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# Pharmacognostic and physico chemical study of stem bark of *Azadirachta Indica*

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**Abstract**
**Background:** The objective of the research was to examine the pharmacognostic and phytochemical characteristics of *Azadirachta Indica* stem bark.
**Materials and Methods:** The study includes Macroscopic, Microscopic, Powder study, Histochemical, Preliminary Phytochemical and Physicochemical evaluation of stem bark of *Azadirachta Indica.*
**Results and Discussion:** The transverse section revealed 18-25 brown colored tangentially elongated and thick-walled cork cells. The cortex region shows 2-3 layers of collenchymatous cell followed by 15-20 rows of thin-walled parenchymatous cell. Lignified pericyclic region. Phloem region contains phloem parenchyma with clusters and prismatic crystals of calcium oxalate and tannins with transversely arranged tri or penta serrate medullary. Powder microscopic analysis showed the presence of suberized thin-walled cork cells, cortical parenchymatous cell and phloem parenchymatous cell with starch grains, parenchyma cells containing prismatic crystals of calcium oxalate. Physico chemical analysis was done by the parameters mentioned in Ayurveda Pharmacopeia of India.
**Conclusion:** Pharmacognostic and phytochemical evaluation can be used as a tool for identifying and confirming the authenticity and quality of *Azadirachta Indica* stem bark. This study helps to provide diagnostic tool for identification of stem bark of *Azadirachta Indica*.

**Keywords:** *Azadirachta Indica* stem bark, Neem bark, nimbin, nimbinin, Pharmacognostic, Phytochemical

## Introduction
Plants have been administered as safe remedies for an array of ailments since the dawn of civilization [1]. Globally plant research has substantially gained recently, and numerous indicators demonstrate to the enormous potential of medicinal plants in various traditional medical practice [2]. It's necessary to assess and standardize the plants used in the formulations by using array of pharmacognostic and phytochemical evaluation tools, such as macros copy, microscopy, powder microscopy, histochemical, phytochemical screening, and physicochemical evaluation of crude medicinal plants [3].
*Azadirachta Indica* belongs to the family Meliacea commonly known as Indian Margosa. It is native to Indian Subcontinent and distributed in the dry forests of Sri Lanka, Thailand, Pakistan, Malaysia, and Indonesia, among other countries in South and Southeast Asia. From Kerala's southernmost point to the peaks of the Himalayas, it is widely grown. Further, neem is commonly found in the tropical regions of Asia, Fiji, Mauritius, northern Australia, Africa, Puerto Rico, the Caribbean, and other South and Central America.
The stem bark contains the compounds nimbin, nimbinin, nimbidin, nimsterol, nimbinone, nimbidiol, margosine, a bitter substance, and 6-desacetyl nimbinene. Additionally, it includes nimbidiol, margosine, nimbicin, and nimbicin [4, 5].
The stem bark of *Azadirachta Indica* is reported to be effective Anti-bacterial, Antioxidant, Anti-malarial, Anti-diabetic, Anti-viral. Anti-inflammatory, Anti-carcinogenic, Antisnake venom, Anti-allergic, Larvicidal, Anti-ulcer [5, 6].

## Materials and Methods
### Procurement of the plant
Barks of *Azadirachta Indica* were procured from Athachi farms and plantations Pvt. Ltd, Palakkad district of Kerala state with voucher specimen APP 101.

### Macroscopic and organoleptic evaluation
*Azadirachta Indica* bark was studied macroscopically for examining its size, shape, texture, colour, odour and taste. Macroscopic examination of crude drug was carried out by naked eye by placing the individual raw materials on a white paper surface and organoleptic characteristics like shape, size, colour, taste, fracture, odour was evaluated [7].

**Microscopic evaluation**
Free hand sections and microtome transverse sections of *Azadirachta Indica* bark was taken. Among the sections thin sections were selected and placed on the microscopic slide and stained with saffranine, mounted in glycerine [8].

**Powder Microscopic evaluation**
*Azadirachta Indica* bark was powdered and sufficient quantity of Powder was taken on a microscopic slide and it is treated with 1-2 drops of safranin. The samples were evenly spreaded over the slide, followed by mounting it in glycerine [9, 10].

**Histochemical evaluation**
*Azadirachta Indica* bark powder was evaluated for the presence of tannins, starch, aleurone grains, lignin and oil globules. Free hand sections and microtome sections of *Azadirachta Indica* bark were taken. Among the sections thin sections were identified and selected and placed on the microscopic slide and stained with specific reagents/stains to determine the specific secondary metabolite.
The sections taken were treated with specific reagents to determine its presence.

**1. Determination of the presence of starch grains:** The sections were stained with Iodine solution. The appearance of blue colour indicated the presence of starch grains in the crude drug.

**2. Determination of the presence of tannins:** The sections were stained with ferric chloride solution. The appearance of Blue or greyish black colour indicated the presence of starch grains in the crude drug.

**3. Determination of the presence of lignin:** The sections were treated with Phloroglucinol solution followed by the addition of one drop of Hydrochloric acid. The Presence of Lignified cells were indicated by the appearance of pink stain.

**4. Determination of the presence of Fixed oils:** The sections were stained with Sudan Red. Appearance of orange pink coloured indicated the presence of Fixed oils in the crude drug.

**Visualisation of Pharmacognostic characteristics:** The microscopic slides of Transverse Section and powder were covered with cover slip and were observed through Leica DM 1000 LED. Trinocular 'Leica' microscope.
The photos of transverse section, powders and histochemical analysis were taken with the help of 'Leica DFC 295' digital camera connected to the computer and Leica Application Software LAS Version 3.6.1 were used.

**Phytochemical evaluation**
Plant-based chemicals that have medicinal uses have been referred to as secondary metabolites. The preliminary phytochemical investigation will shed light on the chemical makeup of the plant. To find the presence of phytoconstituents including alkaloids, carbohydrates and glycosides, flavonoids, tannins, saponins, and terpenoids, a preliminary phytochemical investigation was conducted.

**Physico chemical evaluation**
The parameters employed to analyse dried *Azadirachta Indica* bark were performed as per the guidelines of Ayurveda pharmacopeia of India [11, 12].

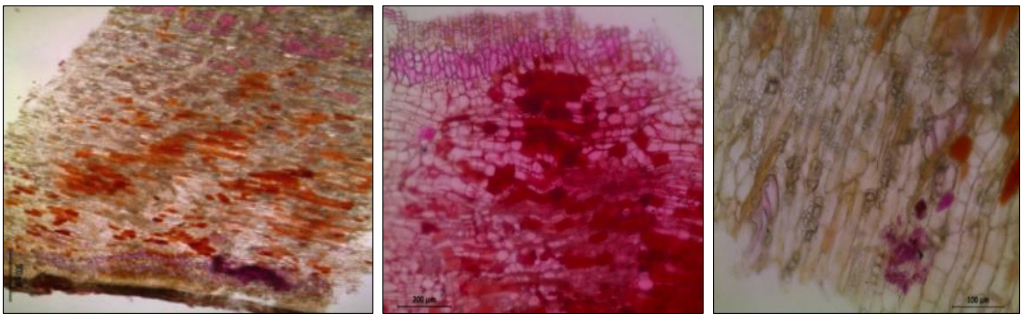
**Results and Discussions**
**Pharmacognostic Study**
Macroscopic and Organoleptic evaluation of Crude *Azadirachta Indica* bark Macroscopic and Organoleptic of crude turmeric sample are shown in Table 1.
The macroscopic examination is the qualitative approach for identifying herbs and it works as an evaluation tool for identifying herbal drugs.

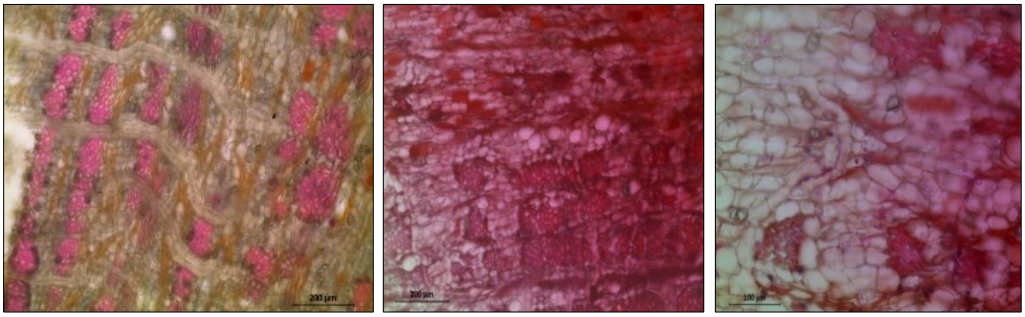
**Table 1:** Macroscopic and Organoleptic of crude *Azadirachta Indica* bark

| Parameters | Observations |
|---|---|
| Appearance | The stem bark is rough, hard, fissured and scaly in appearance. In the outer bark Longitudinal and transverse wrinkles were evident. |
| Colour | Outer bark with Rusty grey to brown whereas inner side was silvery brown |
| Odour | Characteristics |
| Taste | Astringent Bitter |
| Fracture | Fibrous |

**Microscopic evaluation of *Azadirachta Indica* bark**
The transverse section of the stem bark revealed following diagnostic characteristics (Fig 1 to Fig 6).

**Transverse section of *Azadirachta Indica stem bark***





Transverse section of *Azadirachta Indica* bark Showing Fig 1. Cork, Cortical and phloem region, Fig 2. Lignified cells. Fig 3. Crystals and Starch grains. Fig 4. Fibers with Medullary rays Fig 5. Lignified Phloem region with medullary rays Fig 6. Parenchymatous cell containing starch grains, prismatic crystals of calcium oxalate.

**Cork:** 18-25 brown coloured radially arranged, tangentially elongated cork cells with thick wall observed.

**Cortex:** Below cork cell 2-4 rows of collenchymatous cell were seen 15-20 rows of thin-walled parenchymatous secondary cortex were also observed below the collenchymatous layer.

**Pericycle Region:** Long lignified fibers, Lignified walls.

**Phloem:** In the phloem region, phloem parenchyma which contains clusters and prismatic crystals of calcium oxalate and tannins with transversely arranged tri or Penta serrate medullary rays were observed. Thin-walled parenchymatous cells, composed of sieve tubes and companion cells were also evident in the Starch grains are found dissipated in the areas of medullary rays, Cortex and Phloem region.

**Powder microscopy of stem bark of *Azadirachta Indica***

Following features were observed on surface view of powdered Azadirachta (Fig 7 to Fig 12).

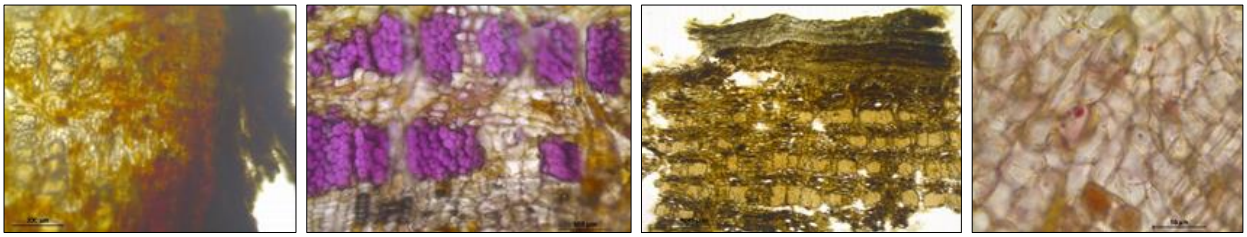
**Powder Microscopic characteristics**

Fig 7 Cork Cells in surface View Fig 8 Cortical Parenchyma with Starch Grains. Fig 9. Fragments of crystal fibres. Fig 10. Stone Cells as clusters. Fig 11.Starch Grains Fig 12. Prismatic Crystals of Calcium Oxalate.

- In the surface view moderately thin-walled fragment of cork cell which are suberized observed.
- Cortical parenchymatous cell with starch grains.
- Fragments of crystal fibres.
- Phloem parenchymatous cell with starch grains.
- Prismatic crystals and starch grains.
- Parenchyma cells containing prismatic crystals of calcium oxalate.
- Stone cells are found as clusters

**Histochemical evaluation of *Azadirachta Indica stem bark***

*Azadirachta Indica stem bark* sections were subjected to histochemical analysis to determine the presence of starch grains, tannins, Oil cells and Lignified cells (Fig 13 to Fig 16), (Table 2).



Histochemical analysis of Curcuma longa for testing the presence of Fig 13. Starch grains Present. Fig 14 Lignin Present Fig 15. Tannins Present Fig 16. Oil Cells Present.

**Table 2:** Histochemical characteristics of *Azadirachta Indica stem bark*

| | Treated with | Observations | Inference |
|---|---|---|---|
| Microtome and Free hand sections of *Azadirachta Indica stem bark* | Iodine solution | Bluish Black Colour | Starch grains detected |
| | Ferric Chloride solutions | Black/Greyish Black Colour | Tannins detected |
| | Phloroglucinol +Hydrochloric acid | Pink Colour | Lignified cell |
| | Sudan red | Orange Pink | Oil cell detected |

**Phytochemical investigation**

Phytochemical investigation of Alcoholic extract of *Azadirachta Indica stem bark.* Performed shows the presence of alkaloid, carbohydrate, glycosides, flavonoids, tannins, terpenoids and Saponins [13] (Table 3).

**Table 3:** Qualitative Phyto chemical screening of *Azadirachta Indica stem bark*

| Test Reagent | Observation | Result |
|---|---|---|
| **Alkaloids** | | |
| Dragendorff's reagent | Reddish brown ppt | + |
| Mayer's reagent | White/cream ppt | + |
| Hager's test | Yellow ppt | + |
| **Flavonoids** | | |
| Shinoda test | Magenta colour | ++ |
| 10% NaOH | Yellow colour | ++ |
| **Tannins** | | |
| 10% K2Cr2 O7 solution | Yellowish brown | ++ |
| 5% FeCl3 solution | Greenish black | + |
| **Anthraquinone Glycoside** | | |
| Bontrager's test | Pink Colour | - |
| **Saponins** | | |
| Froth test | Foaming | +++ |
| **Phenol** | | |
| Ferric Chloride Test | Red colour | +++ |
| **Terpenoids** | | |
| Salkowski's test. | Reddish brown | +++ |

+++ denotes Strong intensity reaction, ++ denotes medium intensity reaction, +denotes Weak intensity reaction, - denotes Not detected.

**Physico chemical analysis of stem bark of *Azadirachta Indica***

The stem bark of *Azadirachta Indica* subjected to arrays of physio chemical tests and the results are tabulated in Table 4.

**Table 4:** Physicochemical investigation of *Azadirachta Indica* stem bark

| Parameters | Observation |
|---|---|
| Foreign matter % w/w | 0.5% w/w |
| Total Ash % w/w | 3.5% w/w |
| Acid insoluble Ash % w/w | 1.1 w/w |
| Water Soluble Extractives % w/w | 19.1% w/w |
| Alcohol soluble extractives % w/w | 15.1 w/w |

Foreign matter is used to determine the percentage of other parts of plants apart from the part mentioned in the plant monograph. Ash value is used to determine the inorganic content present in the crude drug which comprises of silicates, carbonates and oxalates. The acid insoluble ash implies the silica present in the crude drug which indicates the contamination of crude drugs with earthy materials. Extractive values help to determines the chemical constituents present in the herbs and also helps in determination of particular bio constituents in specific solvent.

The macroscopic, Microscopic, histochemical, powder characteristics and Physico chemical evaluation of herbs serves as an important tools for identification and standardisation of herbs. In present study the plant diagnostic techniques helps in the identification and standardisation of Neem stem bark and it can be used as the standardisation parameter to identify the authentic Neem stem bark from its substitutes and adulterants. This could serve as in establishing data's on plant monograph of *Azadirachta Indica* stem bark (14.17).

**Conclusion**

The distinguishing characteristics mentioned in the study can be used as an authentication tool for the *Azadirachta Indica* stem bark. The utilisation of right quality raw materials helps to provide Quality finished formulations.

**Acknowledgement**

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