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Pharmacognostic studies of the fruits of *Terminalia bellirica* (Gaertn.)Roxb

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ABSTRACT
The fruits of *Terminalia bellirica* were studied to analyze its pharmacognostic characters. Organoleptic studies of the air dried powdered fruit rind revealed characters like yellowish brown colour, astringent taste and aromatic odour. The fluorescent analysis of the powder after treatment with different chemical reagents under visible and short UV light showed different colour changes. Microscopic evaluation of the fruit rind revealed epidermal hairs, stone cells, starch grains and needle like crystals of Calcium oxalate as the key identifying features which were confirmed by powder microscopy. Preliminary phytochemical analysis of the aqueous extract revealed the presence of glycosides, flavonoids, tannins, phenols, saponin, carbohydrates and proteins.

Keywords: *Terminalia bellirica*, Powder microscopy, Organoleptic, Stone cells, Fluorescent analysis.

1. Introduction
Plants have been used since time immemorial as a source of medicine to cure different ailments. The ancient Ayurvedic, Unani and Siddha systems of medicine is based on the healing potential of plants. The use of herbal medicine is gaining momentum in this era because of the side effects of synthetic pharmaceutical products and the safety, efficiency and promising potential of plant derived medicine.
Terminalia bellirica (Gaertn.)Roxb is a large tree with broadly elliptic leaves clustered at the ends of branches [1]. The leaves of this plant possess proteins which makes it a promising angiogenic agent [2]. The fruits of this plant are spherical to ovoid, 1.2 to 3.5 cm in diameter and tapering towards both the ends. The dry fruits are grayish brown in colour, pubescent and slightly ridged. It is widely used in Ayurveda, Siddha and Chinese systems of medicine [3]. It has antidiabetic, anticancer and antimicrobial properties [4]. The fruit rind is an important ingredient of Triphala an important Ayurvedic formulation [5]. The fruit extract stimulates the secretion of insulin and enhance its action and inhibits starch digestion [6]. It possess active compounds which can be used to develop antidiabetic drugs [7]. The fruit pulp possess phytosterols, triterpenoids, glycosides, tannins and phenolic compounds which accounts for its anti-inflammatory, analgesic, antimicrobial, antioxidant and antitumor properties [8].
Even though several phytochemical analyses have been carried out on different extracts of the fruit, extensive reports on anatomical evaluation and powder microscopy are lacking which is essential for the authenticity of the drug. The present paper attempts to evaluate the microscopic, organoleptic, fluorescent and preliminary phytochemical characters of the fruit rind of *Terminalia bellirica* (Gaertn.) Roxb.

2. Materials and methods
The official part of *Terminalia bellirica* was collected, washed in running tap water followed by distilled water, shade dried and was subjected to pharmacognostic analysis, the parameters included organoleptic study, microscopic evaluation, fluorescence analysis and preliminary phytochemical analysis.

2.1 Organoleptic study
The colour, odour and taste of the powdered drug were studied [9].

2.2 Microscopic study
Transverse sections of the pericarp of the fruits were taken, and permanent mount was prepared using safranin and fast green stain by double staining technique [10]. The fruits were powdered and the powder was observed under the microscope without staining and after staining with

phloroglucinol and concentrated HCl [11]. The components were observed, noted down and the measurements were taken with the help of a micrometer.

2.3 Fluorescence analysis
The powder was treated with different chemical reagents and observed under visible light and UV light at 254 nm to determine the fluorescence character [12-17].

2.4 Preparation of the extract [18]: 20 g of the powdered sample was extracted with 200 ml distilled water in a soxhlet apparatus at temperature 80 °C for 12 hours. The extract was then concentrated in a boiling water bath to a volume of 40 ml and stored in small air tight brown bottles. The extract was subjected to phytochemical screening for the identification of its constituents.

2.5 Preliminary phytochemical screening [18-21]
2.5.1. Tests for alkaloids
2.5.1.1 Dragendorff's Test: 2 ml of the extract was treated with a few drops of Dragendorff's reagent (solution of potassium bismuth iodide).Formation of orange brown precipitate indicates the presence of alkaloids.

2.5.1.2 Mayer's Test 2 ml of the extract was treated with a few drops of the Mayer's reagent (Potassium mercuric iodide solution). Formation of a cream coloured precipitate indicates the presence of alkaloids.

2.5.2 Test for Glycosides
2.5.2.1 Keller-Kiliani Test The extract was mixed with 2 ml of glacial acetic acid containing 1 or 2 drops of freshly prepared ferric chloride solution. The mixture was shaken well and was carefully poured into a test tube containing concentrated sulphuric acid along the sides. Formation of a brown ring at the junction indicates the presence of cardiac glycosides.

2.5.3 Test for flavonoids
2.5.3.1 Shinoda Test Crude extract was mixed with a few small pieces of Magnesium ribbon for a minute and a few drops of concentrated HCl was added drop wise into this mixture. Development of pink scarlet colour or light red colour after a few minutes indicates the presence of flavonoids.

2.5.3.2 Lead acetate Test: Small quantity of the extract was treated with a few drops of lead acetate solution. Formation of yellow colour or yellow creamy precipitate indicates the presence of flavonoids.

2.5.3.3 Alkaline reagent Test: The extract was mixed with 2% NaOH solution. Intense yellow colouration which loses the intensity on the addition of dilute acid indicates the presence of flavonoids.

2.5.4 Test for tannins
2.5.4.1 Ferric chloride Test: 2 ml of freshly prepared ferric chloride solution was added to 2 ml of the concentrated extract. Formation of dark blue or green or black colour indicates the presence of tannins.

2.5.5 Test for phenols
2.5.5.1 Ferric chloride Test: To 2 ml of the extract, 2 ml of freshly prepared ferric chloride solution was added. The development of blue-green or black colour indicates the presence of phenols.

2.5.6 Test for saponins
2.5.6.1 Froth test: 2 ml of the extract was mixed with 20 ml of distilled water in a graduated test tube and shaken well for 10 minutes. Formation of 1 cm thick froth indicates that the sample contains saponins.

2.5.7 Test for sterols
2.5.7.1 Liebermann-Burchard Test: 2 ml of the extract was mixed with a few drops of acetic anhydride. It was boiled and cooled and concentrated sulphuric acid was added along the sides of the test tube carefully. A brown ring at the junction of two layers and the upper layer turning green indicates the presence of sterols.

2.5.8 Test for diterpenes
2.5.8.1 Copper acetate Test: 2 ml of the extract was mixed with 3-4 drops of copper acetate solution and shaken well. The formation of green colour indicates the presence of diterpenes.

2.5.9 Test for carbohydrates
2.5.9.1 Molisch's test: 2 ml of the extract was taken in a test tube and few ml of Molisch's reagent was added along the sides. Formation of violet ring at the junction indicates the presence of carbohydrates.

2.5.9.2 Fehling's test: 1 ml each of Fehling's solution A and B were mixed and boiled for one minute. Equal volume of the extract was added and then boiled in a water bath for 5 minutes. Formation of reddish brown colour indicates the presence of reducing sugar.

2.5.9.3 Iodine test: 1 or 2 drops iodine solution was added to 1ml of the extract. Formation of dark blue colour indicates the presence of carbohydrates.

2.5.10 Test for proteins and amino acids
2.5.10.1 Ninhydrin test: 3 ml of the extract was boiled with 3 drops of 5% Ninhydrin solution. Formation of blue or violet colouration indicates the presence of amino acids.

2.5.10.2 Xanthoproteic test: The extract was treated with a few drops of concentrated nitric acid. Formation of yellow colour indicates the presence of proteins.

3. Results and discussion
The pharmacognostic characters of the fruits were studied with the help of different parameters. Organoleptic study of the fruit indicated characteristic colour, odour and taste (Table 1).

Table 1: Organoleptic characters

S. No	Parameter	Observation
1	Colour	Yellowish brown
2	Odour	Aromatic
3	Taste	Astringent

The microscopic study of the fruit pericarp showed the following tissue systems (Fig 1)
The epidermis is single layered made of cells having a swollen base and hair like prolongation. The cortex is wide made up of parenchyma cells without intercellular spaces some of which contain rosette like crystals of calcium oxalate. It is intermingled with stone cells of diverse shapes. The stone cells found towards the periphery are tangentially elongated with narrow lumen. The diameter of the stone cells become lesser and the size of the lumen increases towards the inside. The central region is occupied by round or oval stone cells found in groups of upto 15, having broad lumen. Most of the cells of the cortex contain simple or compound starch grains (Fig 2) The vascular bundles are conjoint, collateral and endarch.

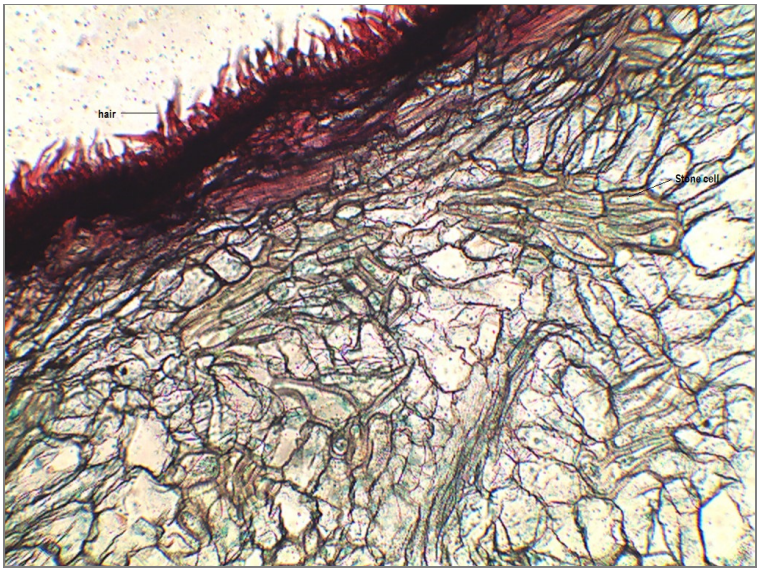

Fig 1: T S of the pericarp - A portion showing epidermal hairs and stone cells.

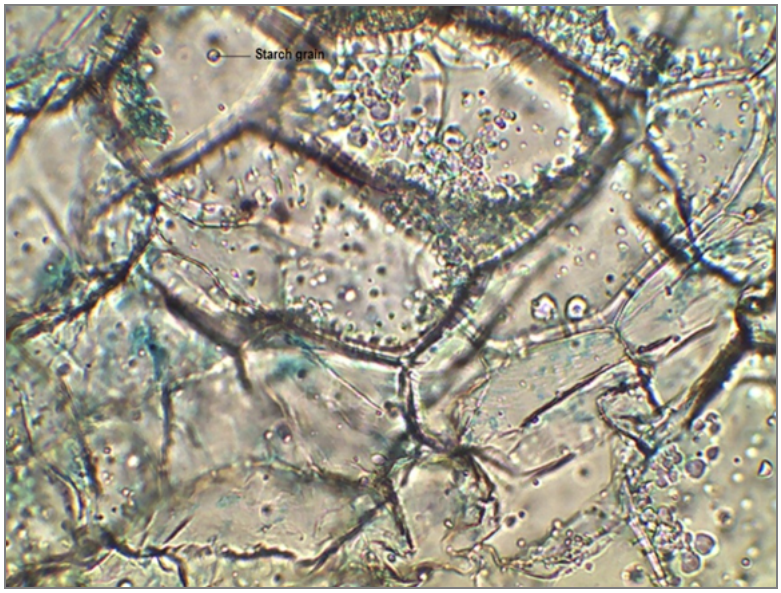

Fig 2: A portion enlarged showing starch grains

The microscopic examination of the powder showed hairs, stone cells, fibres, starch grains, needles, spiral and pitted vessels and pitted tracheids. (Fig 3-8)
Powder characters

Colour - Yellowish brown.
Hairs - Length ranges from 168 µm -91 µm and breadth ranges from 14 - 7 µm, the average length being 128.55 µm and average breadth being 0.95 µm.

Stone cells – found singly or in groups.

Single stone cells – Length ranges from 378-42 µm and breadth ranges from 70-28 µm, the average length being 165.67 µm and average breadth being 38.5 µm.

Group of stone cells – (2-8) – The average length is 260.75 µm and the average breadth is 148.75 µm. The length ranges from 462 µm-112 µm and breadth from 308-70 µm.

Fibres – Average length - 390.83 µm and breadth 1.16 µm. Range in length from 630 µm to 105 µm.

Starch grains – Simple spherical with an average diameter of 30.33 µm, ranging from 35 µm-14 µm or oval with an average length of 29.4 µm and breadth of 24.5 µm, length ranging from 31.5 - 28 µm and breadth ranging from 28 – 21 µm.
Compound starch grains are also found with an average dimension of 85.9 µm to 112 µm.

Xylem –Both spiral vessels having an average diameter of 17.5 µm to 70 µm and pitted vessels with an average diameter of 27.13 µm to 92.16 µm were observed. Pitted trachieds were also observed.
Needle like crystals of Calcium oxalate were also observed.

Fig 3: Powder showing hair

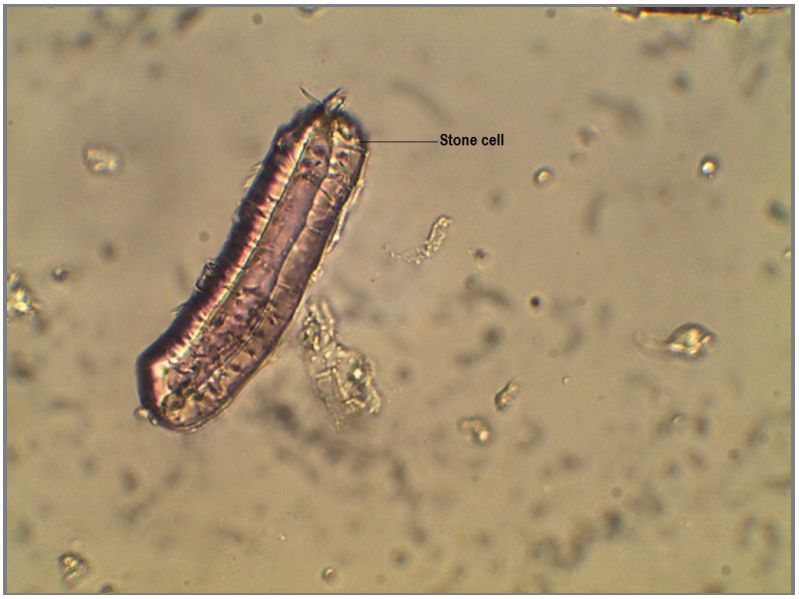

Fig 4: Powder showing stone cells

Fig 5: Powder showing spiral vessel

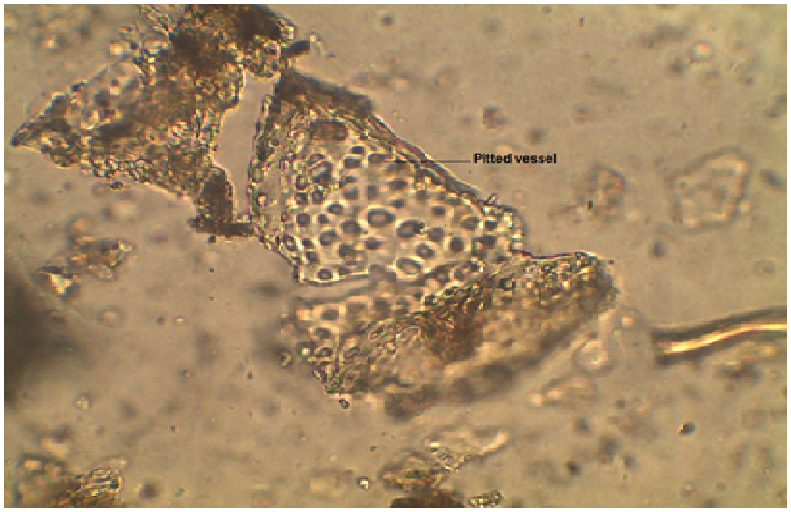

Fig 6: Powder showing pitted vessel

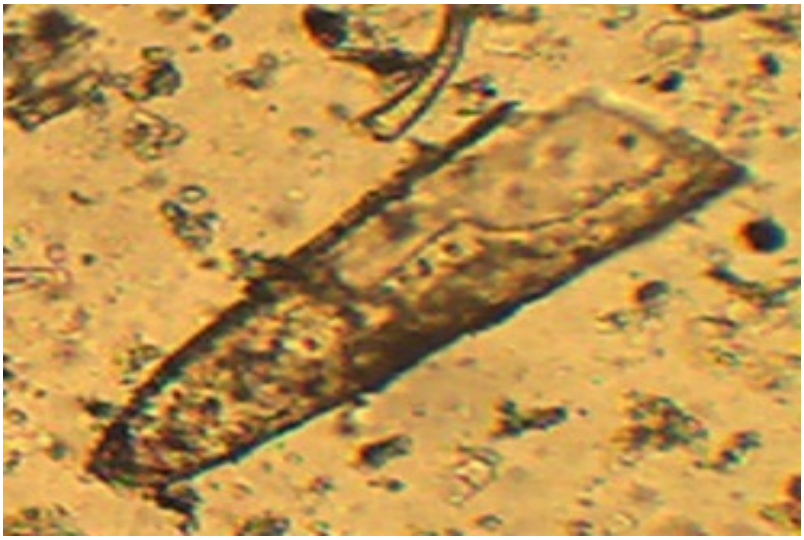

Fig 8: Powder showing pitted tracheid

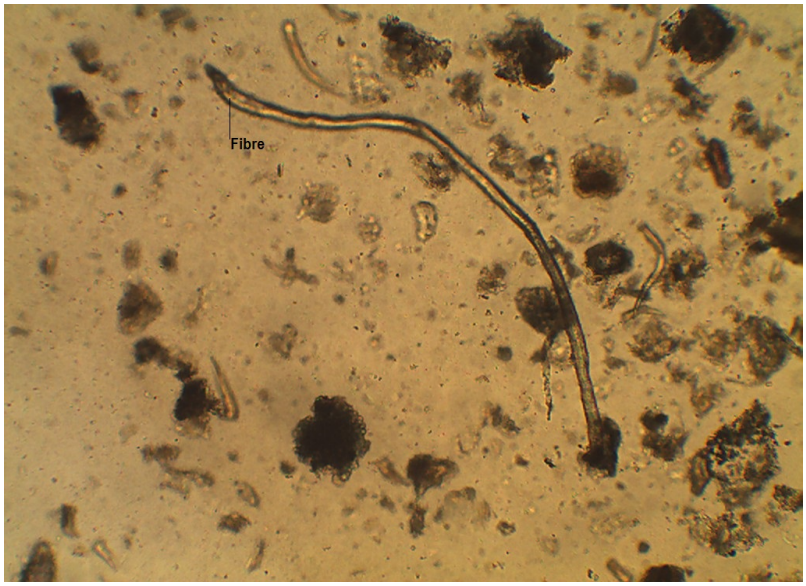

Fig 9: Powder showing fibre

When the powder was treated with different chemical reagents like 1M HCl, Petroleum Ether, Conc HNO_3, Acetic acid, Benzene, Methanol, 50% Ethanol, 1 N NaOH, Picric Acid, Chloroform and $FeCl_3$ different shades of brown, green and black colours were obtained. (Table 2)

Table 2: The fluorescent analysis of the powder of *Terminalia bellirica*

S No	Treatment	Visible light	Short UV(254 nm)
1	Powder+1M HCl	Yellowish brown	Light green
2	Powder+Pet Ether	Light brown	Light green
3	Powder+conc HNO_3	Orange brown	Light green
4	Powder+Acetic acid	Greenish brown	Greenish black
5	Powder+50% H_2SO_4	Light brown	Light green
6	Powder+Benzene	Light brown	Greenish brown
7	Powder+Methanol	Yellowish brown	Light green
8	Powder+50% Ethanol	Yellowish brown	Light green
9	Powder+1 N NaOH	Reddish brown	Dark green
10	Powder+$FeCl_3$	Bluish black	Black
11	Powder+Chloroform	Brown	Green
12	Powder+Picric acid	Yellowish brown	Fluorescent green

Preliminary phytochemical analysis of the aqueous extract revealed the presence of glycosides, flavonoids, tannins, phenols, saponins, diterpenes, carbohydrates and proteins (Table 3).

Table 3: Phytochemical evaluation of aqueous extract of the fruit rind of *Terminalia bellirica*.

Test	Observation
Test for Alkaloids	
Dragendorff's Test	-
Mayer's Test	-
Test for Glycosides	
Keller-Kiliani Test	+
Test for flavonoids	
Shinoda Test	-
Alkaline Reagent Test	+
Lead Acetate test	+

Test for tannins	
$FeCl_3$ test	+
Test for phenols	
$FeCl_3$ test	+
Test for saponins	
Froth test	+
Test for sterols	
Liebermann- Burchard's test	-
Test for diterpenes	
Copper acetate test	+
Test for carbohydrates	
Molisch's test	+
Fehling's test	+
Iodine test	+
Test for proteins and amino acids	
Ninhydrin test	+
Xanthoproteic test	+

4. Conclusion

The organoleptic characters offer a scientific basis for the use of *Terminalia bellerica* in different systems of medicine. The powder when treated with different chemical reagents exhibited different colours which could be used as standardization parameters. The anatomical characters could be used to distinguish this fruit from other myrobalans. The powder microscopy confirmed that the presence of hairs, starch grains, calcium oxalate crystals and stone cells are characteristic of this fruit and can be used as markers to prevent adulteration. Preliminary phytochemical analysis of the aqueous extract revealed the presence of several phytochemicals justifying its use in many Ayurvedic formulations.

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