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Pharmacognostic evaluation of *Nyctanthes arbortristis* bark

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ABSTRACT

Objective: To study detailed Pharmacognosy of the bark of *Nyctanthes arbortristis* Linn (Oleaceae), an important plant in Indian system of medicine. **Methods:** the macroscopy, microscopy, physicochemical analysis, preliminary phytochemical testing of powder of the plant bark and other WHO recommended methods for the standardization was done. **Results:** Trunk bark consists of two distinct regions i.e. outer bark and inner bark. Outer bark consists of broad periderm of a wide phellem and inner phelloderm regions. Inner bark is broader than the outer part and it includes all the secondary phloem tissues. It can be distinguished into 2 zones viz. collapsed secondary phloem and non-collapsed secondary phloem regions. Collapsed secondary phloem region consist of thick blocks of phloem sclereids and radially oblique dark streaks of crushed sieve tubes and dilated axial parenchyma cells. Non-collapsed secondary phloem region is the conducting part of the phloem where the sieve elements are intact. It consists of intact sieve tube members, companion cells, axial parenchyma cells and narrow undilated ray. Calcium oxalate crystals are abundant in collapsed phloem region. **Conclusions:** it can be concluded that the pharmacognostic profile of *N. arbortristis* bark is helpful in developing standards for quality, purity and sample identification.

1. Introduction

Nyctanthes arbortristis Linn (Oleaceae) is one of the well known medicinal plant. It is commonly called as nyctanthes means night flowering and arbortristis means as it loses its brightness during day time. It is common wild hardy large shrub or small tree, native to India, distributed wild in sub-Himalayan regions and southwards to Godavari. It is also planted in Indian gardens for ornamental purpose due to its highly fragrant flowers [1–2]. It is a shrub or small tree up to 10 m in height with gray to greenish rough bark with stiff whitish hairs. Leaves are opposite, ovate, acute or acuminate, entire

or with few large distant teeth, short bulbous hairs rounded or slight cuneate. Flowers are small, delightful fragrant, sessile, slender, and hairy; corolla glabrous, orange colored and lobes are white. Fruits are a capsules of 1–2 m in diameter, long and broad, compressed, 2 celled separating into 2 flat one seeded carpels, reticular veined and glabrous. Leaves are responsible for some CNS activities like hypnotic, tranquilizing and local anesthetics [3–5] and antiasthmatic activity [6]. β -Sitosterol isolated from *N. arbortristis* leaves showed analgesic and anti-inflammatory activity [7]. Iridoid glucosides isolated from this plant has antileishmanial activity [8]. Ethanolic flower extract of this plant is used for the synthesis of gold nanoparticles [9]. Seeds, leaves and flower extract of this plant showed CNS depressant activity [10]. Arbortristoside-A isolated from seeds possesses anti-inflammatory and analgesic activity [11]. Leaf and fruit extracts are useful in the treatment of arthritis [12]. Arbortristoside A and arbortristoside C isolated from plant

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showed antiviral activity [13]. For the standardization and quality assurance purpose, the following three attributes must be verified: authenticity, purity and assay. Hence the objective of present study is to evaluate various pharmacognostic parameters such as macroscopy, microscopy, physicochemical and phytochemical studies of the plant.

2. Materials and methods

2.1. Chemicals and instruments

Phloroglucinol, glycerin, hydrochloric acid, potassium hydroxide and all other chemicals used in the study were of analytical grade. Microtome is used for taking sections.

2.2. Plant material

Bark of *N. arbortristis* was collected from Ahmednagar district of Maharashtra in August 2007 and authenticated by Dr. P.S.N. Rao, Botanical Survey of India, Pune, where a sample specimen (voucher number: Nirmal-1) has been deposited.

2.3. Macroscopic and microscopic analysis

The macroscopy and microscopy of the bark of *N. arbortristis* was studied according to the method of Brain and Turner (1975) [14]. For the microscopical studies, transverse sections were prepared and stained. The powder microscopy was performed according to the methods of Kokate (1994) [15] and Khandelwal (2007) [16].

2.4. Physicochemical analysis

Physicochemical values such as the percentage of ash values and extractive values were determined according to the official methods [17–18] and as per WHO guidelines on quality control methods for medicinal plant materials [19–20].

2.5. Preliminary phytochemical screening

Preliminary phytochemical screening was carried out using the standard procedure described by Kokate (1994) [15].

3. Results

3.1. Macroscopic characteristics

Trunk bark is dark gray or brown in color, rough and firm. Bark surface is dippled due to scaling off of circular barks and patchy due to gray brown colored regions. Scaling off of the bark by circular flakes. Inner bark is creamy white, soft and collapsed and non-collapsed phloem zone distinctly visible (Figure 1–1).

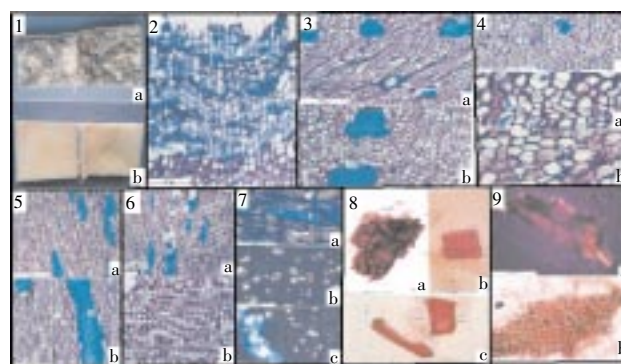


Figure 1. Morphology, histology and powder characteristics of *N. arbortristis* bark.

1–Macroscopic characteristics; a–outer surface; b– inner surface, 2–Structure of the outer bark (periderm), 3– Structure of the inner bark (Collapsed phloem), 4– Structure of the non-collapsed phloem; a– TS of non-collapsed phloem; b– Non-collapsed phloem showing sieve tube members; c– Structure of the perforation plate, 5– TLS of phloem (Collapsed phloem); a– TLS of phloem under low magnification; b– TLS of phloem under high magnification, 6– RLS of phloem; a– RLS of phloem showing sclereids and phloem rays; b–phloem rays enlarged; 7– Crystal distribution in the bark; a–Crystals and sclereids in the heterocellular periderm; b–Crystals in the collapsed phloem; c–Crystals in the phloem ray and sclereids, 8–Powder microscopy in the bark; a–Macroscleireids; b–Square shaped sclereids; c–Rectangular shaped sclereids, 9– Powder microscopy in the bark; a–Sclereids under polarized light microscope; b–Pieces of periderm cell.

Where, Pld– Phelloderm, Pm–Phellem, Pe–Periderm, Scl– Sclereids, CPh– Collapsed phloem, PhP– Phloem parenchyma, PhR– Phloem ray, NCPH– Non-collapsed phloem, ST– Sieve tube members, Pi– Pits, PP– Perforation plate, Cr–Crystal, MScl– Macroscleireid, SScl– Square shaped sclereids, RScL– Rectangular shaped sclereids, W–Wall.

3.2. Microscopic characteristics

Trunk bark consists of two distinct regions i.e. outer bark and inner bark.

Outer bark: Outer bark measures about 600 μ m in width. It consists of broad periderm of a wide phellem and inner phelloderm regions. Phellem measures an average radial width of about 500 μ m and the phelloderm is 100 μ m wide. The outer surface of phellem is uneven with numerous fissures and consists of thin walled, suberised, tangentially oblong

cells as well as lignified narrow tangential bands of phelloids. The phelloderm cells are thin walled, cubical or rectangular and arranged in compact radial files. There are regions where sequent periderm originates in the form of shell shaped bands enclosing secondary phloem tissue. Formation of sequent periderm leads to the compound structure called as rhytidome (Figure 1–2).

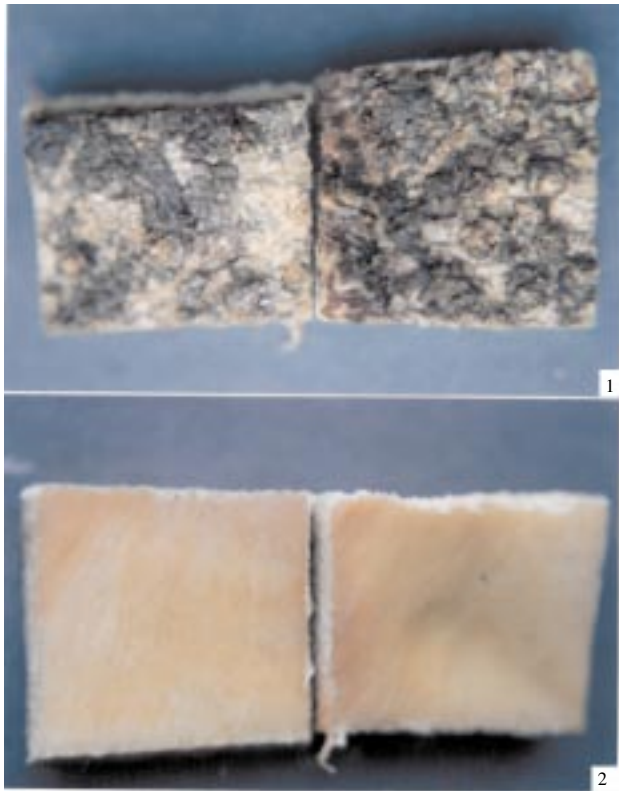


Figure 1. Macroscopic characteristics of *N. arbortristis* bark.
1. Outer surface, 2. Inner surface

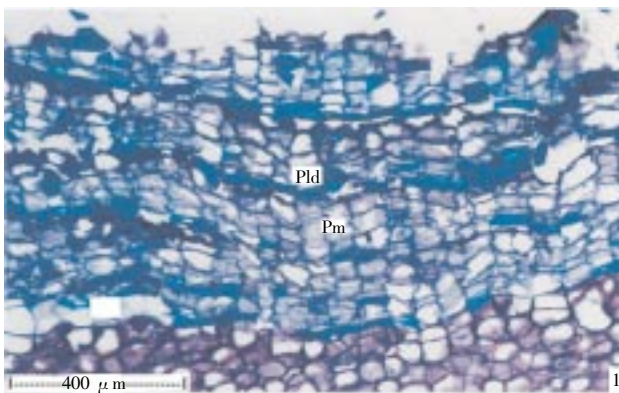


Figure 2. Structure of the outer bark (periderm).
Pld– Phelloid, Pm–Phellem, Pe–Periderm.

Inner bark: Inner bark is broader than the outer part and it includes all the secondary phloem tissues. It can be distinguished into 2 zones viz. collapsed secondary phloem

and non–collapsed secondary phloem regions.

a. **Collapsed secondary phloem region:** It is outer to the inner part of the bark and idle in position. It is the broadest zone. It consists of thick blocks of phloem sclereids and radially oblique dark streaks of crushed sieve tubes and dilated axial parenchyma cells. The phloem rays are narrow with oblong cells. The sclereids are thick walled and occur in small masses and are separated by wide parenchymatous tissue. Ray dilation is less prominent. Collapsed secondary phloem region extends up to the periderm zone appears as pseudo cortex (Figure 1–3).

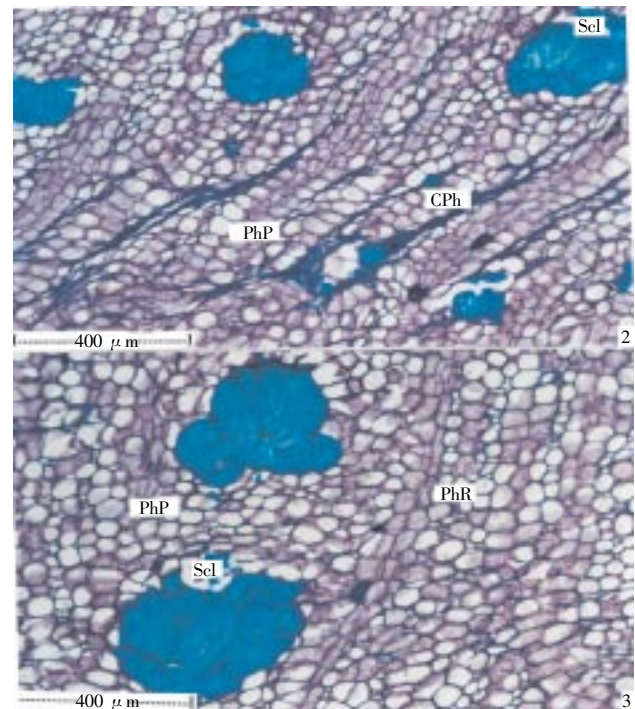


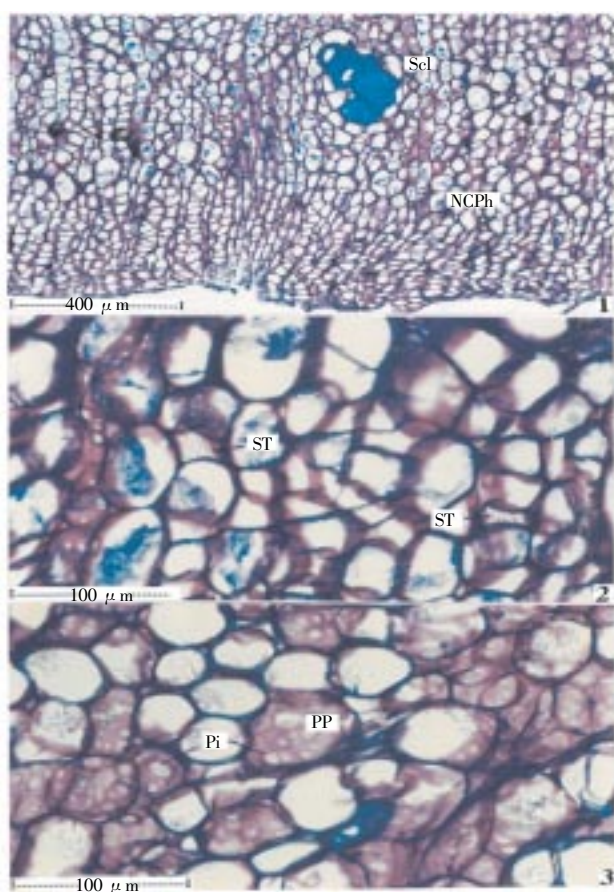
Figure 3. Structure of the inner bark (Collapsed phloem).

Scl– Sclereids, CPh– Collapsed phloem, PhP– Phloem parenchyma, PhR– Phloem ray.

b. **Non–collapsed secondary phloem region:** This zone is the conducting part of the phloem where the sieve elements are intact. It is 400 μ m wide and is next to the cambial zone. It consists of intact sieve tube members, companion cells, axial parenchyma cells and narrow undilated ray. The cells are in regular radial rows in the inner part but radial seriation is distributed and the cells become random in arrangement in older part. The sieve tube members are non–storied and they are polygonal in cross sectional view. The sieve plate is simple with fairly wide sieve pores. The axial parenchyma cells are not abundant. In cross sectional shape, they are more or less similar to the sieve tubes but larger in size (Figure 1–4).

Table 1Preliminary phytochemical screening of *Nyctanthes arbortristis* bark extracts.

Chemical constituent	Chemical test	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
Alkaloid	Dragendorff's test	–	+	–	+	–
	Mayers test	–	+	–	+	–
Steroids	Salkowski test	+	+	–	–	–
	Liebermann–burchard test	+	+	–	–	–
Triterpene	Vanillin–sulphuric acid test	+	+	–	–	–
Tannin	Ferric chloride test	–	–	–	+	+
	Dilute nitric acid test	–	–	–	+	+
Glycoside	Keller–killani test	–	+	–	+	+
Carbohydrate	Molish test	–	–	–	–	+
	Fehling's test	–	–	–	–	+
Flavonoid	Shinoda test	–	–	+	+	–
	Lead acetate test	–	–	+	+	–
Saponins	Foam formation test	–	–	–	–	–
Proteins	Biuret test	–	–	–	–	–
	Millon's test	–	–	–	–	–
Amino acids	Ninhydrin test	–	–	–	–	–

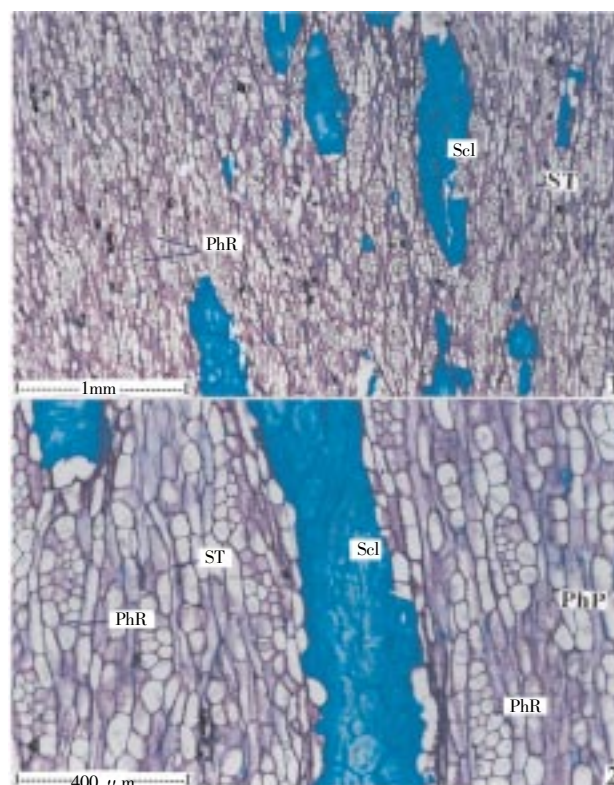
**Figure 4.** Structure of the non-collapsed phloem.

1–TLS of non-collapsed phloem, 2– Non-collapsed phloem showing sieve tube members, 3– Structure of the perforation plate.

Scl– Sclereids, NCPh– Non-collapsed phloem, ST– Sieve tube members, Pi– Pits, PP– Perforation plate.

Tangential longitudinal section (TLS): In TLS the ray seriation and certain characters of sieve tube members can be seen. The phloem rays are exclusively multiserial, broad and high. In the collapsed phloem region, sclereids are abundant and occur

in large masses. The sclereids are thick walled and narrow lumened. The ray cells are vertically elongate, thick walled and homo cellular. The height of the rays range from 200–300 μ m and 50–70 μ m in width. The rays are non-storied. The sieve tube members are either straight or slightly curved and are 250 μ m in height. Sieve plate is simple and slightly oblique. Non-collapsed region is similar to the collapsed region except the sclereids are completely absent in this zone and the sieve tube members are intact (Figure 1–5).

**Figure 5.** TLS of phloem (Collapsed phloem).

1. TLS of phloem under low magnification, 2. TLS of phloem under high magnification.

Php– Phloem parenchyma, PhR– Phloem ray, Scl– Sclereids, ST– Sieve tube members.

Radial longitudinal section (RLS): In RLS the rays show narrow, radially oblong cells of uniform shape and all the cells are procumbent type (Figure 1–6).

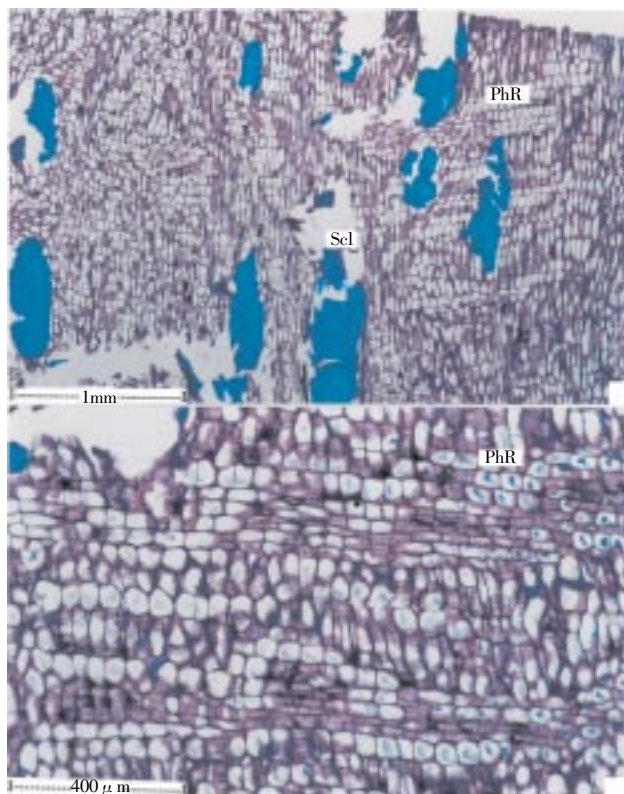


Figure 6. RLS of phloem.

1–RLs of phloem showing sclereids and phloem rays, 2– phloem rays enlarged.

PhR– Phloem ray, Scl– Sclereids.

Crystal morphology and distribution: Presence of crystals and sclereids is a diagnostic feature. In periderm region it consists of thick walled sclereids alternating with thin walled cells. Calcium oxalate crystals are abundant in collapsed phloem region. They are predominant in the ray cells of the phloem. The crystals and sclereids appear bright against dark background under polarized light. The crystals are prismatic type (Figure 1–7).

3.3. Powder microscopic results

Powder of the bark consists of following elements.

Sclereids: Sclerenchyma element, the sclereids are abundant in the powder. They are isodiametric or elongated and rectangular in shape. Sclereids are in small or large masses or broken into individual cells. The squarish sclereids are 70 X 80 μm ; the rectangular sclereids are 50 X 90 μm ; the narrowly elongated sclereids are 20 X 140 μm . The sclereids have thick walls with canal like simple piths; the lumen of the cells is wide. When

the sclereids were viewed under polarized light microscope it appeared bright indicating that sclereids have lignified walls (Figure 1–8).

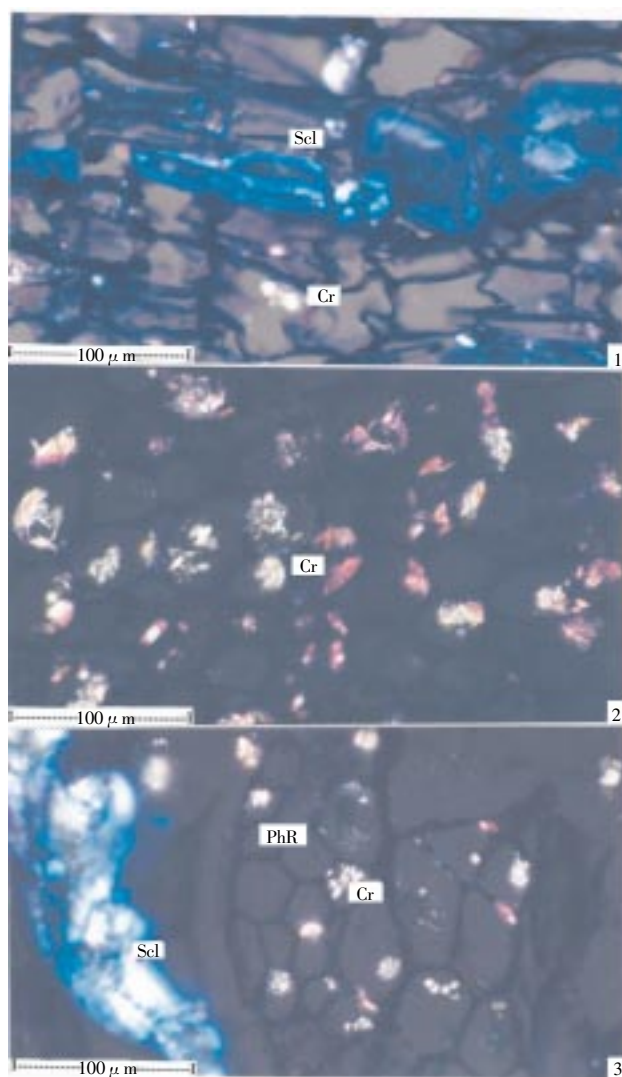


Figure 7. Crystal distribution in the bark; 1. Crystals and sclereids in the heterocellular periderm; 2. Crystals in the collapsed phloem; 3. Crystals in the phloem ray and sclereids.

(Scl– Sclereids, Cr–Crystal, PhR– Phloem ray).

Periderm: Powder contains thick pieces of periderm. It is square or rectangular shaped, thin walled cells; 50 X 50 μm and 30 X 50 μm in size (Figure 1–9).

3.4. Preliminary phytochemical screening

Preliminary phytochemical screening mainly revealed the presence of steroids and triterpenes in petroleum ether extract; alkaloid, steroids, triterpenes and glycosides in chloroform extract; flavonoids in ethyl acetate extract; alkaloid, tannins, glycosides and flavonoids in ethanol extract and tannins,

glycosides and carbohydrates in aqueous extract (Table 1).

3.5. Physicochemical constants

Ash values of the drug gives idea about earthy matter or inorganic composition and other impurities present along with the drug. Various physicochemical parameters such as total ash, water soluble ash and acid insoluble ash of *N. arbortristis* bark was found to be 8.79, 0.09 and 0.17 % w/w, respectively. Moisture content in the bark was found to be 6.87 % w/w. The extractive values are primarily useful for the determination of the exhausted or adulterated drug. Various extractive values such as petroleum ether soluble extract, chloroform soluble extract, ethanol soluble extract and water soluble extract of *N. arbortristis* bark was found to be 1.4, 1.06, 15.76 and 17.5 % w/w respectively.

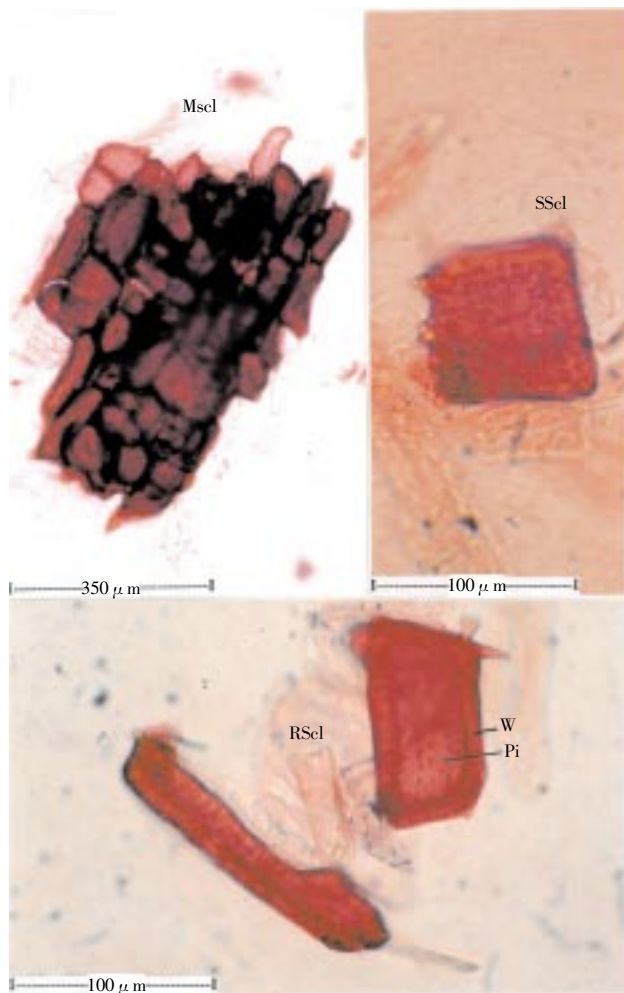


Figure 8. Powder microscopy in the bark;1. Macroscleireids, 2. Square shaped sclereids, 3. Rectangular shaped sclereids (MScl- Macroscleireid, SScl- Square shaped sclereids, RScl- Rectangular shaped sclereids, Pi-Pits, W-Wall)

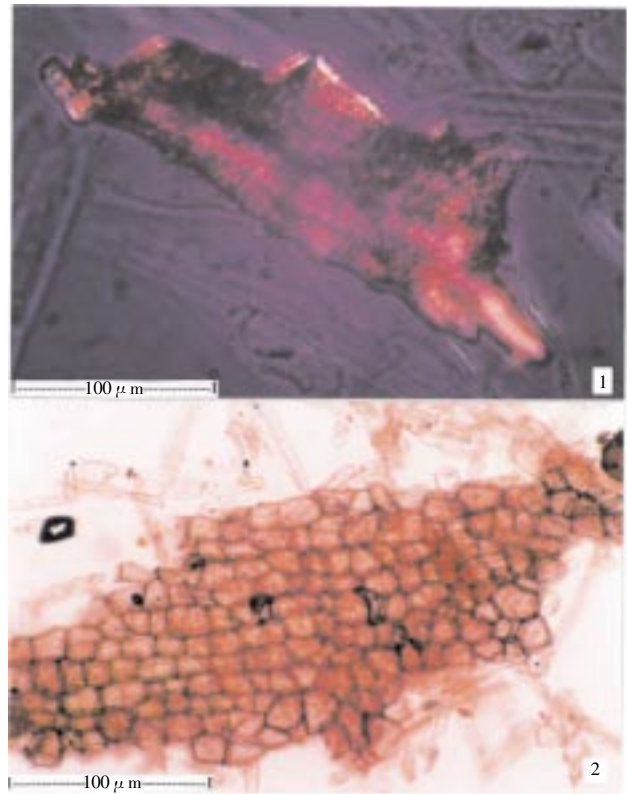


Figure 9. Powder microscopy in the bark;1. Scleireids under polarized light microscope; 2. Pieces of periderm cell.

4. Discussion

Standardization is an essential measure of quality, purity and authenticity. Microscopic method is one of the simplest and cheapest methods to start with establishing the correct identification of the source materials. As there is no pharmacognostic work recorded on this medicinally potent plant, the present work was undertaken to lay down the standards which could be useful for establishing its authenticity. Macro and micro standards are useful identifying parameters for authentication of the drug. The information obtained from the preliminary phytochemical screening will reveal the useful findings about chemical nature of the drugs. Total ash values and extractive values are useful in identification and authentication of the plant material [21-22]. Extractive values are useful to evaluate the chemical constituents of crude drug [23]. Preliminary phytochemical screening mainly revealed the presence of steroids and triterpenes in petroleum ether extract; alkaloid, steroids, triterpenes and glycosides in chloroform extract; flavonoids and flavonoids in ethyl acetate extract; alkaloid, tannins, glycosides and carbohydrates in aqueous extract of the plant bark. T.S. of the bark confirmed the presence of rhytidome, phloem sclereids, multiseriate phloem rays and the ray cells are procumbent

type, and calcium oxalate crystals are abundant in collapsed phloem region and they are prismatic type.

In conclusion, the present work was undertaken with a view to lay down standards which could be useful to detect the authenticity of the medicinally useful plant. Pharmacognostic evaluation can be useful to substantiate and authenticate the drug.

Conflict of interest statement

We declare that we have no conflict of interest.

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