Pharmacognostical study of Ankola seed

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Abstract

Ankola (Alangium lamarckii. Thwaites) an ancient plant described in Vedas, Samhitas and in various nighantus is a very important medicinal plant. Ethnobotanically it is used in many diseases viz. wound healing, diabetes, skin diseases, constipation and various other diseases. It is described in Susruta Samhita in vrana chikitsa for removing the slough of wound. So here we are using it for treatment of vrana and so we are standardizing it for its identity, purity and to prove therapeutic action as well. Pharmacognosy is the study of medicines derived from natural sources. Under this pharmacognosy, pharmacology and phytochemistry are necessary for authentication of crude drug. Research work was carried out to find the phytochemical constituents and its wound healing property. For this macroscopic and microscopic study by microtome process done. Dried seeds of Ankola were taken and then grinded to make fine powder of it, later its petroleum ether extract made in Soxhlet apparatus.

Keywords: wound healing, pharmacognosy, ethnobotanical

Introduction

Pharmacognosy is the study of identification of drugs derived from natural sources. The American Society of Pharmacognosy defines pharmacognosy as “the study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources.” The concept of standardization and quality control of drug can be found in ancient Ayurvedic texts. Assessment of complete and accurate physicochemical value of Ayurvedic herbs not only provides scientific basis of its quality but also helps in globalization of Ayurveda. Under these circumstances, pharmacognosy, pharmacology and phytochemistry are necessary for authentication of crude drug and to prove therapeutic action as well.

Ankola (Alangium lamarckii Thwaites.) is well known plant in Ayurveda. It has been mentioned in Brihatrayi and later it has been described in Nighantu. So a preliminary phytochemical study on Ankola was done\(^1\).

This portion deals with-
Standardization of drug:
1. Macroscopic study
2. Microscopic study
3. Ash Value
4. Extractive value
5. Loss on drying
6. TLC Profile

Materials and Methodology

Plant material: Seeds of Ankola were collected from local area of Ramna near Varnasi U.P.
1. Macroscopic study of seed was done.
2. Microscopic study of seed done by microtome method.
4. Determination of identity, purity and strength by -
   a. Loss on drying at 105 °C/moisture content.
   b. Determination of Total Ash
   c. Determination of Acid Insoluble Ash
   d. Determination of Extractable Matter in water & petroleum ether.

Result and Discussion

Macroscopic study
Seed usually one in a fruit, rounded or elliptic 1- 1.5 cm long and 0.5-1 cm broad, slightly wrinkled with brownish testa. Shiny in nature. In each seed there are two cotyledons, white in colour.
Microscopic study of seed
Microtoming is a method to obtain fine and thin sections of plant tissue ranging from 5-12 μm. It is a multistep process as described below:

Specimen preparation for hard tissue
For hard tissue such as fruit, seed, bark etc. put it in water for 2-3 days so that the tissue get soften. Wash the sample and cut it from sharp knife as per required size and thickness. Seed if small taken as whole.

Fixation
Fix the tissue in different reagents as follows:

- Formaldehyde + Glacial Acetic Acid in 1:1 ratio for 24 hrs.
- 50% alcohol for 24 hrs
- Safranin (stain) 24 hrs
- Blot the tissue on blotting paper and remove extra stain from tissue
- 90% alcohol for 24 hrs
- Absolute alcohol for 24 hrs Solution of Absolute alcohol and Chloroform in which Absolute alcohol is 2/3 part and Chloroform is 1/3 part for 24 hrs
- Solution of Absolute alcohol and Chloroform in which Absolute alcohol is 1/3 part and Chloroform is 2/3 part for 24 hrs
- Chloroform I for 24 hrs
- Chloroform II for 24 hrs

Block Preparation
Put the tissue in melted wax (62 °C) watch effervescence. Do three changes of wax upto coming of effervescence stops. Then put the tissue in fresh wax for 2-3 hrs and make the block. Then stream the block and fix it on wooden block. Then cool it for section cutting.

Section Cutting
Requirements – Sharp knife (Microtome knife), Slides, Brush, Pin, Hot plate, Egg albumin.
Put the egg albumin on slides, make a thin layer. Then section is cut of required thickness from 5 μ to 12 μ. Then put slides immediately on pre heated hot plate so that section get fixed on the slides firmly.

Staining
Slides pre stained so that it could be easily get differentiated under microscope. Slides are put in square coupling jar and put xylene in it and put it in pre heated oven (62°C) to remove the wax completely.

- Keep in Absolute alcohol for 30 min.
- 95% alcohol for 30 min.
- 70% alcohol for 30 min.
- 50 % alcohol for 30 min.
- Wash in double distilled water for 2 changes
- Safranin for 1 hr.
- Wash in double distilled water.
- 30% alcohol for 30 min.
- 50% alcohol for 30 min.
- 70% alcohol for 30 min.
- 95% alcohol for 30 min.
- Fast green for 1 hr.
- Absolute alcohol I for 30 min.
- Absolute alcohol II for 30 min.
- Absolute alcohol III for 30 min.
- Xylene I for 30 min.
- Xylene II for 30 min.
- Xylene III for 30 min.
- Slides are dried in air along with jar
- Mount the slides in DPX.
- Observe under microscope.
Transverse section of seeds shows, testa composed of a layer of palisade like outer epidermis, a layer of large, thin walled somewhat rectangular cells, 2-4 layers of thick-walled parenchyma cells. A few layers of spongy parenchyma having large intercellular space. Cotyledons composed of outer layer of epidermis with cylindrical cells, externally covered with thin cuticle, embryo present in it, filled with oil globules and starch grains in this region.

![Fig 3: Transverse Section of Ankola seed](image)

**Powder study of seed**

Coarse powder of light yellow colour through mustard to brown, coarse and free-flowing; bitter in taste. In microscopic powder study it shows vascular bundles of light pink to brown in colour. Aleurone grains of green colour, shining transparent white crystals of calcium oxalate and oil globules of pinkish blue stain.

![Fig 4: Coarse powder of Ankola](image)

**Identity, purity and strength**

**Loss on drying at 105 °C/moisture content**

Place about 10 gm of drug sample after accurately weighing it in a tared evaporating dish. After placing the above said amount of sample in a tared evaporating dish dry at 105 °C for 5 hours and weigh. After drying tared evaporating dish cool in desiccator for 30 minutes and then weight taken.

\[
\text{The } \% \text{ of loss on drying} = \frac{\text{Difference in weight after heating} \times 100}{\text{Weight of sample taken}}
\]
Determination of Ash
Determination of Total Ash
About 2.0g of powdered drug was incinerated in a tared silica dish at a temperature not exceeding 450 °C until free carbon was left, cooled and final weight was taken. The percentage of ash calculated with reference to the air-dried drug (PASF, 1987).

Determination of acid insoluble ash
The ash obtained as above method was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and collected the insoluble matter on an ash-less filter paper, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash with reference to the air dried drug was calculated (PASF, 1987).

Table 1: Identity, purity and strength

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Result</th>
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<tbody>
<tr>
<td>1.</td>
<td>Foreign matter (w/w %)</td>
<td>1.2%</td>
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<tr>
<td>2.</td>
<td>Moisture content (w/w %)</td>
<td>6.26%</td>
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<td>3.</td>
<td>Total ash (w/w %)</td>
<td>4.35%</td>
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<td>4.</td>
<td>Acid insoluble ash (w/w %)</td>
<td>1.75%</td>
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<td>5.</td>
<td>Petroleum ether soluble extractive (w/w %)</td>
<td>22.5%</td>
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<tr>
<td>6.</td>
<td>Water soluble extractive</td>
<td>34%</td>
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Conclusion
Ankola (*Alangium lamarkii. Thwaites*) is very important medicinal plant with various therapeutic applications. In Pharmacognostical study, in macroscopic study it was seen that seed usually one in a fruit, rounded or elliptic in shape, slightly wrinkled with brownish testa. Shiny in nature. Each seed contains two cotyledons, white in colour.

Microscopic study reveals testa composed of a layer of palisade like outer epidermis, parenchyma cells. A few layers of spongy parenchyma. Powder study shows the presence of Aleurone grains, vascular bundle, oil globules and calcium oxalate crystals. In powder foreign matter 1.2%, moisture content 6.26%, total ash 4.35%,acid insoluble ash 1.75%, petroleum ether soluble extractive 22.5% & water soluble extractive 34%.

References
1. Ayurveda and wound healing by K.N. Dwivedi, Management of wound Healing by Prof. V.K. Shukla.

Determination of extractable matter in water & petroleum ether
About 4.0g of coarsely powdered air dried material, was accurately weighed in a glass stoppered conical flask and macerated with 100ml of the solvent specified for the plant material concerned for 6 hours, shaking frequently, then allowed to stand for 18 hours. Filtered rapidly, taking care not to loose any solvent. The extracted matter was dried at 105 °C for 6 hours, cooled in a dessicator for 30 minutes and then weighed. The percentage extractable matter was calculated.