Pharmacognostic evaluation, physico-chemical and phytochemical analysis of leaves of Badara i.e Ziziphus jujuba Lam

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Abstract
Objective: The present of the study is to evaluate preliminary Pharmacognostic study, Phytochemical constituents and Physicochemical analysis of Drug.

Method: The present paper deals with the estimation of the fresh, powdered and anatomical sections of the leaves of Ziziphus jujuba Lam. - Badara to establish Macromorphological, Micromorphological, Chemomicroscopic, Phytochemical analysis of Drug tests were Loss on drying, Total ash, Acid insoluble Ash, Water soluble extract and Alcohol soluble extract value of Ziziphus jujuba Lam. and Phytochemical constituents tested were Alkaloids, Carbohydrates, Saponins, Aminoacids, Starch, Steroids, Xanthoproteins, Sugars, Proteins, Tannins, Glycosides and pH.

Result: Macro and Microscopical studies indicated presence of simple leaves, alternate, ovate or oblong elliptic, apex round, 3 depressed longitudinal veins at the base, margin slightly dentate, glossay on upper side and pubescent on lower side, venation reticulate, petiole short, Epidermis, Mesophyll and Vascular Bundle. Chemomicroscopic characters include trichomes, rosette and prismatic crystals of calcium oxalate parenchyma cells containing rosette crystals of calcium oxalate few simple oval to spherical starch grains Fragments of Xylem elements, lignified xylem fibres in bundles. Physico-chemical parameters such as Loss on drying (6.48% w/w), Total ash (7.53% w/w), Acid insoluble Ash (0.69% w/w), Water soluble extract (15.77% w/w) and Alcohol soluble extract (15.54%) of Ziziphus jujuba Lam. - Badara. Phytochemical evaluation have been performed which clearly reveals the presence of Alkaloids, Carbohydrates, Sugars, Proteins, Tannins, Glycosides, with pH is 5.31. The results of the study possibly will subsist positive observations in several diagnostic indices designed for the discovery and research of a monograph of the plant.

Conclusion: These studies of Badara leaves are helpful to ensure the Quality, Identity, Purity, Authenticity and Efficacy of the drug and useful for future research.

Keywords: Ziziphus jujuba, pharmacognosy, phytochemical and physico-chemical

Introduction
Various species of Ziziphus are used medicinally in India, China and Japan. The plant Badara - Ziziphus jujuba Lam. is also known as Ber, Jujube. It is taxonomically belongs to the family Rhamnaceae. The leaves used for Hypoglycemc effects, Sweetness inhibitors, Diuretic, Emollient, Expectorant, to promote hair growth, Anticancer, Sedative, Blood purifier and in treatment of Diarrhoea. Chemically Badara - Ziziphus jujuba Lam. contains Flavanoids, Saponins, Tannins, Vitamin A, Vitamin B, Sugars, Calcium, Phosphate and Iron. Leaves of Badara - Ziziphus jujuba Lam. were used as a folklore medicine to treat children suffering from Typhoid fever, Furuncle and Ecthyma in China. Major chemical constituents of Badara - Ziziphus jujuba Lam. leaves are (-)-Catechin, Quercetin-3-O-robinobioside, Rutin. Badara - Ziziphus jujuba Lam. leaves are an ecofriendly green inhibitors of aluminium corrosion in an NaOH solution. Oils extracted from different Badara - Ziziphus jujuba Lam. organs (Pulp, Leaves and Seeds) seem to be rich in Fatty acids, Sterols and Triterpenes. Triterpenes have Anti-inflammatory, Anti-microbial and Anti-oxidant effects. The present study was done to evaluate the Pharmacognostic, Physico-chemical and Phytochemical analysis of Leaves of Badara - Ziziphus jujuba Lam.

Material and methods
Plant material
Fresh leaves of Badara - Ziziphus jujuba Lam., Rhamnaceae were collected from the Tirupati. For the present study Fresh leaves used for Macro and Microscopic studies and Dried leaf powder used for powder analysis were done at the P.G. Department of Dravyaguna, S.V. Ayurvedic college, Tirupati.
**Images of Badara (Ziziphus jujuba Lam.)**

Image 1: Badara Plant

Image 2: Badara Patra

Image 3: Badara Patra cuna

**Macroscopy**

The following macroscopic characters for the fresh leaves were noted: presence of simple leaves, alternate, ovate or oblong elliptic, apex round, 3 depressed longitudinal veins at the base, margin slightly dentate, glossy on upper side and pubescent on lower side, venation reticulate, petiole short.

**Organoleptic Properties**

- **Size:** Length: 2 to 3.5 cm  
  Width: 1.5 to 3 cm
- **Shape:** Ovate or oblong and elliptic
- **Colour:** Pale Green to grey Green
- **Odour:** Not specific
- **Taste:** Mucilaginous and slightly Bitter

**Microscopy**

**Transverse section of Leaf**

Transverse Section of Leaf is done by Free hand Section cutting and Simple staining procedure and findings are as mentioned below.

- **A. Epidermis**
  - Leaf on either sides covered with Epidermal layers i.e Upper Epidermis and Lower Epidermis.
  - Epidermal Layers composed of a single row of tangentially elongated barrel shaped cells.
  - Externally both epidermal layers are covered by a thick cuticle.
  - Lower epidermal layer externally covered with several elongated multicellular, uni -seriate covering trichomes which make the lower surface pubescent.
  - On the lower epidermal layer few sunken stomata are present here and there.

- **B. Mesophyll**
  - In between upper and Lower epidermal layers mesophyll region is present.
  - Mesophyll is distinguished in to 1 to 3 layers of Palisade parenchyma and 2 to 4 layers of spongy parenchyma.
  - Palisade parenchyma cells are arranged compactly without any intercellular spaces and filled with dense chlorophyll pigment.
  - Cells of spongy parenchyma are oval to circular in shape, loosely arranged with intercellular spaces and filled with chlorophyll pigment comparatively less than palisade parenchyma.
  - At the region of midrib the upper epidermis is followed by 4 to 6 layers of collenchymatous hypodermis.
  - Below the vascular bundle towards lower epidermis several layers of polyhedral thin walled, compactly arranged parenchymatous ground tissue is present. In this region large mucilage secreting cavities are present.

- **C. Vascular Bundle**
  - The midrib appear in plano convex shape with flat adaxial surface.
  - In the midrib region Upper epidermis is followed by 4 to 6 layered collenchymatous hypodermis.

**Powder Analysis:**

- **Organoleptic Properties**
  - **Colour:** Brownish dark green
  - **Odour:** Not specific
  - **Taste:** Slightly bitter and mucilagenous
  - **Texture:** Fine Powder

**Microscopic characters**

Numerous elongated, multi cellular, uni-seriate covering trichomes fragments of wavy thin walled epidermal cells in surface view rosette and prismatic crystals of calcium oxalate, parenchyma cells containing rosette crystals of calcium oxalate and few simple oval to spherical starch grains Fragments of Xylem elements, lignified xylem fibres in bundles.
Microscopic study of badara patra

Image 4: T.S of Leaf

Image 5: T.S of Leaf (Midrib region)

Image 6: T.S of Leaf – (Lamina)
Ziziphus jujuba Lam.

Image 7: Leaf powder analysis
Physico-chemical parameters
The various Physico-chemical parameters such as Loss on drying, Total ash, Acid insoluble Ash, Water soluble extract and Alcohol soluble extract.

Aims and Objectives: The Badara (Ziziphus jujuba) leaves is subjected to study Identity, Purity and Strength.

Loss on drying at 105 °C/Moisture content
10 gm of trial drug samples are placed after accurately weighing it in a tarred evaporating dish. After placing the above said amount of sample in a tarred evaporating dish is dried at 105 °C for 5 hours and it is weighed. After drying tarred evaporating dish was allowed to cool in desiccators for 30 minutes and then weighed the remnant material.

\[
\text{Difference in weight after heating} = \frac{\text{Weight of sample taken} - \text{Weight of sample after heating}}{\text{Weight of sample taken}} \times 100
\]

Determination of Ash
About 2.0g of powdered drugs was weighed and placed in three separate previously ignited and tarred silica crucibles. The samples were spread evenly and then ignite or incinerate it to a constant temperature not exceeding 450 °C until it is white indicating the absence of carbon. The crucible then cooled in desiccators and final weighed. The results were then calculated the content of total ash in terms of percentage w/w of the air-dried drug.

Determination of Extractable Matter in Water and Alcohol
About 4.0g of finely powdered air dried samples, was accurately weighed in three glass stoppered conical flask and macerated with 100ml of the solvent (Water, Methanol, Ethanol, Hydro alcoholic, Ethyl acetate, Chloroform, Benzene and Hexane) specified for the plant material concerned for 6 hours, shaking frequently and then allowed to stand for 18 hours. Filtering was done by what man paper, taking care not to lose any solvent, and then transfer 25 ml of filtrate to tarred flat bottomed shallow dish. The extracted matter was dried at 105 °C for 6 hours, cooled in a desiccators for 30 minutes and then weighed. The percentage extractable matter was calculated.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying</td>
<td>6.48% w/w</td>
</tr>
<tr>
<td>Total ash</td>
<td>7.53% w/w</td>
</tr>
<tr>
<td>Acid insoluble Ash</td>
<td>0.69% w/w</td>
</tr>
<tr>
<td>Water soluble extract</td>
<td>15.77% w/w</td>
</tr>
<tr>
<td>Alcohol soluble extract</td>
<td>15.54% w/w</td>
</tr>
</tbody>
</table>

Table 1: Results of identity, purity and strength
It shows that the Total ash (7.53%w/w) would be because of presence of Calcium oxalate, chemical constituents of both Water soluble extract and Alcohol soluble extract, but near to Water soluble extract (15.77%).

Preliminary phytochemical study
Aims and Objectives: The leaves of Badara is subjected to Preliminary Phytochemical screening for the detection of various chemical constituents present in it.

Test for Alkaloids
Mayer's Test: To 1 ml of the extract, 3 ml of Mayer's reagent was added, the formation of full white precipitate confirmed the presence of alkaloids.

Test for Carbohydrates
Molisch Test: To 2 ml of the extract, 1 ml of α-naphthol solution and concentrated sulphuric acid through the sides of test tube were added. Purple or reddish violet colour at the junction of the two liquids revealed the presence of carbohydrates.

Test for Saponins
Saponin Test: About 1 ml of methanol extract was diluted separately with distilled water to 20 ml, and shaken in a graduated cylinder for 15 minutes. 1cm layer of foam indicated the presence of saponins.

Test for Tannins
To 1 ml of the extract, ferric chloride was added, formation of a dark blue or greenish black colour product showed the presence of tannin.
Tests for Proteins
Lead Acetate Test: To the extract, 1ml of lead acetate solution is added. Formation of a white precipitate indicated the presence of proteins.

Tests for Aminoacids
Lead Acetate Test: To the extract, 1ml of lead acetate solution is added. Formation of a white precipitate indicated the presence of proteins.

Tests for Starch
Iodine test: Mix 3 ml of test solution and few drops of dilute iodine solution. Blue colour appears, it disappears on boiling and reappears on cooling.

Tests for Steroids
Salkowski Test: Dissolve the extract in chloroform and equal volume of concentrated sulphuric acid. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer represented the steroid components in the tested extract.

Tests for Glycosides
Keller Killiani Test: The extract was dissolved in acetic acid containing traces of ferric chloride and it was then transferred to a test tube containing sulphuric acid. At the junction, formation of a reddish brown colour, which gradually became blue, confirmed the presence of glycosides.

Tests for Sugars
Benedict’s test: To 5ml of Benedict’s reagent, 1ml of extract solution was added and boiled for 2 minutes and cooled. Formation of a red precipitate showed the presence of carbohydrates.

Tests for Xanthoprotein
Xanthoprotein Test: To 1 ml of the extract, 1ml of concentrated nitric acid was added. A white precipitate is formed, it is boiled and cooled. 20% of sodium hydroxide or ammonia is subsequently added; orange colour is indicated the presence of aromatic amino acids.

<table>
<thead>
<tr>
<th>No.</th>
<th>Phytochemicals</th>
<th>Tests</th>
<th>Badara Patra Cūrṇa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>Mayer’s Test</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Carbohydrates</td>
<td>Molish reagent</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Saponins</td>
<td>Saponin Test</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Tannins</td>
<td>Ferric chloride</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Proteins</td>
<td>Lead Acetate acid</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Aminoacids</td>
<td>Lead Acetate acid</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Starch</td>
<td>Iodine Test</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Steroids</td>
<td>Salkowski Test</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Glycosides</td>
<td>Keller Killiani Test</td>
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</tr>
<tr>
<td>10.</td>
<td>Sugars</td>
<td>Benedict’s Test</td>
<td>+</td>
</tr>
<tr>
<td>11.</td>
<td>Xanthoproteins</td>
<td>Xanthoprotein Test</td>
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<tr>
<td>IX</td>
<td>Acid test (pH)</td>
<td></td>
<td>5.31</td>
</tr>
</tbody>
</table>

Conclusion
Leaves of Badara - Ziziphus jujuba Lam. is subjected to Pharmacognostic, Physicochemical and Preliminary phytochemical analysis. The data generated is helpful in determining the Quality and Purity of the Drug, especially in crude form. The extractive values are being useful for the further extraction of Phytoconstituents from the plant. Macro and Microscopical studies indicated presence of simple leaves, alternate, ovate or oblong elliptic, 3 depressed longitudinal veins at the base, Epidermis, Mesophyll, Vascular Bundle, trichomes, rosette and prismatic crystals of calcium oxalate parenchyma cells. Physico-chemical parameters such as Loss on drying (6.48% w/w), Total ash (7.53% w/w), Acid insoluble Ash (0.69% w/w), Water soluble extract (15.77% w/w) and Alcohol soluble extract (15.54%) of Ziziphus jujuba Lam. which will be very useful for future research. The Phytochemical evaluation clearly reveal the occurrence of the extract of Leaves was found to contain Alkaloids, Carbohydrates, Sugars, Proteins, Tannins, Glycosides, with pH is 5.31, showing it as Mild acidic.

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References