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Research Article

Nyctanthes arbor-tristis-Linn.: Pharmacogenetic, Physicochemical, and Phytochemical Investigations

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Abstract

Nyctanthes arbor-tristis Linn., commonly known as Parijat or night jasmine, belonging to the family Oleaceae, was investigated to establish detailed pharmacognostic, physicochemical, and phytochemical profiles of its leaves. Pharmacognostic evaluation included macroscopic and microscopic analyses, with key findings such as vein-islet number (4), vein termination number (21), and stomatal index (17.85). Additional analyses encompassed powder microscopy and fluorescence characteristics. Physicochemical properties were determined, yielding values for total ash (9.387 ± 0.565%), acid-insoluble ash (0.3067 ± 0.029%), water-soluble ash (4.067 ± 0.088%), alcohol-soluble extractive (16.57 ± 0.4927%), water-soluble extractive (17.33 ± 0.577%), and moisture content (8.48 ± 0.08%). Phytochemical screening of the ethanolic leaf extract revealed the presence of secondary metabolites, including alkaloids, glycosides, flavonoids, saponins, tannins, and steroids. These findings provide a comprehensive pharmacogenetic standard for the identification, authentication, and quality control of Nyctanthes arbor-tristis leaves, facilitating their medicinal and therapeutic applications.

1. Introduction

The goal of achieving "Health for All" remains a universal aspiration for humanity. However, the accessibility of modern pharmaceuticals continues to pose significant challenges, leaving a considerable portion of the global population underserved. This limitation has spurred a growing recognition of the importance of alternative knowledge systems and solutions that can contribute to improving public health on a broader scale [1]. Indian Systems of Medicine, collectively referred to as AYUSH (Ayurveda, Yoga and Naturopathy, Unani, Siddha, and Homeopathy), encompass traditional practices that originated in India and other systems subsequently integrated into Indian healthcare traditions. These systems offer a holistic approach to health, emphasizing prevention, individualized treatment, and the use of natural remedies. In recent years, there has been a resurgence of interest in traditional medicine, both within India and internationally [2]. This renewed focus aligns with the global shift towards integrative healthcare approaches that combine modern medical advancements with time-tested traditional practices. AYUSH systems are being increasingly recognized for their potential in addressing healthcare challenges, particularly in areas with limited access to conventional pharmaceuticals. Their evidence-based integration into primary healthcare frameworks could play a pivotal role in achieving equitable and sustainable health outcomes worldwide [1,3]. It is a terrestrial woody perennial plant found as a shrubs or small plant that grow up to 5-10 meters. Habitat is the outer region of Himalaya, Jammu and Kashmir, East Assam to Nepal, Bengal, U.P. and Central region of the India [4]. The various parts of the plants consist many pharmacological activities and used as folk medicines viz. Leaves are used in chronic fever, sciatica, constipation, haemorrhoids and eczema. The flowers are fragrant, astringent, and stomachic used in dyspepsia, greyness of hair. Different parts of plant like leaves, root, stem, flowers are used in Indian system of medicines for different pharmacological activities like anti- viral, hepatoprotective, antioxidant, anti-inflammatory, anti-fungal activities [5,6]. Establishing detailed pharmacogenetic, physicochemical, and phytochemical profiles is crucial for ensuring the plant's authenticity, quality, and efficacy in medicinal applications. Pharmacogenetic evaluation aids in identifying key structural and microscopic characteristics, while physicochemical analyses provide insight into extractive values, ash content, and moisture levels essential for quality control. Additionally, phytochemical screening identifies the secondary metabolites responsible for its pharmacological activities [5,6]. This study aims to provide a comprehensive pharmacogenetic, physicochemical, and phytochemical profile of *Nyctanthes arbor-tristis* leaves. The findings presented will contribute to the establishment of pharmacognostic standards, aiding in the identification, authentication, and quality assurance of this medicinally significant plant, and facilitating its broader therapeutic applications.

2. Material and Methods

2.1 Plant Collection and authentication

Fresh leaves of *Nyctanthes arbor-tristis* L. were collected from the local area of Prayagraj, Uttar Pradesh, India, in February 2024. The plant material was identified and authenticated by the Botanical Survey of India (BSI), Prayagraj, under Authentication No. 2023-24/887.

2.2 Determination of Morphological features

Morphology refers to the study of the form and structure of an object, while morphography involves the detailed description of that form, particularly when the material is known to occur in a specific shape. The morphological and organoleptic characteristics, including parameters such as color, odor, taste, shape, and size, were observed and evaluated using botanical methods [7,8].

2.3 Determination of Microscopical characters

2.3.1 Leaf microscopy

The leaf portion, including the midrib, was placed between two flat surfaces of pith, typically a piece of potato measuring approximately 3x1x1 cm. A longitudinal slit about 2 cm deep was made in the pith to hold the leaf portion securely. Thin sections were carefully cut and transferred into a watch glass containing water. Toluene was added to the sections, which were then boiled and filtered. The sections were stained using phloroglucinol and hydrochloric acid, mounted in glycerin, and observed under low-power magnification for analysis [5-7].

2.3.2 Powder microscopy

The powdered leaf material was boiled with toluene for 5–10 minutes to enhance the visibility of cellular components. The treated material was then stained with phloroglucinol to highlight lignified tissues and other structural features. The sample was mounted on a slide and observed under high magnification using a microscope to identify microscopic features [7-9].

2.3.3 Stomatal Index

The stomatal index (SI) is calculated to assess the proportion of stomata relative to the total number of epidermal cells, including stomata, on a leaf surface. The procedure involves preparing a leaf peel, either by peeling off a thin layer of the epidermis or by applying clear nail polish and peeling it off after drying. The peel is mounted on a microscope slide with a drop of glycerin and covered with a coverslip. Under a microscope, the number of stomata and epidermal cells in a specific area are counted [7-9]. The stomatal index is calculated using the formula:

$$\text{Stomatal index} = \frac{\text{Number of stomata}}{\text{Number of stomata} + \text{Number of epidermal cells}} \times 100$$

2.4 Extract Preparation

The dried plant material of *Nyctanthes arbor-tristis* was coarsely powdered, and 300 g of the powdered sample was accurately weighed. The powdered material was first defatted using petroleum ether and then subjected to ethanol extraction using a Soxhlet apparatus [9]. The obtained filtrate was concentrated, and the dried extracts were stored in a desiccator. The percentage yield was calculated using the formula:

$$\text{Percentage yield} = \frac{\text{Weight of dried extract}}{\text{Amount of crude drug taken}} \times 100$$

2.5 Physicochemical parameters

The determination of moisture content, total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive, and water-soluble extractive is based on standard procedures described in previous literature and pharmacopoeial methods [10,11]. Moisture content is typically measured by drying the sample at a specified temperature until a constant weight is achieved. Total ash is determined by incinerating the sample at high temperatures to remove organic material, leaving only inorganic residues. Acid-insoluble ash is measured by treating the total ash with dilute hydrochloric acid, filtering, and weighing the residue. Water-soluble ash is determined by dissolving the total ash in water, filtering, and weighing the insoluble residue. Alcohol-soluble extractive and water-soluble extractive are assessed by extracting the sample with ethanol and water, respectively, and calculating the extractive yield based on the weight of the dried residue. These methods provide insights into the purity, quality, and chemical composition of plant materials.

2.6 Phytochemical screening

Phytochemical screening involves the systematic identification of various classes of phytochemicals present in plant extracts. Below is a typical procedure for screening the presence of alkaloids, cardiac glycosides, flavonoids, steroids, saponins, tannins, proteins, and phenolic compounds as per standard literature methods [12-14].

2.7 Chromatographic Studies

The alcoholic extract was analyzed by Thin Layer Chromatography (TLC) to confirm the presence of phenolic compounds. Using a solvent system of ethyl acetate: methanol: water (77:15:8), the separation was carried out. Vanillin-sulfuric acid was employed as a detecting reagent. This method not only validated the presence of phenolic compounds, as indicated by qualitative chemical tests, but also provided insight into the number of distinct compounds present in the extract [15,16].

3. Results and Discussion

3.1 Determination Morphological characters

Determination of morphological characters involves the identification and analysis of the physical traits and structures of an organism, which are used to classify and differentiate species. These characters include aspects like shape, size, color and structure such as leaves, Morphological traits can be either qualitative (e.g., shape or texture) or quantitative (e.g., measurements like length or width), and they provide critical information for taxonomists to distinguish between species or assess evolutionary relationships (See **Figure. 1** and **Table 1**). This process often requires careful observation and comparison, using a variety of tools and techniques to accurately document and interpret the observed features

a) Upper Part b) Lower Part

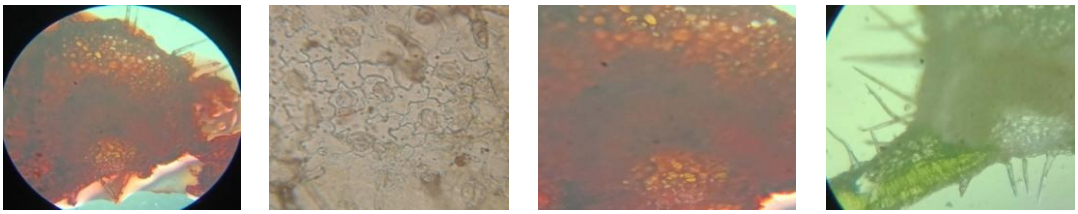
Figure 1: *Nyctanthes arbor-tristis* leaf

Table 1: Macroscopic study of *N. arbor-tristis* Linn. Leaf

S. No	Characters	Observation
1.	Colour	Light green – dark green
2.	Odour	Distinct
3.	Taste	Bitter, astringent
4.	Size	4-12 cm long, 2- 5.6 cm wide
5.	Shape	Cordate, oval
6.	Texture	Rough
7.	Base	Symmetric
8.	Margin	Entire
9.	Apex	Acute
10.	Venation	Reticulate

3.2 Determination of Microscopical Characters

The microscopical characters of *Nyctanthes arbor-tristis* leaf reveal several key features. The epidermis is a single layer of polygonal cells, covered by a cuticle, with stomata predominantly on the lower surface (abaxial), exhibiting anomocytic type with kidney-shaped guard cells (Shown in **Figure 2**). The mesophyll is differentiated into a well-developed palisade layer beneath the upper epidermis, consisting of columnar cells packed with chloroplasts, while the spongy parenchyma beneath contains loosely arranged cells with intercellular spaces for gas exchange. The vascular bundles are scattered throughout the leaf, with xylem and phloem arranged in a central vein. Simple or glandular trichomes may be present on the surface, providing additional protective and secretion functions. These microscopical features aid in the identification and classification of *Nyctanthes arbor-tristis*.



a) T.S. leaves b) Anomocytic stomata c) Vascular bundle d) Trichome

Figure 2: Transverse Section of *Nyctanthes arbor-tristis* leaf

3.3 Determination of Powder Microscopical Characters

Powder microscopy is a technique used to analyze the microscopic characteristics of powdered plant materials. This method involves examining the powdered substance under a microscope to identify its cellular structures, such as trichomes, xylem vessels, phloem, and other anatomical features, which can help in the identification of plant species or assess the quality of the plant material (See **Figure 3**). In the case of medicinal plants, powder microscopy is particularly useful for quality control, ensuring the correct plant is used and verifying the absence of contaminants. Features like epidermal cells, vascular elements, and secretory structures are commonly observed and documented. The technique also helps detect diagnostic features such as the presence of oil cells, tannin cells, or other specialized cells that are unique to certain plant species.

a) trichomes b) Xylem vessels c) Phloem

Figure 3: Powder Characteristics of *Nyctanthes arbor-tristis* leaf.

3.4 Fluorescence analysis

Fluorescence analysis of *Nyctanthes arbor-tristis* (Night-flowering jasmine) leaf powder involves examining the powdered material under ultraviolet (UV) light to observe any fluorescence exhibited by certain components in the leaf. This technique is used to identify specific chemical compounds and features that fluoresce under UV light, providing valuable information about the plants chemical composition and identity. In this analysis, the leaf powder is exposed to UV light (typically at wavelengths of 254 nm or 366 nm), and any fluorescent compounds present in the plant material will emit light at different wavelengths, producing various colors (see **Table 3**). For example, some

plant metabolites, such as alkaloids, flavonoids, and phenolic compounds, may show distinct fluorescence patterns. The fluorescence characteristics of the leaf powder can help in the identification of specific phytochemicals and assist in quality control by ensuring the authenticity of the plant material. Fluorescence analysis is particularly useful in distinguishing *Nyctanthes arbor-tristis* from other species that may have similar morphological characteristics, as the unique fluorescence patterns of its chemical compounds are a distinguishing feature.

Table 3: Fluorescence analysis of *N. arbor-tristis* Linn. Leaf powder.

Treatment	Visible	Long U.V 254 nm	Short U.V 366 nm
Powder	Dark green	Blackish	Black
Powder + water	Yellowish green	Not observable	Not observable
Powder + 5%NaOH	Brown	Not observable	Greenish
Powder + $FeCl_3$	light yellow	dark	greenish-brown
Powder + dil. H_2SO_4	light –brown	Not observable	Greenish
Powder + dil. HCl	Whitish -brown	Not observable	Light green
Powder + dil. HNO_3	Redish black	Dark	Grayish-green
Powder + ethanol	Dark green	Not observable	Not observable
Powder + KOH	Light brown	Reddish-brown	Greenish

3.5 Determination of stomatal index

The ***Nyctanthes arbor-tristis*** **stomatal index theory** likely refers to a concept that examines the relationship between the stomatal index (the number of stomata relative to epidermal cells) and environmental factors in the *Night-flowering Jasmine* (*Nyctanthes arbor-tristis*). Stomatal index is a key indicator of how plants regulate gas exchange, including CO2 intake and water vapor loss, which can be influenced by factors such as atmospheric CO2 concentration, humidity, and environmental stress (See **Figure 4**). The theory might explore how *Nyctanthes arbor-tristis*, as a tropical plant, adapts its stomatal density in response to changing environmental conditions, though this would require specific research to confirm any direct application or detailed study involving the plants stomatal behavior.

Figure 4: Stomatal Index and Vein–islet number of *Nyctanthes arbor-tristis* leaf

Vein islet number and vein termination number are terms used in the study of leaf venation, particularly in plant morphology and physiology. These characteristics can provide insights into the plant's adaptive strategies, as well as its environmental responses. This refers to the number of small, isolated areas (or islets) of vein tissue surrounded by a network of veins in a leaf. These islets are usually found between the main veins and secondary veins. The vein islet number can be used as a measure of leaf venation complexity and is often associated with the leaf's ability to transport water and nutrients. A higher vein islet number may indicate a leaf's adaptation to certain environmental conditions, like increased transpiration or photosynthetic activity. This refers to the number of times a vein ends or terminates in a leaf. Vein terminations are critical to the leaf's functional efficiency, as they are involved in the distribution of nutrients and water to the leaf tissue (see **Table 4**). A higher vein termination number could indicate more complex venation and might correlate with the leaf's ability to efficiently transport water, nutrients, and gases, which are essential for photosynthesis.

Table 4: Leaf surface data of *N. arbor-tristis* Linn. Leaf.

S. No	Leaf surface Parameter	Values
1.	Stomatal Index	17.85
2.	Vein islet per sq mm	4
3.	Vein termination no. per mm sq.	21

3.6 Physicochemical Analysis

The determination of physicochemical parameters in plant materials involves several tests to assess their quality and chemical composition. Moisture content is measured to determine the water present, as excess moisture can affect quality and promote microbial growth. Total ash content reflects the inorganic material, including minerals and salts, while acid-insoluble ash indicates the portion of

minerals resistant to acid dissolution, providing insight into the purity of the sample. Water-soluble ash measures the mineral content that dissolves in water, helping assess the solubility profile. Alcohol-soluble extractive quantifies plant constituents, like alkaloids and essential oils, that dissolve in alcohol, while water-soluble extractive measures hydrophilic compounds such as sugars and proteins. These tests are vital in the standardization of plant-based products, ensuring their purity, identity, and quality. These results, shown in **Table 5**, include the respective percentages for each parameter, and these tests are vital in the standardization of plant-based products, ensuring their purity, identity, and quality.

Table 5: Physicochemical data of *N. arbor-tristis* Linn. Leaf.

S. No	Physicochemical Parameter	Values (% w/w)
1.	Moisture Content	8.48 ± 0.08
2.	Total Ash	9.387 ± 0.565
3.	Acid- Insluble ash	0.3067 ± 0.029
4.	Water soluble ash	4.067 ± 0.088
5.	Alcohol soluble extractive	16.57 ± 0.4927
6.	Water soluble extractive	17.33 ± 0.577

3.7 Phytochemical screening

phytochemical screening of the ethanolic extract reveals the presence of various bioactive compounds, including alkaloids, cardiac glycosides, flavonoids, steroids, saponins, tannins, proteins, and phenolic compounds Shown in **Table 6**. These compounds are known for their potential therapeutic properties, such as anti-inflammatory, antioxidant, antimicrobial, and cardioprotective effects. The detection of these constituents underscores the medicinal value of the plant and supports its use in traditional and modern medicine for various health conditions.

Table 6: Phytochemical screening of Ethanolic extract of *N. arbor-tristis* Linn. Leaf

S. No	Phytochemical test	Ethanolic extract
1.	Starch	+
2.	Reducing sugar	+
3.	Protein/amino acid	+
4.	Alkaloids	++

5.	Tannins	++
6.	Cardiac glycosides	++
7.	Steroids	+
8.	Flavonoids	++
9.	Phenolics	+

3.8 Thin Layer Chromatography (TLC)

The TLC profile of *Nyctanthes arbor-tristis* leaf extract, analyzed using the solvent system Ethyl acetate: Methanol: Water (77:15:8), is shown in **Table 7**. This table includes the distinct spots observed under UV light and after derivatization, with each spot corresponding to a specific phytochemical compound. The Rf (Retention factor) values for each compound are provided, which represent the ratio of the distance traveled by the compound to the distance traveled by the solvent front. This profile is essential for the preliminary identification and standardization of the bioactive constituents in the plant extract.

Table 7. Thin layer chromatography of alcoholic extract of *Nyctanthes arbor-tristis* leaf.

S. No	Solvent system	Solvent front in cm	Distance travelled by solute in cm	Rf value
1.	Ethyl acetate:Methanol:water (77:15:8)	7.4	6.90 6.60 6.20 4.90	0.93 0.89 0.83 0.66

4. Conclusion

In conclusion, the present study provides comprehensive pharmacognostic, physicochemical, and phytochemical data for *Nyctanthes arbor-tristis* Linn. (Parijat or night jasmine) leaves. The detailed macroscopic and microscopic characteristics, along with the physicochemical parameters such as ash values, extractive values, and moisture content, offer essential standards for the identification and quality control of this plant material. The phytochemical screening confirms the presence of valuable secondary metabolites, including alkaloids, glycosides, flavonoids, saponins, tannins, and steroids, which contribute to its therapeutic potential. This study establishes important pharmacognostic standards, aiding in the proper identification and ensuring the quality of *Nyctanthes arbor-tristis* leaves for medicinal and therapeutic use.

Declaration of Competing Interest

The authors declare no competing interest.

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