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PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION OF *NYCTANTHES ARBOR-TRISTIS* STEM

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ABSTRACT: Objective: To study detailed pharmacognostic characters of the stem of *Nyctanthes arbor-tristis* (Oleaceae), along with their physico-chemical parameters, fluorescence analysis and phytochemical screening. **Methods:** The pharmacognostic characters were determined in terms of macroscopy, microscopy, powder microscopy, physicochemical analysis, fluorescence analysis and preliminary phytochemical investigation of plant stem. **Results:** The microscopic study shows the general characteristic of stem. Physico-chemical investigation shows the total ash, acid insoluble ash, water soluble ash were 8.69 ± 0.17% w/w, 0.21 ± 0.11% w/w, and 3.92 ± 0.05% w/w respectively. Phytochemical analysis revealed the presence of various phytochemical groups like alkaloids, glycosides, steroids, phenolic, tannins constituents. **Conclusion:** It can be concluded that the established pharmacognostic profile of *Nyctanthes arbor-tristis* stem will be helpful in developing pharmacopoeial standards for correct identification and quality control.

INTRODUCTION: India has a rich heritage of traditional medicine and traditional health care systems that have been flourishing for many centuries. Now a days, prevalent use of traditional medicines in the developed countries is trending and it has become more popular throughout the world. Traditionally, important large shrub of tropical and subtropical regions of the world have been used to counteract disease [1].

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Nyctanthes arbour - tristis Linn. (Oleaceae) commonly known as Parijat or Harsinghar, a hardy large shrub or small tree up to 5 - 10 m in height, widely occurring in outer Himalayan ranges from Kashmir to Nepal and throughout India up to 1000 - 1500 m altitude [2].

The name 'Nyctanthes' has been coined from two Greek words 'Nykhta' means night and 'anthos' means flower. It is also planted in gardens due to its highly fragrant flowers [3]. It is shrub or small tree, with drooping branches and quadrangular branchlets. Leaves are opposite, ovate, acute or acuminate, entire slight cuneate. Flowers are small, 3 - 7 in head, arranged in trichotomous cymes, delightfully fragrant, sessile, slender, and hairy; corolla glabrous, orange colored and lobes are

white [4, 5]. 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 [6]. Different parts of this plant are used in folk-medicines [7, 8]. The leaves are bitter, useful in chronic fever [9], malarial fever [10], obstinate sciatica, constipation, haemorrhoids [11] and eczema [12]. The flowers are astringent, stomachic, and useful in dyspepsia, greyness of hair and baldness [11]. The plant elaborates different classes of organic compounds of medicinal importance including alkaloids, terpenes, steroids, β-sitosterol, glycosides, iridoid glycosides, arbortristoside-A, B, C, D, E [13, 14, 15, 16, 17, 18]. Different parts of this plant are used in Indian systems of medicine for various pharmacological actions like as anti-leishmaniasis, anti-viral, anti-fungal, anti bacterial, anti-pyretic, antihistaminic, anti-malarial, anti-oxidant, hepatoprotective, and anti-inflammatory activities [19].

The literature survey and scientific data revealed that no systematic pharmacognostical parameter had been carried out on the stem of *Nyctanthes arbor-tristis* Linn. till date. Hence, the objective of present study is to evaluate various pharma-cognostic parameters such as macroscopy, microscopy, physicochemical and phytochemical evaluations of the *Nyctanthes arbor-tristis* Linn. (stem).

MATERIALS AND METHODS:

Chemicals and Instruments: Phloroglucinol, glycerin, hydrochloric acid, potassium hydroxide and all other chemicals used in the study were of analytical grade.

Plant Material: The stem of *Nyctanthes arbortristis* was collected from Dehradun, Uttarakhand, India in the month of August 2016 and authenticated by Dr. S. K. Srivastava, Botanical Survey of India, Northern regional centre, Dehradun, where a voucher specimen (specimen no. 116216) has been deposited.

Macroscopic and Microscopic Analysis: Macroscopic studies were done using simple microscope. The color, shape, size, taste and odour of stem were determined. Microscopic study of fresh stem was carried out by preparing thin transverse section and staining it with concentrated hydrochloric acid: Phloroglucinol (1:1). Photographs sections were carefully taken. The dried stem were powdered and treated with 5% KOH solution followed by staining with concentrated hydrochloric acid - Phloroglucinol (1:1) for 5 minutes and mounted in 50% glycerine solution [20, 21, 22].

Physiochemical Analysis: Physiochemical parameter such as ash values (total ash, acid insoluble ash) and extractive values (water soluble, alcohol soluble extractives) were determined using powdered drug. The moisture content was detected by loss on drying method [23, 24, 25].

Fluorescence Analysis: For the fluorescence analysis of stem powder it was treated with various chemicals and was observed exclusively to different wavelengths of ultra violet (254 nm and 365 nm) and visible light for observing characteristic colour presentation [22, 26, 27].

Preliminary Phytochemical Screening: Preliminary phytochemical screening was qualitatively tested for the presence of phytochemicals as per described standard methods [22, 28, 29, 30].

RESULTS:

Macroscopic Characteristics: Organoleptic characters of stem depicted that the stem was woody in nature having light grey to greenish in colour with characteristic odour and taste. The stem was 1 - 10 m tall, erect and branched.

Microscopic Characteristics:

Stem Microscopy: Section of stem appears quadrangular and revealed the following tissues: The epidermis was single, layered consisting of rectangular cells with a thick continuous cuticle on the epidermis along with many multicellular hairs. Cortex was several cells deep below the four protruded comers while only few layers deep at the other places just beneath the epidermis. It was differentiated into collenchyma and parenchyma. Many intercellular spaces were present and the region extended upto the vascular tissue. Vascular bundles were present in the cortex, each protrudes bulb containing one. The pointed xylem end was faced towards outer side in each of the conical bundle. In other words, the vascular bundle was conjoint, collateral, open and exarch.

The microscopy revealed that the endodermis was not well developed. The pericycle was observed in the form of sclerenchymatous patches. Vascular system was composed of primary phloem, secondary phloem, cambium, primary xylem and secondary xylem. Crushed primary phloem was irregularly present in patches below the pericycle. The secondary phloem consisted of sieve tubes, companion cells, phloem parenchyma and was present in the form of a continuous ring. Cambium was present as one to three cells thick continuous layer in between phloem and xylem. Secondary xylem was present just inner to the cambial ring and consisted of mainly thick walled woody parenchyma and fibres. Trachieds and vessels were also observed. Primary xylem was situated just near the pith in a way facing its protoxylem towards the centre. Pith was found to be thin walled and parenchymatous as shown in **Fig. 1**.

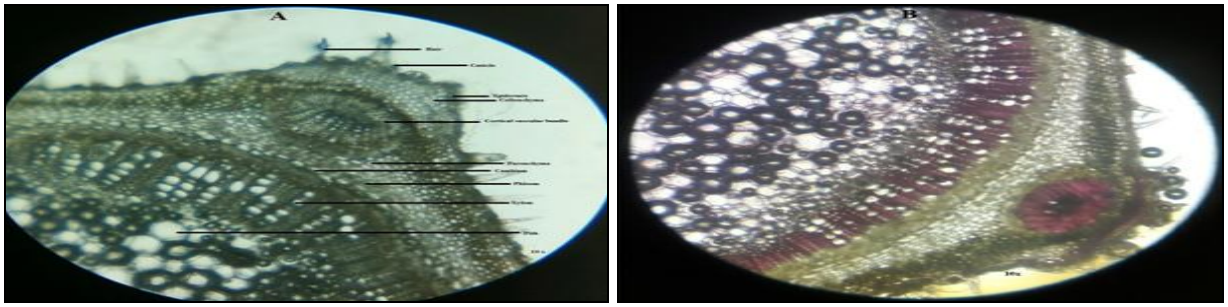

FIG. 1: TRANSVERSE SECTION OF STEM OF *NYCTANTHES ARBOR-TRISTIS* VIEWED AT 10x; A- WITHOUT STAINING REAGENT, B- WITH STAINING REAGENT

Stem Powder Microscopy: Microscopic observation of *Nyctanthes arbor-tristis* stem indicated the presence of parenchyma cells, collenchyma cells, fiber, xylem vessels and calcium oxalate crystal as shown in **Fig. 2**.

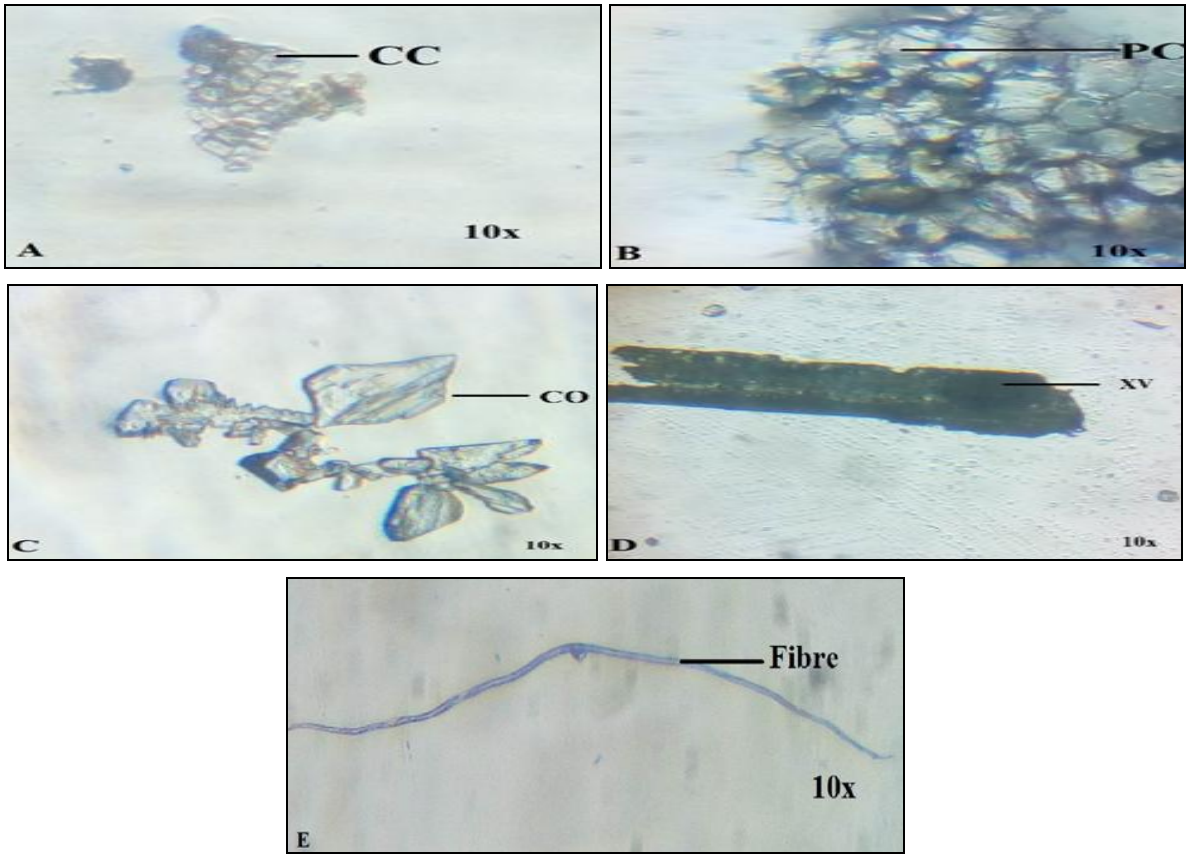

FIG. 2: POWDERED CHARACTERISTICS OF THE STEM PARTS OF *NYCTANTHES ARBOR-TRISTIS* SHOWING A: COLLENCHYMA CELL (CC) B: PARENCHYMA CELLS (PC) C: CALCIUM OXALATE CRYSTALS (CO) D: XYLEM VESSELS (XV) E: FIBER

Physicochemical Parameters: Ash value 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, acid insoluble ash and water soluble ash of *Nyctanthes arbor-tristis* stem were found to be 8.69 ± 0.17, 0.21 ± 0.11 and 3.92 ± 0.05% w/w, respectively. However, 15.93 ± 0.46% w/w alcohol soluble and 18.31 ± 0.46% w/w water soluble extractives were observed. The moisture content of stem powder was nearly 5.49 ± 0.02% w/w **Table 1**.

TABLE 1: PHYSICOCHEMICAL CONSTANTS FOR *NYCTANTHES ARBOR-TRISTIS* STEM

S. no.	Physicochemical parameter	Values (% w/w)
1	Moisture content	5.49 ± 0.02
2	Total ash	8.69 ± 0.17
3	Acid insoluble ash	0.21 ± 0.11
4	Water soluble ash	3.92 ± 0.05
5	Alcohol soluble extractive	15.93 ± 0.46
6	Water soluble extractives	18.31 ± 0.46

Fluorescence Analysis: Fluorescence analysis of stem powder was carried out after treating it with several solvents and chemicals. Fluorescence was observed at 254 and 365 nm comparing its change of colour in visible light. The observations are presented in **Table 2**.

TABLE 2: FLUORESCENCE ANALYSIS OF *NYCTANTHES ARBOR-TRISTIS* STEM

Treatment	Visible light	Under UV light	
		Short wavelength (254 nm)	Long wavelength (365 nm)
Powder	Brown	Light brown	Green
Powder + Methanol	Brown	Light brown	Yellowish green
Powder + 70% ethanol	Brown	Light brown	Green
Powder + Pet. ether	Light brown	Light green	Green
Powder + 50% H_2SO_4	Brown	Greenish brown	Brownish
Powder + 50% HCl	Dark Brown	Green	Green black
Powder + 1N NaOH (aq.)	Light brown	Dark brown	Brownish black
Powder + 1N NaOH (alc.)	Light brown	Dark green	Greenish black
Powder + 50% HNO_3	Brown	Light brown	Light green
Powder + 5% KOH	Brown	Purplish green	Dark purplish green
Powder + Ammonia	Brown	Green	Black
Powder + Picric acid	Yellowish brown	Green	Dark brown

Preliminary Phytochemical Screening: Preliminary phytochemical screening of *Nyctanthes arbor-tristis* is shown in **Table 3**.

TABLE 3: PHYTOCHEMICAL SCREENING *NYCTANTHES ARBOR-TRISTIS* STEM

S. no.	Class of constituents	PEE	CE	EAE	EE	AE
1	Amino acids	-	-	-	-	-
2	Proteins	-	-	-	-	-
3	Carbohydrates	-	-	-	+	+
4	Steroids	+	+	-	-	-
5	Triterpenoids	+	+	-	-	-
6	Alkaloids	-	+	-	+	+
7	Glycosides	-	+	-	+	+
8	Saponins	-	-	-	-	-
9	Flavonoids	-	-	+	+	-
10	Tannins	-	-	-	+	+
11	Phenolic compounds	-	+	+	+	+

PEE- Pet. ether extract, CE- Chloroform extract, EAE- Ethyl acetate extract, EE- Ethanol extract, AE- Aqueous extract, (-) Negative, (+) Positive

DISCUSSION: The wide use of herbal drugs in traditional medicines and herbal formulations, standardization that has been made to an important measure for ensuring and justifying quality, purity and authenticity of the crude drugs [31]. Standardization purpose, morphological and microscopic analysis is one of the simplest and cheapest methods to start with establishing the correct identification of the source materials [32, 33]. As there is no pharmacognostic work available on this medicinally potent plant, the present work was undertaken to lay down the standards which could be useful for establishing its authenticity. Organoleptic and microscopic studies are useful identifying parameters for authentication of the drug [34, 35].

Physicochemical studies acts as a reliable method for detecting adulteration. Physicochemical constants like total ash, acid insoluble ash, and water soluble ash can serve as a valuable source of information which is usually helpful in evaluation of purity and quality of a crude drug. The earthly matter or inorganic composition and other impurities which are present along with the drug are determined by the ash values. Acid-insoluble ash usually indicates the contamination with silicon material like earth and sand. Water-soluble ash was used for the estimation of the amount of inorganic elements [36]. The extractive values give an idea about the chemical constitution of the drug [33].

Fluorescence analysis is an alternative rapid useful method for identification of authentic samples and recognizing adulterants. In this analysis, the crude drugs may be examined as such, in solution or as extracts and in powdered form [37]. Fluorescence characteristics enable the identification and differentiation of plant materials from their adulterants, when physical and chemical methods are scarce. Various chemical constituents present in the plant material exhibit fluorescence on absorbing light. Fluorescence is shown by some of the constituents even in the visible range in daylight. The ultraviolet light produces fluorescence in many natural products *viz.* alkaloids like berberine, which does not show fluoresce in the daylight. With the aid of different reagents, the non fluorescent substances can easily be transformed into their fluorescent derivatives or decomposition products [33].

Phytochemical evaluation and chemo-profiling are useful for the quality assessment of plant materials. Phytochemical compounds in the plant are known to have various therapeutic importances. For instance saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects. Glycosides, flavonoids, tannins and alkaloids have hypoglycemic activities [38, 39]. Flavonoids possess the hepatoprotective, and antioxidant activities [40]. The saponins have hypocholesterolemic and antidiabetic properties [41]. In the animal studies terpenoids tend to decrease blood sugar level. Steroids as well as triterpenoids exhibit the analgesic properties [42]. The steroids and saponins are responsible for CNS activities [43].

CONCLUSION: The present study was focused on establishing pharmacognostic standards for the identification and authentication of the *Nyctanthes arbor-tristis*. Therefore, the outcomes of the above findings will serve as a promising source for laying down pharmacopoeial standards for the future studies and research.

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CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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