Current Trends in Pharmaceutical Research, 2023
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Vol 10 Issue I
ISSN: 2319-4820 (Print)
2582-4783 (Online)

Research Article

PRELIMINARY STUDIES TOWARDS THE VALIDATION OF TRADITIONAL USAGE OF *ZANTHOXYLUM NITIDUM* (ROXB.) DC LEAVES

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Abstract

Background: *Traditionally, North-East India uses the prickly shrub Zanthoxylum nitidum (Roxb.) DC, which is also called Tez-mui in Assamese, for a number of medical conditions, such as fever, rheumatism, toothaches, kidney stones, bleeding gums, pneumonia, etc. Thus, this indigenous plant needs scientific validation and standardization for its use in herbal formulations.*

Objective: *In this study, we aimed to assess Zanthoxylum nitidum leaf extract's phytochemical characteristics, various pharmacognostical criteria as well as the physico-chemical parameters using standard methods.*

Methods: *The pharmacognostical evaluation was done by performing the macroscopical characterization, fluorescence study, and microscopical characterization. Physico-chemical investigation included the determination of extractive values and loss on drying. Phytochemical screening included tests for alkaloids, carbohydrates, fats & oils, flavonoids, glycosides, proteins, phytosterols, saponins, tannins & phenolic compounds, triterpenoids.*

Results and Discussion: *The macroscopic characterization revealed the leaves to be imparipinnate, having a color of glossy green when fresh, and light green when dried with a size of 4.5-10cm long and 2-5cm broad. The leaves had an apiculate apex and rounded base with petioles and were sessile-like. The main microscopic characters included lignan, resins, fibers, and stomata of anisocytic kind. Further, physicochemical analysis of the leaves showed the alcohol and water-soluble extractive values to be 8.8 and 28% w/w respectively. The crude extracts of Z.nitidum revealed the presence of several biologically active phytochemicals.*

Conclusions: *The presented results will be helpful in the proper identification of Zanthoxylum nitidum (Roxb.) (DC) leaves in intact or preserved form and may serve as reference monograph.*

Keywords: *Zanthoxylum nitidum;* North-East India; *Tezmui*; Assam.

1. Introduction

Since the dawn of human civilization, medicinal herbs have played a significant role in society and have been prized for both their culinary and therapeutic qualities. Herbal medicines are known as phytomedicines. Plants rich in phytochemicals are widely used in diet-based therapies for a wide range of illnesses, which has led to an exponential increase in their impact and rate of utilization. Medicinal plants have been a vital source of both curative and preventive medical therapy preparations for human beings, which also has been used for the extraction of important bioactive compounds [1].

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The plant *Zanthoxylum nitidum* Roxburgh (Roxb.) DC (*Z.nitidum*) is an aromatic herb or shrub belonging to the family of Rutaceae. It is commonly known as Shiny-leaf-prickly-ash in English, *Tez-mui* in Assamese, or *Liang Mian Zhen* in Chinese, and is a flowering plant species. It is a morphologically variable plant found in South China, Southeast Asia, Northern Australia, Vietnam, Guinea, and India. In Northeast India, this ethno-medicinally important herbal plant is found in Sikkim, Assam, and Nagaland [2,3]. This shrub can be found all over the world in warm regions such as Guangxi, Guangdong, Yunnan, Fujian, Taiwan, and Japan. It is also frequently seen in forests and shrubs on hills and mountains. The best quality of *Z. nitidum* is cultivated in Guangxi province of China.

It is a woody climber shrub that rises up to a height of 1-2m with prickles on the branchlets, thick, cone-shaped spines on the trunk and older branches, pinnate leaves with five to nine leaflets, and panicles or racemes of white to pale yellow, male or female flowers in leaf axils and on the ends of branchlets. It has 4 basic flowers whose sepals are purplish green, broadly ovate, and about 1 mm wide. Petals of *Z. nitidum* are somewhat yellowish green, oblong, and 3 mm long. The flowering and fruiting period of *Z. nitidum* is from March-May and September-November respectively. The mature seed has dormancy and the seed coat is bright black and rich in oil [2].

The whole plant is used for the treatment of various diseases. Traditionally, different parts of the plants are used for different medicinal purposes. Generally, the roots are used to promote blood circulation, cure snake bites, stomachache, toothache, fever, rheumatism, kidney stones, paresis, boils, and as a pesticide and insecticide. Whereas, fruits are used in the treatment of cough, colic, diarrhea, and as aromatic, stimulants and piscicides. For the treatment of fever, cholera, and diarrhea, the small branches, seeds, and stem bark are used [3]. In Assam, the rural people used to chew the stem and bark for the treatment of gingivitis and toothache. Because of its bitter and pungent flavor and mild properties, it is used for liver and stomach problems. The resin present in *Z. nitidum* has powerful stimulant and tonic properties. The fruits are also used as condiments in India and Nepal. Sometimes the roots, fruits, and leaves show some toxicity depending on their dose while the dose of 40g *Z. nitidum* leaves are considered to be a lethal dose [4]. It has been used for thousands of years as an anti-inflammatory, analgesic, and hemostatic medicine. Traditional Chinese Medicine (TCM) holds that *Z. nitidum* can promotes“*qi*” (a vital life force that flows through everything) circulation to relieve pain, disperse wind to dredge collaterals, and promote blood circulation to remove blood stasis [4,5].

The first step towards ensuring the quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. Despite the modern techniques, the identification of plant drugs by pharmacognostic studies is more reliable. Most medicinal herbs contain a large number of therapeutically important phytochemicals that need to be scientifically validated for medical purposes [5].

Plant Profile:

Taxonomical Classification of *Zanthoxylum nitidum* (Roxb.) DC

- **Scientific name:** *Zanthoxylum nitidum* (Roxb.) DC
- **Kingdom:** Plantae
- **Division:** Tracheophyta
- **Class:** Magnoliopsida
- **Order:** Sapindales
- **Family:** Rutaceae
- **Genus:** *Zanthoxylum*
- **Species:** *nitidum*

Common/English name: Shiny-leaf prickly-ash

Vernacular names: Tez-mui or tejamool (in Assamese); Liang Mian Zhen (in Chinese); Nagarakta (in Hindi)

Zanthoxylum species, also known as *Fagara* species, have a long history of use as sources of food and drugs by locals in different parts of Asia, America, and Africa. In South Africa and Kenya, pastes made from *Zanthoxylum* species are used to suppress pain associated with wounds and to aid wound healing while in Nigeria, *Zanthoxylum* species like *Z. zanthoxyloides* are used for treating rheumatism, sickle cell anemia, toothache, urinary tract infection, and venereal diseases [6].

The Rutaceace family, commonly known as the rue or citrus family, usually placed in the order Sapindales. The family is composed of 160 genera and about 2,070 species. Rutaceae includes woody shrubs and trees (and a few herbaceous perennials) and are distributed throughout the world, especially in warm temperate and tropical regions. The largest numbers are found in Africa and Australia, often in semi-arid woodlands. The family contains several economically important fruit trees as well as several ornamental species [7].

2. Materials and Methods

2.1 Plant Material:

Leaves of *Zanthoxylum nitidum* (Roxb.) DC was collected from the Dibrugarh University campus, Assam, India in September 2023. Good-quality plant stems were selected for the herbarium sheet and were flat-pressed and dried as quickly as possible. The herbarium sheet is shown in Fig. No.1. The plant was identified by Dr. Pankaj Chetia, Associate Professor, Department of Life Sciences, Dibrugarh University, Assam. The leaves were washed with water to remove dust and then shed dried. The dried leaves were then pulverized using a mechanical grinder to a coarse powder and stored in an airtight container free from moisture.

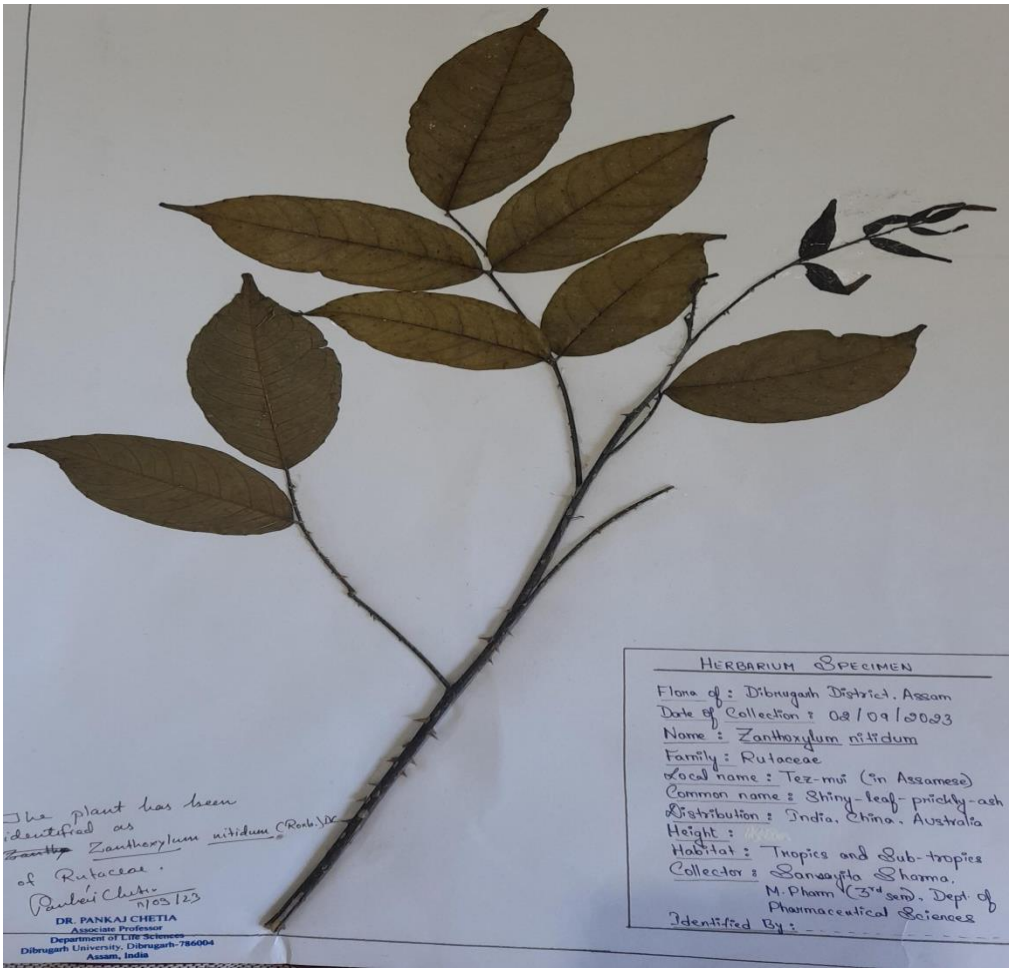


Fig. 1: Herbarium specimen of *Zanthoxylum nitidum* (Roxb.) DC

2.2 Pharmacognostical Evaluation:

2.2.1 Macroscopic characterization of plants:

The macroscopic study is the morphological description of the plant parts which are seen by the naked eye or magnifying lens [8]. Macroscopic evaluation of the leaves of *Zanthoxylum nitidum* (Roxb.) DC was carried out by simple determination techniques like shape, size, color, odour, apex, petioles, and base. The results of macroscopic and organoleptic evaluation are given in Table 1.

2.2.2 Fluorescence Study:

A small quantity (0.5gms) of dried plant powder was placed on a grease-free clean microscopic slide and 1-2 drops of freshly prepared reagent solution were added, mixed by gently tilting the slide, and observed for a few minutes. Then the slide was placed inside the UV chamber and observed the color in visible light, and at 366 nm wavelength. The color observed by the application of different reagents in different radiations was recorded. The results are shown in Table 2.

2.2.3 Microscopic Evaluation:

Microscopic inspection of herbal materials is indispensable for the identification of powdered materials. Microscopic examination of powdered drugs helps in the identification of epidermal trichomes, calcium oxalate crystals, and fibers, etc. which serve as an important diagnostic character for the identification of certain herbal drugs. For effective results, various reagents or stains can be used to distinguish cellular structures [9].

2.2.3.1 Powder microscopy

For microscopic investigation, fine powder of the dried leaves of *Zanthoxylum nitidum* was used. 1-2 drops of mounting agent/staining reagent were placed on a glass slide and the tip of a needle was moistened with water and dipped into the powder. A small quantity of the material that adheres to the needle tip was transferred into the drop of fluid on the slide. It was thoroughly stirred, and a cover glass was applied. The excess fluid was removed from the margin of the cover glass with a strip of filter paper. The slides were then observed under the microscope.

Some of the staining agents along with their application procedure for analysis are given below: -

A. **Phloroglucinol and HCl (for lignans)**
   The powder was moistened on a slide with 1% solution of phloroglucinol in ethanol (90%) and allowed to stand for about 2 minutes. The excess alcohol was removed with a piece of filter paper. 1 drop of conc. HCl was added and a cover slip was applied. All lignified tissues are stained pink color.
B. **Chlor-Zinc iodine solution (for cellulose)**
   The powder was moistened on a slide with 1-2 drops of iodinated zinc chloride and allowed to stand for a few minutes. The excess reagent was removed with a strip of filter paper and 1 drop of sulphuric acid was added. The cellulose cell walls are stained blue.
C. **Sudan IV (for cellulose cell walls, fats, volatile oils, and resins)**
   The powder was moistened on a slide with 1-2 drops of Sudan red solution and allowed to stand for a few minutes. The cuticular cell walls are stained orange-red or red.
D. **Iodine solution (for starch)**
   The powder was moistened on a slide with a small volume of iodine (0.02mol/L). The starch grains attain a blue or reddish-blue color.
E. **1% of methylene blue solution (for cell walls)**
   The powder was moistened on a slide with 1-2 drops of methylene blue. It turns cell walls blue except cutinized cells.
F. **Ruthenium red (for mucilage and gum)**
   The powder was moistened on a slide with 1-2 drops of 0.08% ruthenium red in 10% lead acetate. The mucilage and gums are stained pink.
G. **50% of glycerine in water (for calcium oxalate crystals)**
   A small quantity of powdered drug was taken into a clean glass slide. 1-2 drops of glycerine-water mixture was added. The cover slip was placed and observed under high magnification.

The results of the microscopic studies are given in Fig No. 2(a) to 2(f) and Fig. No.3.

2.3 Physico-chemical Analysis:

Physico-chemical constants such as the percentage of water and alcohol soluble extractive and loss of weight on drying were calculated based on standard procedures mentioned in the Indian Pharmacopoeia 2018 Edition. Water and alcohol-soluble extractive values were determined by the cold maceration method as per WHO guidelines. Each study was performed in triplicate; mean values with standard error of the mean (SEM) were calculated.

2.3.1 Determination of Extractive values:

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be rapidly estimated by any other means. This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for material for which no suitable chemical or biological assay exists [10].

Method:

a. About 5g of coarsely powdered air-dried material was weighed accurately and placed in a glass-stoppered conical flask. It was macerated with 100ml of the specified solvent (usually ethanol and chloroform-water) for 6 hours, shaking frequently, and then allowed to stand for 18 hours.
b. The solvent was then filtered rapidly; taking care not to lose any solvent, 25ml of the filtrate was then transferred to a tarred flat-bottomed dish and evaporated to dryness on a water bath.
c. It was dried at 105°C for 6 hours, cooled in a desiccator for 30 minutes, and weighed without any delay.
d. The content of extractable matter was then calculated in mg per g of air-dried material.

$$\text{Percentage of the extractable matter (\%w/w)} = \frac{y-x}{z} \times 100$$

Where, x is the weight of the empty petri dish,
y is the weight of the petri dish + extract,
z is the weight of the crude drug taken.

The results of the content of extractable matter are given in Table 3.

2.3.2 Determination of Loss on Drying (LOD):

The percentage of active chemical constituents in crude drugs is mentioned on air-dried basis. Hence the moisture content of a drug should be determined and should also be controlled. The moisture content of a drug should be minimized in order to prevent the decomposition of crude drugs either due to chemical changes or microbial contamination [11]. Loss on drying is the loss in weight in %w/w resulting from water and volatile matter of any kind that can be driven off under specified conditions.

Method:

a. 2g of the air-dried powdered material was accurately weighed in a dried and tarred petri dish.
b. The sample was kept in hot air oven at 100-105°C and cooled in a desiccator.
c. The loss in weight was recorded.

$$\%\ Loss\ on\ Drying\ = \frac{\text{Loss in weight}}{\text{Weight of crude drug taken}} \times 100$$

The results of determination of loss on drying are given in Table 4.

2.4 Extraction Procedure:

Coarsely powdered leaves were extracted by subjection to successive extraction using the Soxhlation method. Solvents used for the extraction were in increasing order of polarity, i.e., Petroleum ether (40-60°C), Chloroform, Ethyl acetate, and Methanol. Each extract was concentrated by distilling off the solvent and evaporated to dryness in petri dish on a water bath. The crude extracts were stored in a desiccator to prevent moisture absorption. Physical characteristics of leaf extract of *Zanthoxylum nitidum* (Roxb.) DC is given in Table 5.

2.5 Phytochemical Investigation:

The different extracts were separately tested for the presence of various plant constituents such as alkaloids, flavonoids, glycosides, steroids, tannins, phenolic compounds, carbohydrates, saponins, triterpenoids, proteins, anthraquinones, and fatty acids. The procedures followed for the different phytochemical tests were as follows:

i. Tests for Alkaloids:

A small portion of the extract was stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was then subjected to the following reagents:

a. **Mayer's Reagent:** To 3ml of the filtrate, few drops of Mayer's reagent (potassium mercuric iodide solution) were added and observed for the formation of white or cream colored precipitate.
b. **Dragendroff's Reagent**: To 3ml of the filtrate, few drops of Dragendroff's reagent (potassium bismuth iodide solution) were added and observed for the formation of orange-brown precipitate.
c. **Hager's Reagent:** To 3ml of the filtrate, few drops of Hager's reagent (saturated aqueous solution of picric acid) were added and observed for the formation of yellow precipitate.
d. **Wagner's Reagent:** To 3ml of the filtrate, few drops of Wagner's reagent (iodine in potassium iodide) were added and observed for the formation of red-brown precipitate

ii. Tests for Carbohydrates:

A small quantity of extract was dissolved in 5ml of distilled water and filtered. The filtrate was then subjected to the following tests to detect the presence of carbohydrates.

Molisch's Test (General Test): To 3ml of the filtrate, few drops of α-naphthol (20% in ethyl alcohol) added. The mixture was shaken well. Then about 1ml of concentrated sulfuric acid was added along the sides of the test tube and observed for the formation of reddish-violet colored ring at the junction of two layers.

iii. Tests for Fats and Oils:

a. **Saponification Test:** Few drops of 0.5N alcoholic potassium hydroxide were added to a small quantity of the extract along with a drop of phenolphthalein. The mixture heated on a water bath for 1-2 hours. The formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

b. **Spot Test:** A small quantity of the extract was pressed between two filter papers. The appearance of oil stains on paper indicated the presence of fixed oils.

iv. Tests for Flavonoids:

a. **Shinoda Test:** Small quantity of the extract was taken in 5ml of alcohol (95% ethanol), treated with few drops of concentrated hydrochloric acid, and 0.5g of magnesium turnings was added, and observed for the formation of pink color.

b. **Lead Acetate Test:** To the small quantity of extract, a few drops of 10% lead acetate solution were added and observed for the formation of yellow colored precipitate.

v. Tests for Glycosides:

A small portion of the extract was hydrolyzed with dilute hydrochloric acid for 1 hour over water-bath, filtered and the filtrate was then subjected to the following tests:

a. <u>Tests for Anthraquinone glycosides:</u>

Borntrager's test for Anthraquinone glycosides: To 2ml of the filtrate, 3ml of chloroform was added and shaken, chloroform layer was separated. 10% ammonia solution was added to it and observed for the formation of reddish-pink color in ammonical layer.

Modified Bortrager's Test for C-Glycosides: To 5ml of the extract, 5ml 5% FeCl3 and 5ml dilute HCl were added and heated for 5min on boiling water bath, cooled and benzene (or any organic solvent) was added. It was shaken well and the organic layer was separated and an equal amount of dilute ammonia was added and observed for the formation of pink red color.

b. <u>Tests for Cardiac Glycosides:</u>

Legal's test (Test for Cardenolide): To the filtrate, 1ml of pyridine and 1ml of sodium nitroprusside solution was added and observed for the formation of pink to red color, indicating the presence of glycoside.

Liebermann's test (Test for Bufadenolide): The extract was dissolved in 3ml of acetic anhydride and the mixture was heated and cooled, then few drops of concentrated sulfuric acid were added and observed for the formation of blue color.

Test for De-oxy Sugars (Keller-Killiani Test): To 2ml of filtrate, a few drops of glacial acetic acid, one drop of 5% ferric chloride, and concentrated sulfuric acid were added. Observed for the formation of reddish brown color at the junction of two liquid layers and the upper layer appeared bluish green in the presence of glycosides.

c. <u>Test for Cyanogenetic Glycosides:</u>

Grignard reaction or sodium picrate test: A filter paper strip was first soaked in 10% picric acid, then in 10% sodium carbonate and dried. Moistened powder drug was placed in a conical flask and it was corked, the above filter paper strip was placed on the slit in the cork. The filter paper was observed for brick red color.

d. <u>Tests for Saponin Glycosides:</u>

Foam Test: The drug extract was shaken vigorously with water and observed for the formation of persistent foam.

vi. **Tests for Proteins:**

a. **Biuret Test:** To 3ml of test solution, 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution was added and observed for the formation of pinkish or purple violet color.

b. **Millon's Test:** 1ml of test solution acidified with sulfuric acid added to Millon's reagent and boiled this solution and observed for the formation of a white precipitate. After warming, observed for precipitate turned to brick red or the precipitate dissolved giving red colored solution.

c. **Test for protein containing sulphur:** 5ml of the test solution was mixed with 2ml of 40% NaOH and two drops of 10% lead acetate solution. The solution was boiled and observed for the formation of black or brownish color solution due to PbS formation.

d. **Xanthoprotein Test:** To 1ml of the extract, add 1ml of concentrated nitric acid, a white precipitate was formed, it was boiled and cooled. Then 20% sodium hydroxide or ammonia was added. Then it was observed for formation of orange color due to the presence of aromatic amino acids.

e. **Precipitation Test:** The test solution was observed for white colloidal precipitate with 5% CuSO4 solution and 5% Lead Acetate solution.

vii. **Tests for Phytosterols:**

Extracts were treated with chloroform and filtered. A few drops of acetic anhydride were added to the filtrate which was then boiled and cooled followed by the addition of concentrated sulfuric acid. Observed for the formation of brown ring at the junction that will indicate the presence of phytosterols.

viii. **Tests for Saponins:**

a. **Froth test:** Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15minutesand observed for the formation of layer of foam which will indicate the presence of saponin.

b. **Foam test:** 0.5g of extract shaken with 2ml of water. If foam persists for 10minutes, it indicates the presence of saponins.

ix. **Tests for Tannis and Phenolic Compounds:**

To 3ml of the aqueous or alcoholic extract, few drops of the following reagents were added and observed for the mentioned change in color or colored precipitate:

a. **5% Ferric Chloride solution:** Deep blue or black color

b. **Lead Acetate solution:** White precipitate

c. **Acetic acid solution:** Red color solution

d. **Potassium dichromate:** Red precipitate

e. **Dilute iodine solution:** Transient red color

f. **Dilute HNO3:** Reddish to yellowish color

g. **Dilute Potassium Permanganate solution:** Decolouration

x. **Tests for Triterpenoids:**

Salkowski Test: The extracts were treated with a few drops of concentrated sulfuric acid. Observed for the formation yellow colored lower layer which indicates the presence of triterpenoid [12-14].

The results of the phytochemical investigation are given in Table 6.

3. Results& Discussion

3.1 Pharmacognostic Evaluation:

Table 1: Organoleptic and macroscopic characters of *Zanthoxylum nitidum*(Roxb.) DC leaves

Sl. No.	Characteristic	Observations
1.	Color	**Fresh:** Upper surface is glossy green and lower surface is dull green. **Dried powder:** Light green
2.	Size	4.5cm to 10cm long, 2cm to 5cm broad
3.	Odour	Characteristic
4.	Taste	Bitter and pungent
5.	Shape	Leaves are imparipinnate
6.	Texture	Smooth and glossy in the upper surface, lower surface contains hook like prickles along the midrib
7.	Petioles	Sessile like with length of 2-5mm
8.	Apex	Apiculate
9.	Base	Rounded

Table 2: The fluorescence produced in Visible/daylight & UV radiation (366nm)

Sl. No.	Particulars of the treatment	Visible/Daylight	UV 366nm
1.	Powder as such	Dark Green	Black
2.	Powder + Methanol	Dark Green	Black
3.	Powder + 50% H2SO4	Light Green	Black
4.	Powder + 1N HCl	Brown	Dark Brown
5.	Powder + Dragendroff's reagent	Reddish Brown	Black
6.	Powder + 5% HNO3	Brown	Light Brown
7.	Powder + Ammonia	Light Green	Dark Brown
8.	Powder + 5% Iodine	Dark Brown	Dark Brown
9.	Powder + Acetone	Green	Black
10.	Powder + Acetic acid	Brown	Brown
11.	Powder + Conc. H2SO4	Brown	Black
12.	Powder + Picric acid	Yellow	Light Yellow
13.	Powder + H2O	Brown	Green
14.	Powder + Ethanol	Green	Black
15.	Powder + Ethyl acetate	Brown	Black

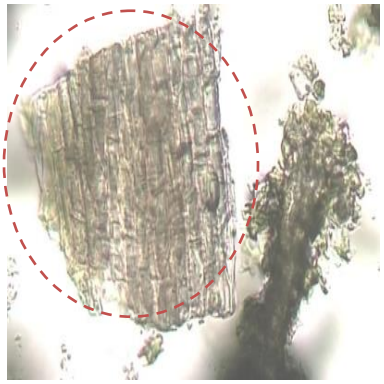


Fig. 2(a)

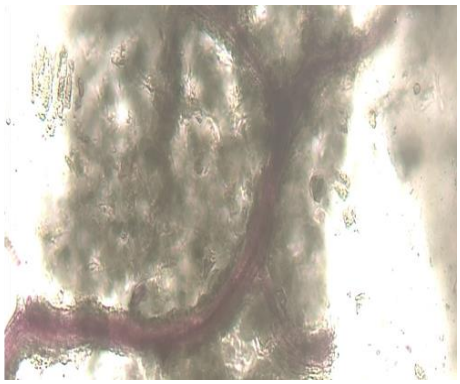


Fig. 2(b)

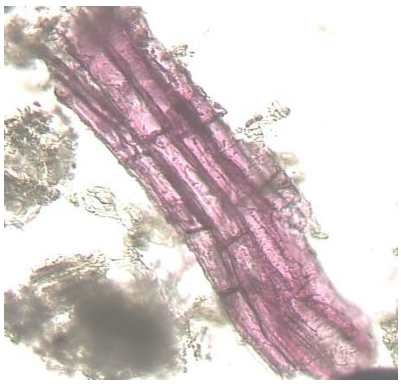
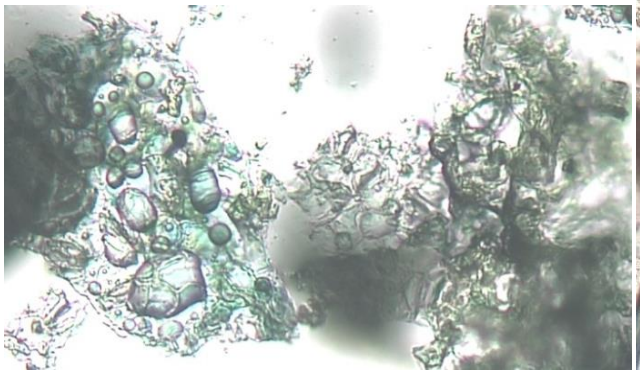


Fig. 2(c)



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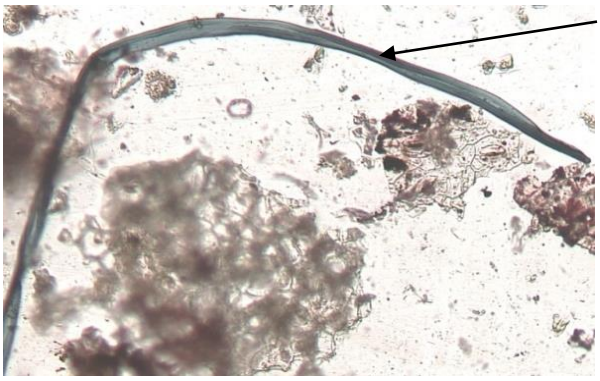

Fig. 2(e)

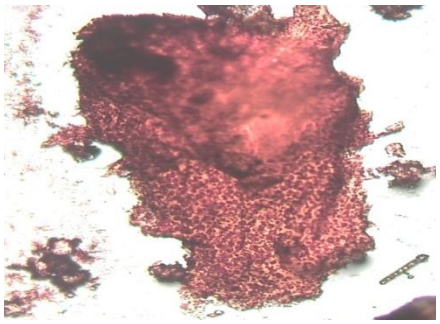

Fig. 2(f)

Fig. 2(a)-(f): Microscopic characteristics as observed during powder microscopy of the leaves of *Zanthoxylum nitidum* (Roxb.) DC.(a) Mesophyll Tissue, (b) Lignan vein, (c) Lignan, (d) Cell wall in Blue, (e) Fiber, (f) Resin.

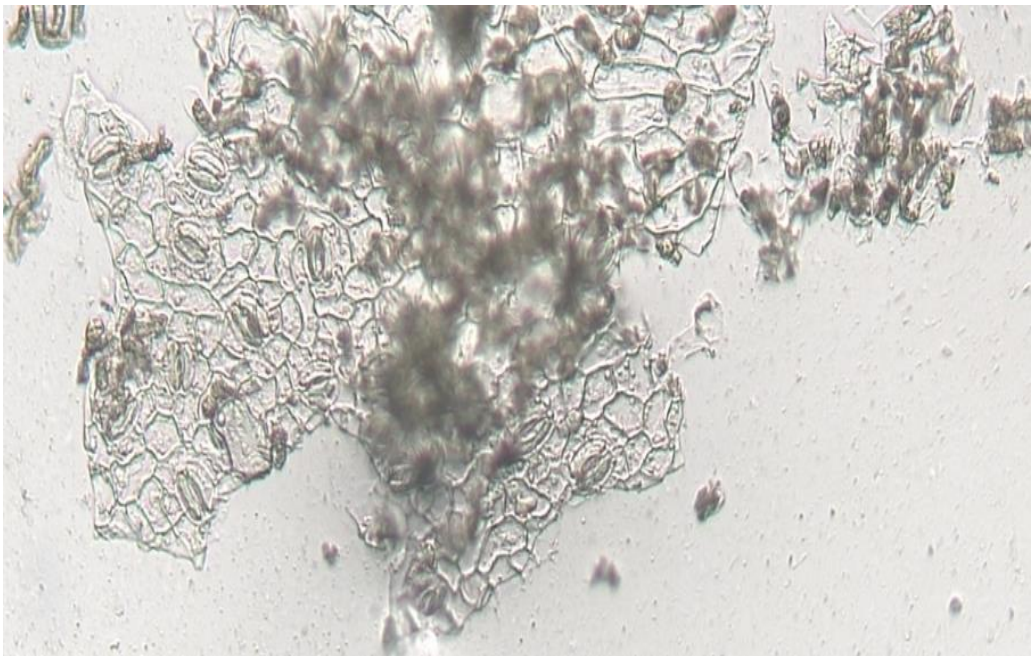

Fig. 3: Anisocytic Stomata

3.2 Physico-chemical Evaluation:

Table 3: Results of Extractive Value Determination

Sl. No.	Parameter	Results (%w/w)	Mean ± S.D. (%w/w)
1.	Alcohol soluble extractive	8.56	8.8 ± 0.24
		9.04	
		8.82	
2.	Water soluble extractive	28.24	28.4 ± 0.16
		28.56	
		28.41	

It was observed that the water-soluble extractive value was higher compared to alcohol soluble extractive value. Thus, it indicates that the constituents of the drug are more extracted by water when compared to alcohol.

Table 4: Results of Loss on Drying

Sl. No.	Drug taken (in g)	Results (%w/w)	Mean ± S.D. (%w/w)
1.	2	12.32	12.5 ± 0.180
		12.68	
		12.52	

3.3 Physical Characteristics of the Extracts:

Table 5: Physical characteristics of leaf extract of *Zanthoxylum nitidum* (Roxb.) DC

Sl. No.	Extract	Consistency	Color	Odour	Yield (%w/w)
1.	Petroleum ether	Sticky semisolid	Dark Green	Characteristic	1.54
2.	Chloroform	Sticky semisolid	Blackish Green	Characteristic	1.99
3.	Ethyl acetate	Sticky semisolid	Blackish Green	Characteristic	1.04
4.	Methanol	Sticky semisolid	Reddish Brown	Characteristic	3.65

The yield value of Methanol extract of the leaves of *Zanthoxylum nitidum* (Roxb.) DC was found to be high as compared to the yield of different solvent extracts. This indicates that more phytoconstituents are extracted by Methanol. Thus, methanolic extract was chosen for further studies.

3.4 Phytochemical Investigation:

Table 6: Phytochemical screening of different solvent extracts of *Zanthoxylum nitidum* (Roxb.) DC leaves

Sl.No.	Phytoconstituent	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract
1.	Alkaloids	-	-	-	-
2.	Carbohydrate	-	-	+	+
3.	Fats & oils	-	-	-	-
4.	Flavonoids	+	+	+	-
5.	Glycoside	-	-	-	-
6.	Proteins	-	-	-	-
7.	Saponins	-	-	-	+
8.	Phenols	-	-	-	-
9.	Triterpenoid	-	-	-	-
10.	Phytosterols	+	+	+	+

* "+" represents Positive; "-'' represent Negative

4. Conclusions

To ensure the reproducible quality of herbal products, proper control of starting material is of utmost essential. Thus, in recent years there has been an emphasis on the standardization of medicinal plants of therapeutic potential [15]. According to the World Health Organization (WHO), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken. Medicinal plants have provided copious leads to combat diseases, from the dawn of civilization. Substitution of herbal drugs and adulteration is the major drawback in the promotion of herbal products. Like the quality control of allopathic medicine, the standardization of herbal medicines is also necessary to assure the quality of the drug. This systemic approach will help in the confirmation and detection of the identity, quality, purity, and safety of crude drugs for human use [16].

The majority of the information on the identity, purity, and quality of the plant material can be obtained from its macroscopy, microscopy, physicochemical, and phytochemical parameters [17].

After present investigation, it can be concluded that the histological and physico-chemical studies of *Z. nitidum* leaves yielded a set of qualitative pharmaco-botanical parameters or standards that can serve as an important source of information to ascertain the identity and determine the quality and purity of the plant material in future studies. As previously mentioned, *Z. nitidum* being a morphologically variable species, this information will also be helpful to differentiate *Z. nitidum* from the closely related other species and varieties of *Zanthoxylum*. In powder microscopy of *Zanthoxylum nitidum* (Roxb.) DC leaves, lignans, resins, fibers were found. Loss on drying is a component of crude drug which helps in its preservation. The preliminary phytochemical screening of the leaves extracts revealed the presence of different phytoconstituents.

The presented results will be helpful in the proper identification of *Zanthoxylum nitidum*(Roxb.) DC leaves in intact or in preserved form and may serve as reference monograph for the said plant.

Acknowledgement

NA

Conflict of Interest

The authors declare no conflicting interests.

Funding

NA

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How to cite this article:

Sharma S, Bhuyan B. Preliminary studies towards the validation of traditional usage of *Zanthoxylum nitidum* (Roxb.) DC leaves, *Curr Trends Pharm Res*, 2023; 10 (1): 22-36.