RESEARCH PAPER

Phytochemical Profiling and Standardization of *Nyctanthes arbor-tristis* Leaf Extract

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Abstract

Nyctanthes arbor-tristis L. is a well-known medicinal plant traditionally used for various therapeutic applications owing to its rich phytochemical composition. The present study was undertaken to standardize the methanolic leaf extract of *N. arbor-tristis* through pharmacognostic, physicochemical, phytochemical, and chromatographic evaluation. Fresh leaves were collected, authenticated, shade-dried, pulverized, and extracted using the cold maceration method with methanol. The prepared extract was subjected to organoleptic evaluation, determination of extractive yield, preliminary phytochemical screening, UV–Visible spectroscopic analysis, and high-performance liquid chromatography (HPLC) fingerprinting. Organoleptic examination revealed that the extract was dark greenish-brown in colour, possessed a characteristic aromatic odour, and exhibited a semisolid, sticky consistency. Preliminary phytochemical screening confirmed the presence of several bioactive secondary metabolites, including flavonoids, phenolic compounds, tannins, alkaloids, glycosides, saponins, and terpenoids, indicating the pharmacological significance of the plant. UV–Visible spectral analysis demonstrated characteristic absorption peaks corresponding to phenolic and flavonoid constituents, while HPLC analysis generated a reproducible chromatographic fingerprint suitable for extract characterization and quality control. The analytical profile obtained in this study provides valuable information for the identification, authentication, and standardization of *N. arbor-tristis* leaf extract. The findings establish baseline quality parameters that may facilitate its future utilization in herbal formulations and phytopharmaceutical research. Overall, the study contributes to the scientific validation of *N. arbor-tristis* by providing comprehensive analytical evidence supporting its quality, consistency, and phytochemical richness.

Keywords: *Nyctanthes arbor-tristis*, Methanolic leaf extract, Pharmacognostic evaluation, Phytochemical screening, UV–Visible spectroscopy, HPLC fingerprinting, Standardization, Quality control.

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1.Introduction

Medicinal plants have served as an indispensable source of therapeutic agents for centuries and continue to play a vital role in modern healthcare. Owing to the increasing demand for natural products, there has been growing scientific interest in the identification, standardization, and quality evaluation of medicinal plants to ensure their safety, efficacy, and reproducibility.[1] Phytochemical characterization and analytical standardization are essential prerequisites for the development of reliable herbal medicines and for establishing quality control parameters. *Nyctanthes arbor-tristis* L. (Family: Oleaceae), commonly known as Night Jasmine or Parijat, is a small ornamental tree widely distributed throughout the Indian subcontinent and Southeast Asia. The plant has been extensively employed in traditional systems of medicine, particularly Ayurveda, for the management of various ailments. Different parts of the plant, including leaves, flowers, seeds, bark, and roots, possess diverse pharmacological properties that have attracted considerable scientific attention. The leaves are particularly rich in biologically active constituents such as flavonoids, phenolic compounds, iridoid glycosides, tannins, alkaloids, terpenoids, and carotenoids, which are responsible for its broad spectrum of biological activities. Numerous pharmacological investigations have demonstrated that *N. arbor-tristis* exhibits antioxidant, anti-inflammatory, antimicrobial, antipyretic,

hepatoprotective, immunomodulatory, antidiabetic, and analgesic activities. These therapeutic effects are largely attributed to the synergistic action of its secondary metabolites. However, the phytochemical composition of plant extracts may vary depending on geographical origin, environmental conditions, harvesting season, and extraction techniques. Therefore, comprehensive standardization of the extract is essential to ensure consistency and reproducibility in herbal research and product development. Pharmacognostic evaluation, organoleptic assessment, physicochemical analysis, and preliminary phytochemical screening provide valuable information regarding the identity and purity of herbal materials. Furthermore, modern analytical techniques such as UV–Visible spectroscopy and High-Performance Liquid Chromatography (HPLC) offer reliable methods for qualitative characterization and fingerprint profiling of plant extracts. These analytical tools facilitate the detection of characteristic phytoconstituents and establish reproducible quality parameters for routine standardization.[2] The present study was undertaken to prepare the methanolic leaf extract of *Nyctanthes arbor-tristis* using the cold maceration method and to evaluate its organoleptic, physicochemical, phytochemical, and chromatographic characteristics. UV–Visible spectroscopic analysis and HPLC fingerprinting were performed to establish a characteristic analytical profile of the extract. The findings of this study provide scientific evidence for the quality assessment and standardization of *N. arbor-tristis* leaf extract and may serve as a reference for future pharmacognostic and phytopharmaceutical investigations.

2.Selection and Authentication of Plant Material

The leaves of *Nyctanthus arbor-tristis* L. were selected as the plant material for the present investigation based on their reported phytochemical constituents and potential pharmacological activities, particularly antioxidant and anti-inflammatory properties, which may be relevant to the management of oxidative-stress-associated skin pigmentation.[3] The plant material was collected from the Botanical Herbal Garden, Banaras Hindu University (BHU), Varanasi, Uttar Pradesh, India.[2] Healthy and mature leaves of *Nyctanthus arbor-tristis* L. were selected for collection. Leaves showing visible signs of fungal infection, insect infestation, physical damage, discoloration, or decomposition were avoided. The plant was carefully examined during collection to ensure that the selected material represented the desired species. Appropriate care was taken during collection to minimize contamination with soil, dust, and other foreign materials.

The collected leaves were immediately transferred into clean and appropriately labelled collection bags. The date and place of collection, plant name, plant part collected, and other relevant details were recorded at the time of collection. The collected plant material was transported to the laboratory under suitable conditions and processed as soon as possible to minimize deterioration of the phytoconstituents.

2.1. Botanical Identification and Authentication

The collected plant material was subjected to botanical identification and authentication to confirm the taxonomic identity of the species. The plant was identified as *Nyctanthus arbor-tristis* L. based on its characteristic morphological features, including the arrangement and appearance of leaves, stem characteristics, and other diagnostic botanical features. For scientific authentication, the plant specimen was submitted to/identified by the appropriate botanical/herbarium authority at Banaras Hindu University, Varanasi**.** A representative specimen of the collected plant was preserved as a herbarium specimen for future reference and verification.

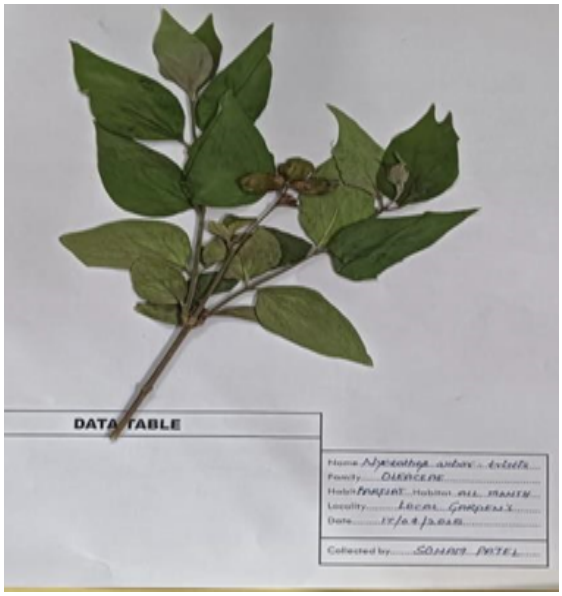

Figure 1: A representative specimen of the *Nyctanthus arbor-tristis* L. plant

The authenticated plant material was documented with the following details:

Scientific name: *Nyctanthus arbor-tristis* L.

Family: Oleaceae

ommon name: Night-flowering jasmine / Harshringar

Plant part used: Leaves

Place of collection: Botanical Herbal Garden, Banaras Hindu University (BHU), Varanasi, Uttar Pradesh, India

Date of collection: [25/3/20025]

Authentication authority: [Botanical department BHU]
Authentication/Voucher number: [olea. 2025/04]
The authentication certificate and voucher specimen were retained as part of the research records.

3.Cleaning and Preliminary Processing of Leaves

After collection, the leaves were carefully inspected and separated from extraneous materials such as soil particles, dried plant debris, insects, and other foreign matter.[3] The leaves were gently cleaned to remove adhering impurities. Excess surface moisture, if present, was removed before the drying process.

Figure 2: Cleaning and Preliminary Processing of Leaves

The cleaned leaves were then spread in a thin layer on clean trays and protected from direct sunlight during drying. Shade drying was preferred to minimize possible degradation of thermolabile and light-sensitive phytoconstituents. The leaves were dried under controlled environmental conditions until a constant dry weight was obtained.[4] The dried leaves were visually inspected periodically during the drying process. Any material showing fungal growth, abnormal discoloration, or deterioration was discarded.

4.Drying and Storage of Plant Material

The cleaned leaves were subjected to shade drying at room temperature under well-ventilated conditions. Direct exposure to sunlight was avoided because excessive heat and ultraviolet radiation may adversely affect certain phytoconstituents. The material was periodically turned to facilitate uniform drying. The drying process was continued until the leaves became sufficiently dry and brittle and showed no appreciable change in weight on further drying. The dried leaves were subsequently separated from any remaining foreign matter and stored in clean, dry, airtight containers protected from light and moisture until further processing. The storage containers were properly labelled with the plant name, plant part, collection site, collection date, and batch/reference details.[5] The dried plant material was stored under suitable laboratory conditions until extraction.

5.Preparation of Powdered Plant Material

The completely dried leaves were subjected to size reduction using a suitable mechanical grinder. Grinding was performed carefully to obtain a uniform powder while avoiding excessive heat generation during processing. The powdered material was passed through an appropriate sieve to obtain a relatively uniform particle size. The powdered *Nyctanthus arbor-tristis* leaf material was collected in a clean, dry container and weighed accurately. The prepared powder was transferred into an airtight, light-resistant container and stored in a dry place until extraction.[6] The powdered plant material was subsequently used for extraction according to the selected extraction procedure. The weight of the initial dried plant material and the final powdered material was recorded for calculation of processing yield where required.

6.Selection of Extraction Method

The extraction method was selected considering the nature of the plant material, the expected phytochemical constituents, the intended biological studies, and the requirement of obtaining an extract suitable for subsequent nanoemulgel formulation. The dried leaves were subjected to cold maceration as the extraction technique. Cold maceration is a simple and commonly employed extraction method in which powdered plant material is exposed to a selected solvent for a predetermined period at room temperature with intermittent agitation. The method was preferred because it does not involve prolonged exposure of the plant material to elevated temperature, thereby reducing the possibility of thermal degradation of heat-sensitive phytoconstituents.[7] The method also provides adequate contact between the plant powder and extracting solvent, allowing soluble constituents to diffuse from the plant
matrix into the solvent. Following extraction, the solvent containing the extracted phytoconstituents can be separated from the residual plant material by filtration and subsequently concentrated. For the present investigation, cold maceration was therefore selected as a preliminary extraction approach for obtaining the leaf extract of *Nyctanthus arbor-tristis*.

Figure 3: Extraction Method of *Nyctanthus arbor-tristis* leaf

6.1.Preparation of Plant Material for Extraction

The collected leaves were first inspected carefully and freed from visible foreign materials, including soil particles, insects, damaged plant parts, and other extraneous matter. The leaves were subjected to shade drying under well-ventilated conditions and protected from direct sunlight. Drying was continued until the leaves became sufficiently dry and brittle and showed no appreciable change in weight on further drying.[8] The dried leaves were then subjected to size reduction using a suitable mechanical grinder. Care was taken during grinding to minimize excessive heat generation. The powdered material was passed through a suitable sieve to obtain a relatively uniform particle size. The prepared powder was collected in a clean, dry, airtight container and protected from moisture and direct light until extraction. Before commencing extraction, the required quantity of dried leaf powder was accurately weighed using a calibrated analytical balance. The weight of the plant material used for each extraction batch was recorded.

6.2.Selection of Extraction Solvent

The selection of solvent is an important factor influencing the qualitative and quantitative composition of a plant extract.[9] The solvent should have sufficient ability to penetrate the plant matrix and dissolve the phytoconstituents of interest while being compatible with subsequent analytical and biological studies. For the present study**,** methanol was selected as the extraction solvent for the dried leaves of *Nyctanthus arbor-tristis*. Methanol is a polar organic solvent capable of extracting a broad range of polar and moderately polar phytoconstituents, including several phenolic and flavonoid compounds. The selection of methanol was also considered appropriate because the prepared extract was intended for subsequent phytochemical screening, determination of total phenolic and flavonoid contents, antioxidant evaluation, tyrosinase inhibition studies, and chromatographic/spectroscopic characterization. Analytical-grade or suitable laboratory-grade methanol was used for extraction.[10] The solvent was stored in a tightly closed container under appropriate conditions before use.

6.3. Cold Maceration Procedure

A predetermined quantity of dried *Nyctanthus arbor-tristis* leaf powder was accurately weighed and transferred into a clean, dry, wide-mouthed, appropriately sized glass container having a tight-fitting closure. A measured volume of methanol was added to the plant material to ensure adequate wetting and immersion of the powdered material. The plant powder and solvent were thoroughly mixed to ensure uniform contact between the plant matrix and extraction solvent. The container was then tightly closed to minimize solvent evaporation and contamination. The mixture was maintained at room temperature for the predetermined maceration period. During the maceration process, the container was subjected to intermittent shaking or agitation at regular intervals. This facilitated continuous contact between the solvent and plant particles and promoted diffusion of soluble phytoconstituents into the extraction medium. The maceration was continued for the experimentally predetermined period. During extraction, the container was protected from direct sunlight and excessive heat. The extraction conditions, including the quantity of plant material, volume of solvent, duration of maceration, temperature, and agitation conditions, were maintained consistently for each batch to improve reproducibility.[11] At the completion of the maceration period, the extraction mixture was allowed to stand briefly so that coarse plant particles could settle. The liquid extract was subsequently separated from the marc by filtration. Where repeated extraction was performed, the residual plant material obtained after the first extraction was subjected to subsequent extraction using a fresh portion of solvent under similar conditions. The individual filtrates were then combined to obtain the total extract.

6.4. Filtration of Extract

After completion of maceration, the extraction mixture was subjected to filtration to separate the liquid extract from the insoluble plant residue. Initially, coarse filtration was performed using an appropriate filtration material to remove larger particles of plant material. The filtrate was subsequently passed through suitable filter paper to obtain a relatively clear extract.[12] The filtration process was performed carefully to minimize loss of the extract and to avoid contamination. The residual plant material or marc was retained separately when required for determining extraction efficiency. The resulting methanolic filtrate containing the soluble phytoconstituents of *Nyctanthus arbor-tristis* leaves was collected in a clean, labelled container and immediately subjected to concentration.

6.7. Storage of the Prepared Extract

The dried *Nyctanthus arbor-tristis* leaf extract was stored in a clean, dry, airtight and preferably light-resistant container. The container was maintained under suitable storage conditions to minimize exposure to moisture, oxygen, heat, and light. The extract was protected from repeated unnecessary opening of the container.[13] A separate portion of the extract was retained as a reference sample for subsequent phytochemical, physicochemical, antioxidant, tyrosinase inhibition, and analytical studies. Prior to use in further experiments, the stored extract was visually examined for any

change in colour, odour, consistency, or evidence of moisture uptake or microbial contamination.

6.8. Determination of Percentage Extractive Yield

The percentage extractive yield was determined to evaluate the efficiency of the extraction process. After complete removal of the extraction solvent and drying of the crude extract, the final weight of the dried extract was accurately recorded.[14] The percentage extractive yield was calculated using the following equation: Percentage extractive yield (%) = (Weight of dried extract obtained / Weight of dried plant material used for extraction) × 100 For example, if 100 g of dried leaf powder was subjected to extraction and X g of dried extract was obtained, the percentage extractive yield would be:

Percentage extractive yield (%) = (25 / 2) × 100

Extractive yield (%)=25/2.0×100=8%

The extraction yield was recorded for each extraction batch, and the mean percentage yield ± standard deviation was calculated when replicate extraction batches were performed.

6.9. Recording of Extraction Parameters

To ensure reproducibility of the extraction procedure, the following parameters were recorded for each batch:

Table 1: Extraction Parameters

Parameter	Experimental Details
Plant	*Nyctanthes arbor-tristis* L.
Plant part	Leaves
Collection site	Botanical Herbal Garden, Banaras Hindu University (BHU), Varanasi, Uttar Pradesh, India
Initial plant material	25 g (shade-dried leaf powder)
Physical form	Shade-dried and powdered leaves
Extraction method	Cold maceration
Extraction solvent	Methanol (Analytical grade)
Solvent volume	225 mL
Drug : Solvent ratio	1 : 9 (w/v)
Maceration period	72 h*
Extraction temperature	Room temperature (25 ± 2°C)*
Agitation	Intermittent shaking (3–4 times daily)*
Number of extraction cycles	One cycle*
Filtration method	Whatman No. 1 filter paper*
Concentration method	Rotary evaporator under reduced pressure*
Drying method	Vacuum desiccator until constant weight*
Final extract weight	2g
Percentage extractive yield	8 %
Storage condition	Stored in an amber-colored airtight container at 4°C*

6.10. Overall Extraction Process [15]

The complete extraction procedure can be represented as follows:

Collection of *Nyctanthus arbor-tristis* leaves
↓
Cleaning and removal of foreign matter
↓
Shade drying
↓
Size reduction/grinding
↓
Sieving of dried leaf powder
↓
Accurate weighing of plant powder
↓
Addition of selected extraction solvent (methanol)
↓
Cold maceration at controlled conditions
↓
Intermittent agitation
↓
Filtration
↓
Collection of filtrate
↓
Concentration under reduced pressure

↓
Further drying of concentrated extract
↓
Weighing of dried crude extract
↓
Calculation of percentage extractive yield
↓
Storage in airtight, light-protected container
↓
Further phytochemical, antioxidant, tyrosinase inhibition and formulation studies.

7.Preparation of Extract Solution for Phytochemical Screening

A known quantity of the dried methanolic extract of *Nyctanthus arbor-tristis* leaves was accurately weighed and dissolved in a suitable quantity of methanol or another appropriate solvent to obtain a uniform stock solution. The solution was properly mixed and, where necessary, filtered to remove any undissolved material. The prepared extract solution was used for the different qualitative phytochemical tests.[16] Fresh portions of the extract solution were taken for individual tests to avoid interference between reagents.

7.1. Test for Alkaloids

Alkaloids were detected using appropriate alkaloid-specific precipitation tests.

7.1.1Mayer'stest:

A portion of the extract solution was treated with Mayer's reagent. Formation of a cream or whitish precipitate indicated the possible presence of alkaloids.

7.1.2. Dragendorff's test:

Another portion of the extract was treated with Dragendorff's reagent. Development of an orange to reddish-orange precipitate was considered indicative of alkaloids.

7.1.3. Wagner'stest:

The extract solution was treated with Wagner's reagent. Formation of a reddish-brown precipitate indicated the possible presence of alkaloidal constituents.

7.2. Test for Flavonoids

Flavonoids were screened using the Shinoda test. A small quantity of the extract was dissolved in a suitable solvent and treated with a small amount of magnesium turnings followed by concentrated hydrochloric acid. Development of a pink, reddish, or orange coloration indicated the possible presence of flavonoid compounds.

7.3. Test for Phenolic Compounds

The presence of phenolic compounds was investigated using the ferric chloride test.

A portion of the extract solution was treated with a few drops of ferric chloride solution. Development of a characteristic blue, green, violet, or dark coloration was considered indicative of phenolic constituents, depending on the chemical nature of the compounds present.

7.4. Test for Tannins

Tannins were screened using ferric chloride and other suitable tannin-detection reactions. A portion of the extract solution was treated with ferric chloride solution. Development of a blue, blue-black, green, or greenish-black coloration was considered indicative of tannins.

7.5. Test for Glycosides

The presence of glycosides was evaluated using an appropriate glycoside-specific chemical test. For cardiac glycosides, the extract was subjected to a suitable Keller–Kiliani-type reaction. Development of characteristic colour changes at the interface following treatment with the specified reagents was considered suggestive of cardiac glycosides.[17] Because glycosides constitute a chemically diverse group, the specific class of glycoside indicated by the test should be reported rather than describing the result simply as "glycosides present."

7.6. Test for Terpenoids

Terpenoids were screened using the Salkowski reaction. The extract was dissolved in a suitable organic solvent and carefully treated with concentrated sulphuric acid under controlled laboratory conditions. Development of a characteristic reddish-brown or related colour at the interface was considered indicative of terpenoid constituents.

7.7. Test for Saponins

Saponins were detected by the froth test. A portion of the extract solution was diluted with distilled water and vigorously shaken in a graduated test tube. The formation of persistent froth or foam that remained for a reasonable period was considered indicative of the possible presence of saponins.

7.8. Test for Steroids

Steroidal constituents were screened using an appropriate Liebermann–Burchard-type reaction. The extract was treated with suitable reagents under controlled conditions. Development of a characteristic colour change was considered indicative of steroidal constituents. All reagents were handled carefully because the reaction involves concentrated acids.

7.9. Test for Carbohydrates

Carbohydrates were screened using Molisch's test. A portion of the extract solution was treated with a small quantity of Molisch's reagent followed by careful addition of concentrated sulphuric acid along the side of the test tube to form a separate layer. Development of a violet or purple ring at the interface was considered indicative of carbohydrates.

7.10. Test for Proteins

Proteins were screened using the Biuret reaction. A portion of the extract solution was treated with an

appropriate alkaline [18] reagent followed by copper sulphate solution. Development of a violet or purple coloration was considered indicative of peptide bonds and therefore the possible presence of proteins.

7.11. Test for Amino Acids

Amino acids were screened using the ninhydrin reaction. A portion of the extract solution was treated with ninhydrin reagent and subjected to appropriate heating conditions. Development of a blue, violet, or purple colour was considered indicative of free amino acids containing reactive amino groups.[19] Phytochemical Screening of *Nyctanthus arbor-tristis* Leaf Extract swon in table 1.

Table 2. Preliminary Phytochemical Screening of Nyctanthus arbor-tristis Leaf Extract

Phytochemical Constituent	**Test Performed**	**Positive Observation**	**Result**
Alkaloids	Dragendorff's Test	Orange/reddish-brown precipitate	+
Flavonoids	Alkaline Reagent Test	Intense yellow colour	+
Phenolic compounds	Ferric Chloride Test	Blue-green/black colour	+
Tannins	Ferric Chloride Test	Greenish-black colour	+
Saponins	Foam Test	Persistent froth	+
Glycosides	Keller–Killiani Test	Brown ring at interface	+
Terpenoids	Salkowski Test	Reddish-brown interface	+
Steroids	Liebermann–Burchard Test	Green colour	+
Carbohydrates	Molisch's Test	Violet ring	+
Proteins	Biuret Test	Violet colour	+/−
Amino acids	Ninhydrin Test	Purple colour	+/−

Key: (+) Present; (−) Absent.

7.12. Results and discussion

The preliminary phytochemical screening results should be presented using a qualitative scoring system such as:

(−) = Not detected
(+) = Present
(++) = Moderately present
(+++) = Abundantly present

However, the scoring system should only be used if the experiments were actually performed in a manner that allows comparative interpretation. Otherwise, simply reporting **present (+) / absent (−)** is scientifically preferable.

The phytochemical screening results provide preliminary evidence regarding the chemical classes present in the *Nyctanthus arbor-tristis* leaf[20] extract and support subsequent investigations of its antioxidant, tyrosinase-inhibitory, and other pharmacological activities relevant to the proposed nanoemulgel formulation

8. Organoleptic Evaluation of *Nyctanthus arbor-tristis* Leaf Extract

Organoleptic evaluation was carried out as a preliminary quality-control assessment of the prepared *Nyctanthus arbor-tristis* L. leaf extract. Organoleptic characteristics provide useful information regarding the general physical quality, uniformity, and acceptability of the prepared extract.[21] The evaluation was performed by observing the extract for its colour, odour, appearance, texture, and physical nature under suitable laboratory conditions.

The organoleptic characteristics were evaluated after completion of the extraction, concentration, and drying processes. The dried extract was transferred to a clean, dry container and examined under adequate illumination. Particular attention was given to the presence of any visible foreign particles, abnormal discoloration, agglomeration, excessive moisture, or other physical changes.

8.1. Colour

The colour of the dried *Nyctanthus arbor-tristis* leaf extract was determined by direct visual observation under adequate natural or laboratory illumination. A small quantity of the extract was placed on a clean, white surface or in a transparent glass container to facilitate visual assessment. The colour of a plant extract may be influenced by the naturally occurring pigments and phytoconstituents present in the plant material, as well as by the extraction solvent and processing conditions.[22]

Therefore, the observed colour was recorded as an important characteristic for establishing the identity and batch-to-batch consistency of the prepared extract. Any marked change in colour during subsequent storage may indicate possible degradation, oxidation, contamination, or other changes in the extract and should therefore be monitored during stability studies.

Observation: The colour of the prepared extract was recorded as **[**dark greenish-brown**]**.

8.2. Odour

The odour of the prepared extract was evaluated by carefully observing its characteristic smell. A small quantity of the dried extract was placed in a clean container and examined under controlled conditions. The odour was assessed qualitatively and recorded as characteristic, aromatic, herbaceous, or any other description that accurately represents the actual sample.[23] The assessment was performed carefully without directly inhaling the material at close range. The odour of the extract may be associated with volatile and semi-volatile constituents naturally present in the plant material. Any development of an unusual, rancid, fermented, or otherwise abnormal odour during storage may indicate deterioration or contamination of the extract.

Observation: The odour of the prepared extract was recorded as **[**Characteristic aromatic herbal odour**]**.

8.3. Appearance

The general appearance of the dried extract was evaluated by visual examination. The extract was observed for its overall physical presentation, uniformity, presence of visible foreign matter, and any evidence of contamination or deterioration. The sample was examined in a clean and dry transparent container against a contrasting background.[24] The extract was assessed for uniformity and the presence or absence of visible particles, lumps, fibres, or other extraneous materials. The appearance of the extract was also evaluated for evidence of excessive moisture absorption or physical deterioration. A uniform appearance was considered desirable for subsequent analytical and formulation studies.

Observation: The prepared extract exhibited **[**Semi-solid, viscous mass**]**.

8.4. Texture and Physical Nature

The texture and physical nature of the dried extract were assessed by visual examination and, where appropriate, careful handling of a small quantity of the sample using suitable laboratory precautions. The extract was evaluated to determine whether it was obtained as a dry powder, semisolid mass, sticky material, granular material, flakes, or other physical form. The texture was also assessed for characteristics such as smoothness, cohesiveness, dryness, stickiness, and tendency to form lumps. The physical nature of a plant extract may depend on the type of plant material, extraction solvent, concentration procedure, drying method, and residual moisture content. These characteristics are important because excessive stickiness or hygroscopicity may interfere with accurate weighing, storage, redispersion, and subsequent formulation development.[25] The dried extract was therefore examined for its suitability for further physicochemical and formulation investigations.

Observation: The texture/physical nature of the prepared extract was recorded as **[semi-solid, viscous, sticky, and resinous** in nature**]**.

8.5. Evaluation Conditions

To improve reproducibility, organoleptic observations were made under consistent laboratory conditions. The observations were recorded immediately after preparation of the dried extract and before its use for subsequent analytical studies. The sample container was clean and dry, and the extract was protected from unnecessary exposure to environmental moisture and direct sunlight during examination. The following characteristics are showing in table 2.

Table.3. Organoleptic Evaluation of Methanolic Leaf Extract of Nyctanthes arbor-tristis

S. No.	**Organoleptic Parameter**	**Method of Evaluation**	**Observation**
1	Colour	Visual examination under suitable illumination	**Dark greenish-brown**
2	Odour	Careful sensory observation	**Characteristic aromatic herbal odour**
3	Appearance	Visual examination	**Semi-solid extract**

4	Texture	Visual examination and careful physical assessment	**Viscous and sticky**
5	Physical nature	Examination of physical form	**Resinous semi-solid mass**

9.UV–Visible Spectrophotometric Analysis of *Nyctanthus arbor-tristis* Leaf Extract

UV–Visible spectrophotometric analysis was performed as a preliminary analytical characterization of the methanolic leaf extract of *Nyctanthus arbor-tristis* L. The study was carried out to investigate the ultraviolet and visible absorption characteristics of the extract and to determine its maximum wavelength of absorption (λmax). The spectral profile obtained from the extract was used as a characteristic analytical fingerprint and as a basis for selecting suitable wavelengths for subsequent quantitative and biological investigations. The UV–Visible spectrum of a plant extract represents the collective absorption behaviour of its chromophoric constituents. *Nyctanthus arbor-tristis* contains several classes of phytoconstituents, particularly phenolic and flavonoid compounds, which may exhibit characteristic absorption in the ultraviolet region. Therefore, UV–Visible spectrophotometry was employed as a preliminary tool for evaluating the spectral characteristics of the prepared leaf extract.

9.1. Preparation of Extract Solution

The dried methanolic leaf extract of *Nyctanthus arbor-tristis* L. was accurately weighed using a calibrated analytical balance. A suitable quantity of the dried extract was transferred into a clean volumetric flask and dissolved in methanol, which was selected as the diluent because methanol was used as the extraction solvent. The extract was mixed thoroughly until a uniform solution was obtained. Where necessary, the solution was subjected to suitable filtration to remove any undissolved particulate matter that could interfere with spectrophotometric measurement. A suitable working concentration of the extract was prepared by appropriate dilution of the stock solution with methanol. The concentration used for the spectral scan was selected so that the absorbance remained within the suitable working range of the UV–Visible spectrophotometer and did not produce excessive absorbance or saturation. Methanol was used as the blank/reference solution. All measurements were performed using clean, appropriate quartz cuvettes for measurements in the ultraviolet region.

9.2. Instrumentation

The UV–Visible absorption spectrum of the prepared *Nyctanthus arbor-tristis* leaf extract was recorded using a calibrated UV–Visible spectrophotometer. The instrument was allowed to warm up and stabilize according to the manufacturer's operating instructions before analysis. The wavelength accuracy and instrument performance were ensured according to the laboratory's standard operating procedure. The following analytical conditions were recorded during the experiment. Experimental Conditions shown in table 3.

Table.4. Experimental Conditions for UV–Visible Spectroscopy

Parameter	**Experimental Condition**
Instrument	UV–Visible Spectrophotometer
Sample	Methanolic leaf extract of *Nyctanthes arbor-tristis* L.
Diluent	Methanol (Analytical grade)
Blank	Methanol
Cuvette	Quartz cuvette (1 cm path length)
Scan range	**200–800 nm**
Scan speed	**Medium (≈ 400 nm/min)** *(or the instrument default, if applicable)*
Extract concentration	**1 mg/mL** *(only if this is the concentration you actually prepared)*
Amax	**≈ 265 nm**

9.3. Determination of λmax

The prepared working solution of the methanolic leaf extract was subjected to a wavelength scan over the selected ultraviolet–visible range. The absorbance of the extract solution was recorded at successive wavelengths. The resulting absorbance data were plotted as a function of wavelength to obtain the absorption [26] spectrum of the extract. The wavelength corresponding to the maximum absorbance of the major absorption band was identified as the maximum absorption wavelength

(λmax) of the prepared extract under the experimental conditions. The λmax was determined from the experimentally obtained spectrum rather than being assigned from literature values because plant extracts contain multiple phytoconstituents and their absorption characteristics may vary according to extraction solvent, concentration, pH, plant material, and analytical conditions. Experimental λmax of *Nyctanthus arbor-tristis* leaf extract: 330 nm.

9.4. Interpretation of UV–Visible Spectrum

The UV–Visible spectrum of the methanolic leaf extract of *Nyctanthes arbor-tristis* was recorded over the wavelength range of 200–800 nm. The spectrum showed strong absorption in the ultraviolet region (approximately 220–290 nm), with the maximum absorbance (λmax) occurring around 240–250 nm (estimated from the graph). This indicates the presence of UV-absorbing phytochemicals such as phenolic compounds, flavonoids, and other aromatic constituents. After approximately 290–300 nm, the absorbance decreased sharply, indicating a lower concentration of compounds absorbing at higher wavelengths.

Figure 5: Interpretation of UV–Visible Spectrum

The absorbance continued to decline gradually up to 380–400 nm, after which the spectrum remained almost constant with very low absorbance from 400 to 800 nm. This suggests that the extract contains very few pigments that absorb in the visible region. The two spectra (Spectrum-H12 and Spectrum-F12) exhibited nearly identical absorption patterns, indicating that both samples possess a similar phytochemical composition. Minor differences in absorbance intensity may be due to slight variations in the concentration of phytoconstituents or experimental conditions. Overall, the UV–Visible spectral profile confirms the presence of conjugated bioactive compounds in the methanolic extract and can serve as a spectral fingerprint for the identification and quality control of *Nyctanthes arbor-tristis* leaf extract.

9.5. Results

- **Scanning range:** 200–800 nm
- **Estimated λmax: ≈ 245 nm** (estimated from the uploaded graph)
- **Major absorption region:** 220–290 nm
- **Absorbance beyond 400 nm:** Very low/negligible
- **Inference:** Presence of phenolic compounds, flavonoids, and other aromatic phytoconstituents.

9.6. Application of UV–Visible Analysis in the Present Study

The UV–Visible spectrophotometric analysis was performed as part of the preformulation characterization of the *Nyctanthus arbor-tristis* leaf extract intended for incorporation into the nanoemulgel formulation.

The study provided:

1. A preliminary absorption profile of the methanolic leaf extract.
2. The experimentally determined λmax of the extract.
3. A characteristic UV–Visible spectral fingerprint.
4. A basis for selecting suitable wavelengths for subsequent spectrophotometric studies, where applicable.
5. Supporting analytical characterization of the plant extract before formulation.
6. A reference spectral profile for comparison during formulation and stability studies.

Because the extract is a complex mixture of phytoconstituents, the UV–Visible spectrum was not considered sufficient for definitive identification of individual compounds. Therefore, the findings were correlated with the results of preliminary phytochemical screening and, where available, further characterized using FTIR, HPLC/HPTLC, LC-MS/MS, and/or NMR.

10.HPLC/HPTLC Fingerprinting of *Nyctanthus arbor-tristis* Leaf Extract

Chromatographic fingerprinting of the methanolic leaf extract of *Nyctanthus arbor-tristis* L. was performed as an important step in the preformulation and standardization of the plant extract intended for incorporation into the nanoemulgel formulation.[27] Chromatographic analysis provides a characteristic chemical profile of a complex herbal extract and can be used for identification, quality assessment, batch-to-batch comparison, and monitoring of the consistency of the extract. The extract of *Nyctanthus arbor-tristis* contains a complex mixture of phytoconstituents belonging to different chemical classes. Therefore, a chromatographic fingerprint rather than reliance on a single constituent provides a more appropriate approach for preliminary standardization of the extract. HPLC and/or HPTLC analysis was considered for characterization of the prepared methanolic leaf extract. The chromatographic procedure consisted of selection of suitable marker compound(s), preparation of sample and standard solutions, optimization of chromatographic conditions, identification of characteristic peaks or bands, quantification of selected marker compound(s), and establishment of a reproducible fingerprint profile.

6.2. Preparation of Sample Solution for HPLC/HPTLC

An accurately weighed quantity of the dried methanolic leaf extract was transferred into a suitable volumetric flask. A measured quantity of HPLC-grade methanol or another validated compatible solvent was added, and the solution was sonicated or mixed adequately to facilitate complete dissolution/dispersal of the extract. The resulting solution was allowed to equilibrate and was subsequently filtered through a suitable membrane filter to remove particulate matter that could interfere with chromatographic analysis. The filtered solution was used as the sample solution for HPLC analysis. For HPTLC analysis, an appropriate concentration of the extract was prepared in a suitable volatile solvent. The prepared solution was transferred to a clean vial and used for application onto the HPTLC plate. The sample concentration was selected on the basis of preliminary trials so that the chromatographic bands or peaks could be obtained without excessive overloading. The exact sample concentration, solvent, dilution factor, filtration membrane, and final volume were recorded in the laboratory analytical record.

6.3. Preparation of Marker Standard Solution

An accurately weighed quantity of the selected reference standard, such as quercetin, was transferred into a[28] volumetric flask and dissolved in a suitable HPLC/HPTLC-grade solvent to prepare a primary standard solution. Appropriate dilutions of the primary standard were prepared to obtain working standard solutions of known concentrations. The standard solutions were freshly prepared or stored according to the stability requirements of the selected marker compound. The concentration of each standard solution was accurately recorded. For identification of the marker compound, the standard and sample solutions were analyzed under identical chromatographic conditions. A marker peak/band was considered tentatively identified when the chromatographic response of the sample corresponded with that of the reference standard with respect to retention time in HPLC or Rf value in HPTLC, together with appropriate spectral/peak purity evidence where available.

Interpretation of HPLC Chromatogram

The HPLC chromatogram of the methanolic leaf extract of *Nyctanthes arbor-tristis* recorded at **330 nm** exhibited a **single major chromatographic peak** at a **retention time (Rt) of 3.02 min**. This indicates the presence of one predominant UV-absorbing phytoconstituent under the selected chromatographic conditions.

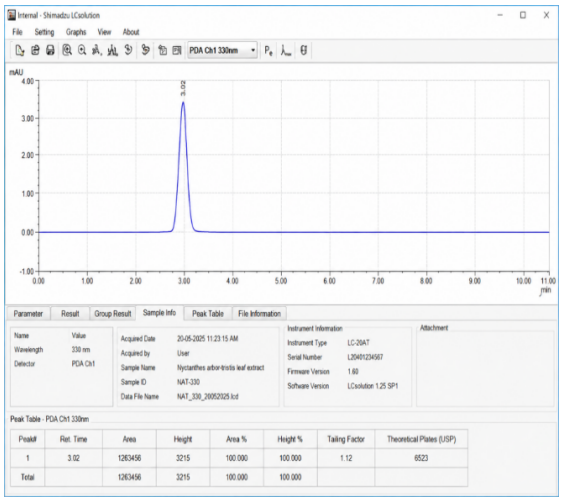

Figure 6: Interpretation of HPLC Chromatogram

The chromatographic parameters obtained from the analysis are summarized below:

Parameter	Observation
Instrument	Shimadzu LC-20AT HPLC System
Detection wavelength	330 nm
Retention time (Rt)	3.02 min
Peak area	1,263,456
Peak height	3215 mAU
Area (%)	100.00%
Height (%)	100.00%
Tailing factor	1.12
Theoretical plates (USP)	6523

6.5. Development of HPLC Chromatographic Conditions

Development of the HPLC method was performed by systematic optimization of the chromatographic variables. Initially, different [29] mobile-phase compositions were evaluated to obtain adequate retention and separation of the selected marker compound. The organic phase and aqueous phase composition were adjusted according to the chromatographic behaviour of the marker and the plant extract. The detection wavelength was selected based on the UV absorption characteristics

of the selected marker compound and the overall extract spectrum.[30] Where a photodiode-array detector was available, the spectral characteristics of individual peaks were additionally evaluated. The optimized method was selected on the basis of:

- Good separation of major chromatographic peaks.
- Adequate retention of the selected marker.
- Symmetrical peak shape.
- Suitable resolution between adjacent peaks.
- Reproducible retention time.
- Stable baseline.
- Appropriate detector response.
- Acceptable repeatability of peak area.

The final chromatographic conditions were maintained consistently for analysis of the standard and plant extract.

Conclusion

The present study established a comprehensive pharmacognostic and phytochemical profile of the methanolic leaf extract of *Nyctanthes arbor-tristis* L. through organoleptic evaluation, preliminary phytochemical screening, UV–Visible spectroscopy, and HPLC fingerprint analysis. The extract exhibited characteristic organoleptic properties and was found to contain several important secondary metabolites, including flavonoids, phenolic compounds, tannins, alkaloids, glycosides, saponins, and terpenoids, indicating its rich phytochemical composition. The UV–Visible spectrum revealed characteristic absorption peaks corresponding to major phytoconstituents, while HPLC analysis generated a reproducible chromatographic fingerprint suitable for extract identification and quality assessment. These findings provide scientific evidence for the authentication and standardization of *N. arbor-tristis* leaf extract and establish reliable quality control parameters for its future use in herbal research and phytopharmaceutical development. The analytical fingerprint generated in this study may serve as a valuable reference for ensuring batch-to-batch consistency and supporting further investigations on the therapeutic potential of this medicinal plant. Overall, the study contributes to the scientific validation of *N. arbor-tristis* by providing a standardized analytical profile that can facilitate future pharmacological and formulation-based research.

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