash value was calculated for crude drug by using the below mentioned formula:

$$\text{Percentage of Total ash} = \frac{(\text{Weight of ash})}{(\text{Weight of sample taken})} \times 100$$

Acid insoluble ash value

The total ash value revealed of a drug was decided by adding 45 ml of 1:5 hydrochloric acid to a dish, boiling for 5 minutes, and filtering. Insoluble matter was collected on ashless filter paper, washed with distilled water, and transferred to the original dish. The filter paper was dried, ignited to constant weight, and the weight was taken after cooling the dish in desiccators [11]. The percentage (%) of acid-insoluble ash value was calculated for drug using the below mentioned formula.

$$\text{Percentage of Acid insoluble ash} = \frac{(\text{Weight of acid insoluble residue remained})}{(\text{Weight of total ash value sample taken})} \times 100$$

Extractive Value Determination

Plant's substances consists of water, mineral and variety of organic compounds (Metabolites) which can be defined as both primary and secondary metabolites. These substances are obtained by extraction using a range of solvents and in varying ratios. The extractive value refers to the quantity of active compounds that can be obtained from a specified amount of medicinal plant material using solvents.

Alcohol soluble extractive value determination

The five grams of air-dried powder drug were measured, added to 100 ml of ethanol, and shaken for 6 hours and permitted to stand for 18 hours. After 18 hours, the plant extract was filtered and 25 ml of filtrate were pipetted out. The obtained filtered sample was subsequently evaporated using water bath, and then kept in a hot air oven set at 105 °C for a duration of six hours. Once cooled, the weight of remaining residue was measured [12]. The percentage (%) of alcohol-soluble extractives was calculated for drug using the below mentioned formula:

$$\text{Alcohol soluble extractive value (\%)} = \frac{(\text{Weight of the extract})}{(25 \times \text{Weight of sample taken})} \times 100 \times 100$$

Water-soluble extractive

The powered stem of plant was air-dried and then measured, added to 100ml of chloroform water, and shaken for 6 hours. After allowing it to 18 hours, the mixture was filtered, and 25 ml of obtained filtrate pipetted out. The obtained filtered sample was subsequently evaporated using water bath, and then kept in a hot air oven set at 105 °C for six hours. Once cooled, the weight of remaining residue was measured [13]. The percentage (%) of water-soluble extractive matter was calculated using the below mentioned formula:

$$\text{Water soluble extractive (\%)} = \frac{(\text{Weight of the extract})}{(25 \times \text{Weight of sample taken})} \times 100 \times 100$$

Determination of Moisture Contents

Moisture contents indicates the loss of weight due to water and volatile matter under specific conditions. A 2 g air-dried powder drug was accurately weighed, heated in an oven at 105 ºC for 5 hours, and then cooled in a desiccator before being weighed again until a stable weight was achieved. (14) Moisture contents was determined using the following formula:

$$\text{Percentage of Loss on drying at 105 °C} = \frac{(\text{Loss in weight of the sample})}{(\text{Weight of sample taken})} \times 100$$

Determination of pH Value

The pH of a solution / aqueous liquid is measured with a glass electrode attached with a pH meter. The coarse powdered herbal drugs are incorporated into 100 ml of water followed by filtered, and pH is assessed at 25 °C. The pH value was expressed as the reciprocal of hydrogen ion concentration [15].

Preparation of Ethanolic Extract of Dried Stem of *Balanites roxburghii* (EEBR)

The dried stem of *Balanites roxburghii* was powdered, boiled, filtered, and centrifuged to extract a concentrated solution. The supernatant was collected and concentrated, resulting in a volume one-fourth of the original volume. The solution was autoclaved at 121 °C and 15 lbs pressure, and stored at 4 °C. For solvent extraction, the air-dried powder was placed in ethanol, plugged with cotton wool, and shaken at 190-220 rpm for 24 hours. The supernatant was collected, and the solvent was evaporated to create the final volume, which was stored at 4 °C in an airtight bottle.

Qualitative estimation of the Phytochemical of the Extract

The analyses performed were aimed at determining the presence of carbohydrates, proteins, alkaloids, flavonoids, glycosides, saponins, tannins, and essential oils in extracts, following standards methodologies [16].

Quantitative Estimation of Phytoconstituents

Total Phenolic Content

Total phenolic contents of plant's extract was measured using spectrometry [17]. Folin-Ciocalteu's reagent was mixed to a sample, tannic acid (10-100 µg/ml), sodium carbonate (75 g/l), and distilled water. The mixture was stirred for two hours at room temperature (25-30 ˚C), followed by centrifuged at 2000 rotation per minutes for five minutes. The absorbance was measured at a wavelength of 760 nanometer, and a standard curve was created using various concentration of tannic acid. Results were stated as mg of tannic acid equals per gram of extract.

Total Flavonoids Content

The total flavonoid content of extracts may be assessed by using a aluminum chloride colorimetric assay [18]. The sample or standard quercetin solution was mixed to a 10 ml distilled water contained in volumetric. Afterward, 5% $NaNO_2$, 10% $AlCl_3$, and 1 M of NaOH were introduced. The solution was combined, and the absorbance was recorded at a wavelength of 510 nm. The total flavonoid content was represented as milligrams of quercetin equivalents for each gram of the extract [19].

3. Results

Organoleptic Characters

The *Balanites roxburghii* is spiny tree or shrubs and its height is ranging from 3m to 5m. Spines of plants are unbranched but rarely branched.

Stem: The fresh stem or twig of *Balanites roxburghii* is small spiny green in colour have branches and each branch contain hard and ascending spines and two obovate or elliptical bifoliate leaves. The immature stem of this plant is odorless and bitter in taste. The mature stem of this plant is yellowish colour with rough wrinkles, odorless and bland or astringent taste, contain less spines as compared to immature stems.

Fig 3: Immature/young stems of *Balanites roxburghii* Planch

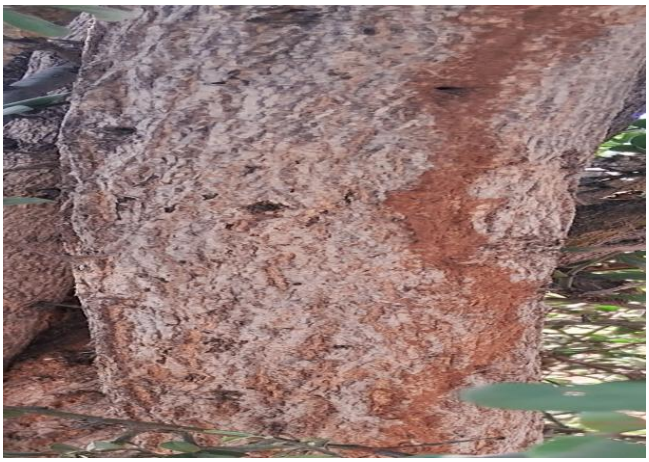

Fig 4: Mature stem of *Balanites roxburghii* Planch

Microscopic Characters

Transverse section of stem of a *Balanites roxburghii* is made up of the following parts when observed under microscope:

- **Epidermis**: Outer most layer of section consists of anticlinal cutinized epidermal cells and unicellular, non-glandular, straight, reversed comma shaped trichome. parenchymatous cells is the main part of the section. It is rectangular or tubular, thickened one cell arranged compactly and don't have intercellular spaces.
- **Hypodermis**: Hypodermis is underneath part of the epidermis which contains 3-4 rows of lamellar collenchyma identified by the presence of tannins. Cells are compactly arranged and don't have any cellular space.

- **Cortex**: It is just below region of hypodermis and made up of 5-10 rows of parenchyma. Cells present in cortex are spherical or isodiametric or oval in shape. Schizogenous glands is present in the cortex region.
- **Pith**: It is the innermost and major part of section, characteristic feature of dicot stem and contains parenchymatous loose cells. This section contains vascular bundles (which consists of xylem, phloem and vascular cambium), as well as medullary rays, medulla (medulla is the centre part of the pith) and pericycle.

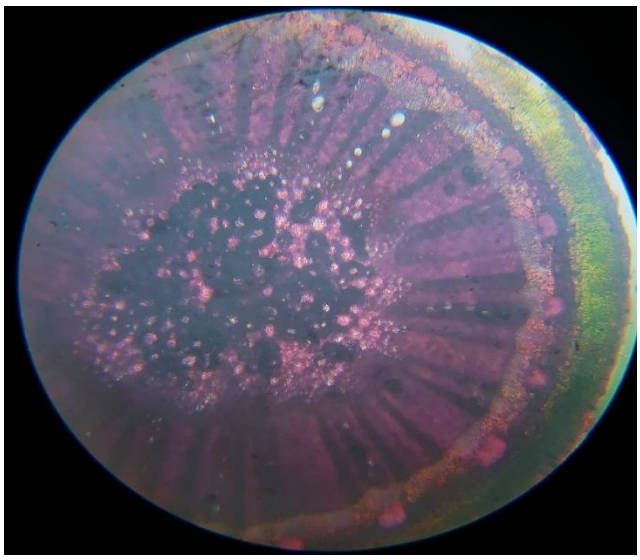

Fig 5: Transverse section of *Balanites roxburghii* stem observed under compound microscope.

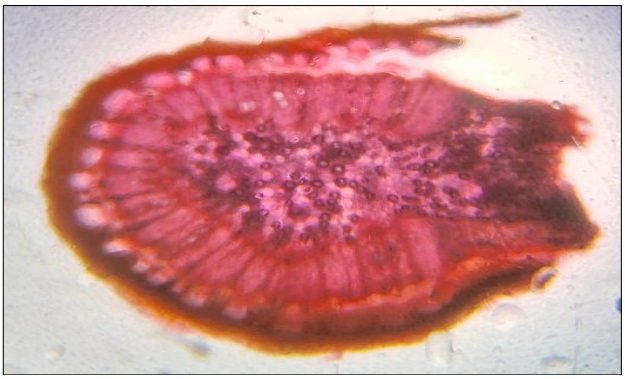

Fig 6: Transverse section of *Balanites roxburghii* stem observed under digital microscope (QUASMO Microscope).

Determination of Physico-Chemical Parameters

The Physico-chemical properties of the crude drug, includes evaluating the level of ash, extractive value, moisture content and pH, align with pharmacopoeial standard and previous reports, indicating the drug meets all prescribed parameters and suggests appropriate quality of drugs. (Table 1).

Table 1: Physico-Chemical Properties of *Balanites roxburghii*'s stem

S. No.	Standardization Parameters	Results
1	Total Ash content	9.451±0.05623
2	Acid insoluble ash	5.909±0.02866
3	Alcohol Soluble Extractive Value	18.980±0.54543
4	Water Soluble Extractive Value	31.446±0.45002
5	Loss on Drying	13.059±0.14158
6	pH value	8.0130±0.06028

Phytochemical Evaluation of the Ethanolic Extract of Dried Stem of *Balanites roxburghii* (EEBR)

The analysis of the plant extracts revealed that they contained different alkaloids, proteins, amino acids, saponins, flavonoids and phenolic compounds. However, glycosides, sterols, carbohydrates, and volatile oils were found to be absent. The quantitative analysis of phyto-chemical constituents including total flavonoids, total phenolics and saponins, indicated that EEBR was particularly high in total flavonoids measured 195.09±1.132 mg/g expressed as quercetin equivalents. However, the total phenolic content of EEBR was lower, measured as 45.13±1.104 mg/g expressed as tannic acid equivalents. (Table 2).

Table 2: Quantitative Estimation of Phytoconstituents

S. No.	Standardization Parameters	Results
1	Total Flavonoids Content	195.09±1.132 mg/g quercetin equivalents of EEBR
2	Total Phenolic Content	45.13±1.104 mg/g tannic acid equivalents of EEBR

4. Discussions

Pharmacognostical standardization of medicinal plants is crucial for ensuring their safety, efficacy, and quality. *Balanites roxburghii* Planch, a significant plant in traditional medicine, has garnered attention for its therapeutic potential. This plant is commonly found in several regions of Asia and Africa and is utilized in treating various ailments such as inflammation, infections, and gastrointestinal disorders. The stem of *Balanites roxburghii* is particularly noteworthy due to its rich phytochemical profile, which warrants a thorough pharmacognostical evaluation. The first step in the standardization process involves morphological and anatomical studies of the stem. Detailed examination using microscopy can reveal critical features such as vessel arrangement, fiber types, and cellular structures that contribute to the plant's medicinal properties. Additionally, organoleptic evaluations provide insights into the sensory attributes like color, texture, and taste that may influence therapeutic applications. Such foundational knowledge forms the basis for identifying authentic samples versus adulterants or substitutes. Following anatomical studies, phytochemical analysis becomes essential to determine the presence of bioactive compounds within the stem. Establishing a profile of these compounds not only aids in validation but also enhances understanding of their pharmacological mechanisms. Furthermore, this information can be pivotal for regulatory compliance and quality control measures in herbal medicine manufacturing.

5. Conclusions

In conclusion, the Pharmacognostical standardization of *Balanites roxburghii*'s stem plays a vital role in promoting its safe use within traditional healing practices and modern therapeutics alike. By combining morphological assessments with phytochemical profiling, researchers can establish comprehensive standards that ensure both efficacy and safety for consumers while preserving traditional knowledge systems.

6. Conflict of Interest

None

7. Acknowledgements: We would like to acknowledge the contributions of Dr. Rajshree Dahiya, Mr. Bhuvanesh Baniya, Ritika Dadhich, Assistant Professor, J. N. V. University, Jodhpur (India) for their expertise and efforts in reviewing the literature and writing this article."

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