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Original Article

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

Nishant Kumar[1], Pooja Puri[2], Navdeep Kaur[3], Jasreen Uppal[1], Apporva Chawla[1], Monika[3], Prince Ahad Mir[1,*]

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, of 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.

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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-hydroxydictamnine, dictamnine, epieudesmine, magnoflorine, eudesmine, xanthoplanine, sikimmianine, γ-fagarine, armatamide, (+) sesamin, (-) sesamin, pluviatide, lupeol, and vanillic acid have been reported.[3-4] Traditionally the stem bark is used as an anti-inflammatory, carminative, stomachic and anthelmintic. 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), Uttrakhand 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. Rankem (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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Figure 1: Stem-bark of *Zanthoxylum alatum* Roxb.

Macroscopical and Microscopical Evaluation

The bark of *Zanthoxylum alatum* Roxb was investigated using the procedures outlined in Trease and Evans Pharmacognosy.[8-10] 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_2SO_4.[11]

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.[12] 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).

Other Physicochemical Analysis

Physicochemical parameters like ash value,[13] loss on drying,[14] swelling index, foaming index[15] and Flourescence analysis[16] of *Zanthoxylum alatum* Roxb were studied using standard procedures.

Preliminary Phytochemical Screening

Preliminary phytochemical analysis of various extracts and fractions of *Zanthoxylum alatum* Roxb was carried out by using standard procedure described.[17]

Quantative Estimation of Alkaloids

The powdered material (80 g) previously defatted with petroleum ether was extracted with methanol (90%) and the extract obtained was concentrated under vacuum. The crude methanolic extract (10.5 g) was treated with 5% HCl and partitioned against diethyl ether in a separating funnel. The diethyl ether phase was separated which contains neutral compounds and the aqueous acid phase was made alkaline with ammonium hydroxide and partitioned against chloroform. The chloroform layer was concentrated *in vacuo* which must contains primary, secondary and tertiary alkaloids whereas the aqueous layer was also concentrated which have quaternary type of alkaloids.[18]

Quantative Estimation Flavonoids

The methanolic extract (15g) was dissolved in distilled water and extracted 2-3 times with chloroform to remove the coloring matter, to the aqueous layer, 10% NaCl was added drop wise in order to precipitate out the tannins. Then the resulting solution was subjected to centrifugation. The precipitates formed are tannins and the supernatant liquid contains flavonoids. Then the supernatant liquid was partitioned with ethyl acetate. The ethyl acetate layer was evaporated to dryness to get the crude flavonoids.[19]

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

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

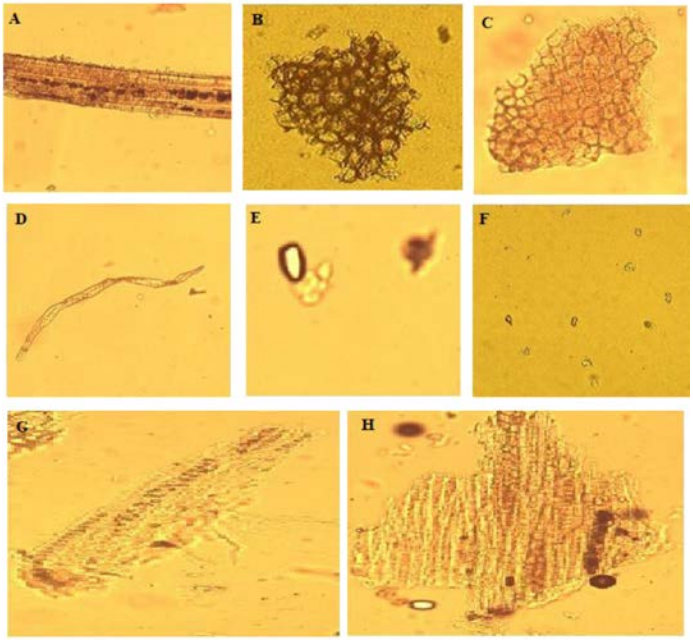

Figure 2: A: Lignified vascular bundles, B: Cork cells, C: Collenchymatus cells, D: Fibrous sclereids, E: Oil cells, F: Prismatic calcium oxalate crystals, G and H: Crystal fibers.

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

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

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 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	_	_	_
	Millons'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% $FeCl_3$ test	--	+	+
	Lead acetate test	--	+	+

The phytochemical analysis of various extracts and fractions of stem bark of *Zanthoxylum alatum* Roxb revealed the presence of various phytoconstituents and are shown in Table (3, 4). The fluorescence analysis of drug when treated with various reagents was studied under visible light and UV light and the results are shown in Table 5.

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 critera.[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

Table 4: Results of Phytochemical Screening of various fractions of aqueous extract of *Zanthoxylum alatum* stem- bark.

Phytoconstituents	Test	Chloroform fraction	Ethylacetate fraction	*n*-butanolic fraction	Aqueous fraction
Carbohydrates	Molisch's test	–	+	–	–
	Fehling's test	–	+	–	–
	Benedict's test	–	+	–	–
Proteins	Biuret test	–	–	–	–
	Millons'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% $FeCl_3$ 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 + Iodine	Brownish	Dark brown	Black
Drug + $FeCl_3$	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 + $CHCl_3$	Golden brown	Golden brown	Dark brown
Drug + Ethylacetate	Golden brown	Dark brown	Dark brown
Drug + Methanol	Greyish brown	Dark brown	Dark brown
Drug + Conc. HNO_3	Greyish brown	Dark brown	Black
Drug + Conc. HCl	Greyish brown	Dark brown	Black
Drug + $K_2Cr_2O_7$	Browinsh	Dark brown	Black
Drug + Acetone	Light brown	Dark brown	Black
Drug + Conc. H_2SO_4	Greyish brown	Dark brown	Black
Drug + ammonia	Dark brown	Black	Black
Drug + dil. HCl	Dark brown	Dark Brown	Black
Drug + dil. H_2SO_4	Dark brown	Dark Brown	Black
Drug + dil. HNO_3	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_2SO_4:** sulphuric acid; **HNO_3:** Nitric acid; **KOH:** Potassium hydroxide; **UV:** ultra violet.

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GRAPHICAL ABSTRACT

SUMMARY

The first step in identifying a medicinal plant's identity and purity is to perform macro- and microscopy on the specimen. Histological characteristics of the drugs are uncovered by examinations of powdered plant material. Powdered sample moisture content may be measured using the loss-on-drying technique. Water-soluble ash is used to measure the amount of inorganic components in medicines. Carbonate, oxalate, and silicate are among the pollutants that have been detected. Some chemicals' fluorescence may be seen even in broad daylight. This is the 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. The findings of this research may serve as a springboard for future efforts to learn more about this medicinal plant.

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