Pharmacognostical Standardization, Phytochemical Characteristics of Stem-bark of *Zanthoxylum alatum* Roxb

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ABSTRACT

**Background:** *Zanthoxylum alatum* Roxb (ZA) also known as ‘Indian Prickly Ash’ is a member of the Rutaceae family. It is native to the Himalayas, Jammu and Kashmir, Andhra Pradesh, and various other parts of India and is an important medicinal plant species. The stem bark of the plant is known to be a particularly rich source of medicinal compounds, and it is frequently utilized as an anti-diabetic, antioxidant, and anti-inflammatory agent. Despite the high therapeutic efficacy of bark, nothing is known about the requirements for its standardization. Since the quality control and standardization qualities of the product must be thoroughly documented, the stem bark of *Zanthoxylum alatum* Roxb was produced as a result of the current research, which may be seen here. As part of this research, the stem bark of *Z. alatum* was harvested, dried in the shade, and then pulverised. The establishment of pharmacognostical standards was accomplished via the use of techniques such as micro- and macroscopy, physicochemical parameters, extractive values, and fluorescence analysis.

**Results:** There were numerous distinguishing traits in the stem bark of *Z. alatum* Roxb that were discovered using macroscopic, microscopic, and physical-chemical criteria. **Conclusion:** This is the first research to provide a comprehensive pharmacognostic profile of the stem bark of *Z. alatum* Roxb, and it will be a helpful source of information in the development of pharmacognostic criteria for identification, purity, quality, and categorization of *Z. alatum* Roxb stem bark.

**Keywords:** Zanthoxylum alatum, Standardization, Indian Prickly Ash, Microscopical features, Phytochemical screening.

INTRODUCTION

The *Zanthoxylum alatum* Roxb. (Family: Rutaceae) is commonly known as Timru, toothache tree, Nepali Dhaniya. It is an armed scandent, erect shrub, small tree 6 m tall or more, with dense foliage and found in hot valleys of Himalayas.[1] According to Indian traditional System, the seeds and fruits are used in fever as an aromatic tonic, cholera and dyspepsia. The seeds possess antiseptic, disinfectant and deodorant potency and used in the treatment of toothache and scabies.[2] Various pharmacologically active phytoconstituents have been isolated from the different parts of *Z. alatum*. From the stem bark, wood and roots, various phytoconstituents like β-sitosterol, berberine, 8-hydroxycitramine, dictamine, epieudesmine, magnoflorine, eudesmine, xanthoplane, sikkimianine, γ-fagarine, armamatide, (+) sesamin, (-) sesamin, pluviatide, lupelo, and vanillic acid have been reported.[3,4] Traditionally the stem bark is used as an anti-inflammatory, carminative, stomachic and anthelminitic. It also exhibited antiproliferative activity against growth and multiplication of human keratinocytes, [5] and hepatoprotective activity.[6-7]

METHODS

**Plant Material Collection and Verification**

For the present study, collection of *Zanthoxylum alatum* Roxb bark was carried out from Tihri (Garhwal), Uttarakhand in the month of October – November (Figure 1). Authentication was done by Dr. H. B. Singh from department of Raw Material Herbarium and Museum, National Institute of sciences Communication and Information Resources, New Delhi under Ref. NISCAIR/ RHMD/Consult/-2009-10/1324/127. The stem-bark was powdered and stored in air tight containers for further use.

**Chemicals and Reagents**

All reagents and reagents were of analytical grade and procured from different companies. Ranbaxy (Ranbaxy Fine Chemicals), CDH (Central Drug House), QualiKems (QualiKems Fine Chemicals), Merck (Merck Ltd. Mumbai), S.D. Burgoyne, Qualigens (Qualigens Fine Chemicals, Mumbai) and Lobal Chemi, Sigma Aldrich.

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Macroscopical and Microscopical Evaluation

The bark of *Zanthoxylum alatum* Roxb was investigated using the procedures outlined in Trease and Evans Pharmacognosy. An extremely little quantity of powder was used for powdered drug microscopy, which was then stained in a 1:1 solution of strong hydrochloric acid in a watch glass using a phloroglucinol solution. For approximately 3 min, it was well combined and left to rest. Mounted in 50% Glycerin, the specimen was examined under a microscope. Also, a weak iodine solution was used to identify starch granules in the powder. Calcium oxalate crystals were detected using a powder treated with conc. H$_2$SO$_4$.

Extraction of Plant Material

The coarsely powdered drug (700 g) was defatted with dichloromethane for 72 hr via Soxhlet apparatus. After defatting the marc obtained was extracted with methanol (90%) for 72 via soxhlet apparatus. The extract obtained was concentrated under vacuum rotary evaporator. For aqueous extract, triple maceration process was carried out and the extract was concentrated under vacuum rotary evaporator. Yields were calculated on the basis of percentage w/w.

Fractionation of Aqueous Extract

The liquid-liquid extraction technique was used to prepare the various fractions. For the sake of clarity, 30 g of water-soluble extract were diluted in 70 mL of distilled water and filtered. Chloroform, ethyl acetate, and n-butanol were used to separate the prepared solution in a separating funnel. For now, the aqueous extract and various solvents were preserved in desiccators. *Zanthoxylum alatum* stem bark extracts were analysed for colour, consistency, and w/w yields (Table 1).

Table 1: The percentage yield of the extracts and fractions of *Zanthoxylum alatum* Roxb. stem-bark.

| Sl. No | Extract      | Method of extraction | Colour          | Consistency | Yield (%w/w) |
|-------|--------------|----------------------|-----------------|-------------|--------------|
| 1     | Dichloromethane | Soxhlet extraction   | Greenish black  | Semi–solid  | 7.40         |
| 2     | Methanol (90%) | Soxhlet extraction   | Reddish brown   | Semi–solid  | 13.50        |
| 3     | Aqueous      | Maceration           | Reddish brown   | Solid       | 19.20        |

| Sl. No | Fraction | Method of extraction | Colour          | Consistency | Yield (%w/w) |
|-------|----------|----------------------|-----------------|-------------|--------------|
| 1     | Chloroform | Liquid-liquid extraction | Greenish brown  | Semi–solid  | 3.75         |
| 2     | Ethyl acetate | Liquid-liquid extraction | Reddish brown   | Semi–solid  | 2.5          |
| 3     | n-butanol | Liquid-liquid extraction | Reddish brown   | Semi–solid  | 12.95        |
| 4     | Aqueous   | Liquid-liquid extraction | Dark Reddish brown | Solid       | 64.15        |

RESULTS

Macroscopic Features

**Colour:** dark brownish (Externally), golden brown (internally)

**Shape:** curved,

**Size:** 4-7 cm in length

**Taste:** Astringent

**Odour:** aromatic and pungent

**Texture:** Rough with prominent large circular prickles or circular depressions

**Fracture:** Short and fibrous

Microscopic Study

**Powder Microscopy:** Powder microscopic of stem-bark revealed the presence of polygonal to rectangular thick-walled cork cells, prismatic...
crystals of calcium oxalate, fibrous sclereids, fragments of crystal fibres, radially and longitudinally cut medullary rays crossing the crystal fibres and cortex cells (Figure 2).

**Physicochemical analysis of Stem barks of Zanthoxylum alatum Roxb.**

For the evaluation of Pharmacognostic parameters of stem bark of *Zanthoxylum alatum* Roxb, the proximate analysis was used as the results are described in Table 2.

**Extractive values:** The stem-bark of *Zanthoxylum alatum* Roxb was extracted with dichloromethane, methanol and aqueous solvent. Further the aqueous extract is fractioned with different solvent and the results are given in Table 2.

**Table 2:** The percentage ash values of *Zanthoxylum alatum* Roxb. Stem-bark.

| Type                          | Result (w/w) |
|-------------------------------|--------------|
| Total ash value               | 8.20         |
| Acid insoluble ash value      | 2.50         |
| Water soluble ash value       | 1.03         |
| Sulphated Ash value           | 3.23         |
| Loss on drying                | 8.54         |
| Foreign matter                | 0.062        |
| Swelling Index                | 3.28         |
| Foaming Index                 | Less than 100|
| Total alkaloid content        |              |
| In Chloroform layer           | 1.5          |
| In aqueous layer              | 13.5         |
| Total flavonoid content       | 2.4          |

**DISCUSSION**

A medicinal plant’s quality and pharmacognostic criteria must be defined before it can be assessed. According to the WHO, the first step in identifying a medicinal plant’s identity and purity is to perform macro- and microscopy on the specimen. This has to be carried out prior to any testing being done.[20]

Identification of the plant’s origins needs a close look under a microscope. It is possible to identify species, genera, and even families using anatomical traits. It is also possible to assess the quality and standardization of herbal remedies using structural characteristics like cork cells, cortex, secondary phloem, and fibres. Histological characteristics of the drugs are uncovered by examinations of powdered plant material, which reveals structural details about the medicines’ source materials. Cell inclusions and secretory cells like pollen, starch, and calcium oxalate crystals are employed in the powdered examination of herbal material because they are cytomorphological criteria.[21]

These physical and chemical features are critical for standardization and quality control of herbal medicines. Herbal medications’ purity may be checked using a foreign matter analysis of powdered pharmaceuticals. Powdered sample moisture content may be measured using the loss-on-drying technique. Medication should be stored in a dry environment with a moisture content of not more than 5 percent.[14] Ash values may be

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**Table 3:** Results of phytochemical screening on various extracts of *Zanthoxylum alatum* stem-bark.

| Phytoconstituents          | Test                  | Dichloromethane extract | Methanolic extract | Aqueous extract |
|----------------------------|-----------------------|-------------------------|--------------------|----------------|
| Carbohydrates              | Molisch’s test        | –                       | +                  | +              |
| Fehling’s test             |                       | –                       | +                  | +              |
| Benedict’s test            |                       | –                       | +                  | +              |
| Proteins                   | Biuret test           | –                       | –                  | –              |
| Millon’s test              |                       | –                       | –                  | –              |
| Amino acids                | Ninhydrin test        | –                       | –                  | –              |
| Alkaloids                  | Dragendorff’s test    | –                       | +                  | +              |
| Mayer’s test               |                       | –                       | +                  | +              |
| Hager’s test               |                       | –                       | +                  | +              |
| Wagner’s test              |                       | –                       | +                  | +              |
| Saponin                    | Foam test             | –                       | –                  | –              |
| Steroids                   | Salkowski test        | +                       | –                  | --             |
| Liebermann burchard test   |                       | +                       | --                 | --             |
| Flavonoids                 | Shinoda test          | --                      | +                  | +              |
| Phenolics and tannins      | 5% FeCl₃ test         | --                      | +                  | +              |
| Lead acetate test          |                       | --                      | +                  | +              |
Table 4: Results of Phytochemical Screening of various fractions of aqueous extract of Zanthoxylum alatum stem bark.

| Phytoconstituents | Test                | Chloroform fraction | Ethylacetate fraction | n-butanol fraction | Aqueous fraction |
|-------------------|---------------------|---------------------|-----------------------|-------------------|-----------------|
| Carbohydrates     | Molisch's test      | -                   | +                     | -                 | -               |
|                   | Fehling's test      | -                   | +                     | -                 | -               |
|                   | Benedict's test     | -                   | +                     | -                 | -               |
| Proteins          | Biuret test         | -                   | -                     | -                 | -               |
|                   | Millon's test       | -                   | -                     | -                 | -               |
| Amino acids       | Ninhydrin test      | -                   | -                     | -                 | -               |
| Alkaloids         | Dragendorff's test  | +                   | -                     | +                 | +               |
|                   | Mayer's test        | +                   | -                     | +                 | +               |
|                   | Hager's test        | +                   | -                     | +                 | +               |
|                   | Wagner's test       | +                   | -                     | +                 | +               |
| Saponin           | Foam test           | -                   | -                     | -                 | -               |
| Steroids          | Salkowski test      | -                   | -                     | +                 | -               |
|                   | Liebermann burchard test | -               | -                     | +                 | -               |
| Flavonoids        | Shinoda test        | -                   | +                     | +                 | +               |
|                   | Lead acetate test   | -                   | +                     | +                 | -               |
| Phenolics and tannins | 5% FeCl₃ test       | -                   | +                     | +                 | -               |
|                   | Lead acetate test   | -                   | +                     | +                 | -               |

Table 5: Fluorescence analysis of Zanthoxylum alatum Roxb with various chemical reagents under visible light, short and long wave length*.

| Drug Treatment        | Visible light | short UV (254nm) | long UV (360nm) |
|-----------------------|---------------|------------------|-----------------|
| Drug as such          | Dark brown    | Dark brown       | Black           |
| Drug with distilled water | Brownish       | Dark brown       | Black           |
| Drug + FeCl₃          | Golden brown  | Brown            | Black           |
| Drug + Picric acid    | Light brown   | Dark brown       | Black           |
| Drug + NaOH           | Light brown   | Greenish brown   | Dark brown      |
| Drug + Pet. ether     | Dark brown    | light brown      | Black           |
| Drug + CHCl₃          | Golden brown  | Golden brown     | Dark brown      |
| Drug + Ethylacetate   | Golden brown  | Dark brown       | Dark brown      |
| Drug + Methanol       | Greish brown  | Dark brown       | Dark brown      |
| Drug + Conc. HNO₃     | Greyish brown | Dark brown       | Black           |
| Drug + Conc. HCl      | Greyish brown | Dark brown       | Black           |
| Drug + K₂Cr₂O₇        | Brownish      | Dark brown       | Black           |
| Drug + Acetone        | Light brown   | Dark brown       | Black           |
| Drug + Conc. H₂SO₄    | Greyish brown | Dark brown       | Black           |
| Drug + ammonia        | Dark brown    | Black            | Black           |
| Drug + dil. HCl       | Dark brown    | Dark Brown       | Black           |
| Drug + dil. H₂SO₄     | Dark brown    | Dark Brown       | Black           |
| Drug + dil. HNO₃      | Dark brown    | Dark Brown       | Black           |
| Drug + glacial acetic acid | Golden Brown | Dark brown       | Black           |
| Drug + alcoholic KOH (10%) | Greyish brown | Dark brown       | Black           |

used to determine the quality and purity of a crude medicine. Carbonate, oxalate, and silicate are among the pollutants that have been detected. To measure the number of inorganic components in medicines, water-soluble ash is utilised. The acid insoluble ash is dominated by silica, which indicates the presence of earthy minerals.[22] It is possible to identify the raw drug's chemical components based on its pH values. Saponins are either nonexistent or present in very low concentrations in all of the sampled species, as indicated by a foaming index below 100. The presence of gums and mucilage, hemicellulose, or pectin in natural medicine is indicated by a high swelling index. Estimates of how much active plant components may be extracted from a given volume of plant material using a certain solvent are known as extractive values. There are many different phytoconstituents that may be extracted from a crude medication by utilising a specific solvent. The chemical composition of these components is determined by the kind of drug and the solvent used. It also serves as a measure of how much of the raw medicine is left. An important pharmacognostic factor is fluorescence analysis. Some chemicals' fluorescence may be seen even in broad daylight. UV light is invisible in the daylight, yet many natural materials seem to glow in the dark. Various reagents may quickly transform a chemical that isn't fluorescent into one that is. Due to the importance of this criterion in pharmacognostic evaluations, crude medications are often assessed in this way.[23]  

**CONCLUSION**

The findings of this research may serve as a springboard for future efforts to learn more about this medicinal plant. It is the initial stage in determining a plant's identification and purity by conducting pharmacognostic research. This is the very first study on the pharmacognostic profile of Zanthoxylum alatum, and it will be beneficial in future research for accurate identification and authenticity of the species.
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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

ABBREVIATIONS
ZA: Zanthoxylum alatum Roxb; CDH: central drug House; HCl: hydrochloric acid; H$_2$SO$_4$: sulphuric acid; HNO$_3$: Nitric acid; KOH: Potassium hydroxide; UV: ultra violet.

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