Review on pharmacognostic, proximate analysis, purification process, traditional uses, and pharmacological uses of whole plant parts of Nyctanthes arbor-tristis Linn. – A sacred ornamental plant (The Wonder of Ayurveda)

Saheli Chakraborty<sup>1</sup>, Sindhuja Sengupta<sup>2</sup>, Partha Sen<sup>3</sup>, Amartya Sen<sup>4</sup>, Shamim Sultana<sup>2</sup>, Abhilekha Baruah<sup>2</sup>, Momtaz Begum<sup>2</sup>, Paramjyoti Adhikary<sup>2</sup>, Sourav Chakraborty<sup>5</sup>, Souvik Chattopadhyay<sup>5</sup>, Debaprotim Dasgupta<sup>2</sup>, Souvik Biswas<sup>6</sup>, Mrinmoy Nag<sup>2\*</sup>

<sup>1</sup>Department of Pharmacognosy, Himalayan Pharmacy Institute (HPI), NH10,

Majhitar, Sikkim 737136

<sup>2</sup>NEF College of Pharmaceutical Education & Research,

Nagaon-782001, Assam, India

<sup>3</sup>BCDA COLLEGE OF PHARMACY AND TECHNOLOGY 78/1 Jessore Road,

Hridaypur, Barasat, Kolkata – 700127

<sup>4</sup>ADAMAS University, Adamas Knowledge City, Barasat - Barrackpore Road, Jagannathpur,

Kolkata, West Bengal 700126

<sup>5</sup>Pandaveswar School of Pharmacy, Pandaveswar – 713346,

West Bengal, India

<sup>6</sup>Bharat Technology, Uluberia, Howrah – 711316,

West Bengal, India

\* Corresponding author

Dr. Mrinmoy Nag

Associate Professor

NEF College of Pharmaceutical Education & Research,

Nagaon, 782001 Assam, India

Abstract: Nyctanthes arbor-tristis also known as the sacred night jasmine is a beautiful ornamental plant found in many regions of tropical and subtropical Asia. Its flowers bloom at night giving it its common name. This plant is highly valued for its abundance of bioactive compounds. In this comprehensive review we will focus on the pharmacognostical evaluation of Nyctanthes arbor-tristis including parameters such as ash and extractive values as well as standardization methods like TLC and HPLC for purifying different plant parts like seeds leaves flowers bark and stem. These parts of the plant have long been used in traditional medicine to treat a variety of ailments. In fact, Nyctanthes arbor-tristis is a key ingredient in Ayurvedic Siddha-Ayurvedic and Yunani medicines where

it is used as a diuretic digestive aid laxative mild tonic anti-venom, and expectorant. The aim of this review is to provide a thorough understanding of the pharmacognostic properties proximate analysis purification techniques and traditional therapeutic uses of Nyctanthes arbor-tristis Linn.

**Keywords**: Nyctanthes arbor-tristis Linn, physicochemical screening, proximate analysis, standardisation process, Traditional uses, Pharmacological uses

### 1. Introduction

Traditional medicines have been commonly utilized to treat a variety of ailments however they often come with numerous negative consequences<sup>1</sup>. In recent times there has been a growing global preference for natural remedies derived from plants due to their minimal adverse reactions and lower levels of toxicity<sup>2,3</sup>. Based on the World Health Organization's statistics a significant 25% of modern medicines are derived from plants traditionally used while 75% of herbal remedies have been discovered through research. Across the world an estimated 21000 herb species are utilized by indigenous healers for medicinal purposes<sup>17</sup>.

During the period of 4500 to 1600 BC the ancient text 'Rigveda' documented the utilization of plants with healing properties for both preventing and treating illnesses. As time progressed the Ayurvedic Unani system of medicine a traditional method introduced novel perspectives on the significance of herbal remedies in promoting well-being<sup>4</sup>. These natural products contain a variety of phytochemicals including tannins alkaloids carbohydrates terpenoids steroids and flavonoids all of which possess medicinal properties that can impact human health<sup>5,6</sup>.

The tropical Nyctanthes arbor-tristis is a versatile plant with popular medicinal benefits. It is known by several names such as night jasmine Coral Jasmine Parijat Harsinghar and queen of the night<sup>7</sup>. This sacred perennial tree is sought after for its fragrant flowers. Various parts of the plant contain diverse bioactive compounds that possess specific properties for treating numerous pharmacological ailments including laxative diuretic antivenom digestive mild bitter tonic expectorant antioxidant antidepressant cytotoxic antitussive mucolytic stomachic carminative astringent and antibilious effects<sup>2,8</sup>.

The vernacular name of *Nyctanthes arbor-tristis* is mentioned in Table 1.

## Synonyms<sup>9</sup>

Table 1. Vernacular name of Nyctanthes arbor-tristis

Assamese	Sewali, Shewali
English	Coral Jasmine, Night Jasmine
Hindi	Paarijat, Shefali, Harsingar
Malayalam	Parijatam
Manipuri	Singarei
Sanskrit	Parijata, Parijath, Sephalika
Gujarati	Jayaparvati, Parijatak
Bengali	Sephalika, Seoli
Kannada	Goli, Harsing, Parijata
Marathi	Kharbadi, Parijatak, Kharassi, Khurasi

Tamil	Manjhapu, Pavala-malligai
Telugu	Kapilangadustu, Pagadamalle, Parijat

#### Taxonomy:

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Eudicots
Clade: Asterids
Class: Eudicots
Division: Angiosperm
Order: Lamiales
Family: Oleaceae
Genus: Nyctanthes
Species: arbor-tritis

Geographical source:

The tropical regions of southern Asia are home to the Night Jasmine plant which can commonly be found in places like Bangladesh Indo-Pak subcontinent and South-East Asia<sup>10</sup>. This species is known to thrive in rocky terrain on dry hillsides as well as in the undergrowth of deciduous forests. In India it is cultivated across the outer Himalayas Jammu Tripura East Assam Maharashtra Punjab and Bengal extending all the way to the Godavari region in the

South<sup>11</sup>.

### **Cultivation:**

The Night Jasmine also known as the "Moonflower" thrives in loamy terrain and is commonly found in gardens with a pH level of 5.6 to 7.5. To unleash its full potential this plant needs at least six hours of sunlight and some shade every day. Overwatering should be avoided as it can hinder its growth<sup>12</sup>. At an elevation of 1500m above sea level this tree can withstand different levels of rainfall and is most vibrant from July to October when it's adorned with delicate blooms<sup>13</sup>.

## **Propagation:**

The propagation of plants can be achieved through the use of seeds or by taking cuttings from various parts. This process can also be accomplished by isolating the immature embryos found in cotyledons hypocotyls roots leaves and internodes of plantlets which are then regenerated in a medium containing thiadizuron (TDZ) and 6-benzyladenine. Alternatively, ex-vitro methods involve rooting through shoot regeneration with the aid of indole-3-butyric acid (IBA) followed by transplanting the new growth into sterile soil<sup>14</sup>.

### Mythological origin:

The plant is commonly called "Parijata" stemming from its birth in the Samudra (Sea) due to (Parinaha). This herb appears in multiple ancient writings under various names<sup>13</sup>.

The position of Parijata in different literature is mentioned in Table 2.

Table 2: position of Parijata in different literature

Name of literature	Name of verga
Hridaya – deepika Nighantu	Ekakapada Verga
Shaligram Nighantu	Pushpa Verga
Bhavaprakasha Nighantu	Guduchayadi Verga
Nighantu Aadarsha	Jatyadi Verga

Priya Nighantu	Hartiakyadhi Verga	

## Plant description:

N. arbor tristis a magnificent plant reaching up to 10 meters in height boasts a striking appearance with its grey or verdant complexion and rugged flaking bark. The outer layer of the bark is unevenly textured featuring mottled patterns of grey and brown. As for the inner bark, it exudes a softness and is characterized by both collapsed and uncollapsed regions of phloem tissue in a smooth creamy white hue<sup>15</sup>. The leaves of this plant measure between 6 to 12 cm in length and span a width of 2 to 6.5 cm all sporting a continuous edge. Its flowers boast a 5 to 8-lobed corolla adorned with immaculate white petals that emit a delightful fragrant aroma. Meanwhile at the center of each flower sits a vibrant reddish-orange coloration typically arranged in clusters numbering between two to seven. When this plant bears fruit which is often shaped like a heart and displays a rich brown color each section contains one seed measuring about 2 cm in diameter<sup>16</sup>.

## Pharmacognostical studies

The different parts of the plant are shown in Figure 1.

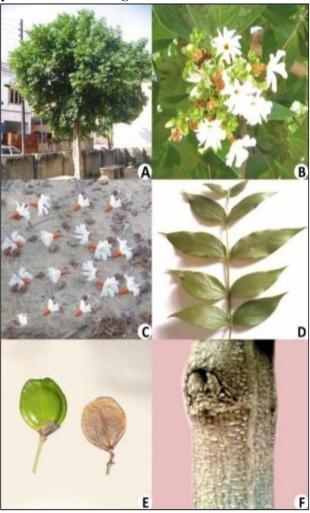


Figure 1. Different parts of the plant

A: Whole plant, B: Flowers and Buds C: Flowers, D: Leaves, E: Fruits,

F: Bark

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## Organoleptic character:

Bark<sup>25</sup>

Colour- creamish grey

Odour- characteristic

Taste- bitter

Shape- concavo- convex or planoconvex

Size- 10 meter

Surface-rough

Leaf<sup>27</sup>

Colour - green

Odour- indistinct

Taste- bitter and astringent

Shape- ovate

Size- 5-15 cm long

2.5 - 7.5 cm width

Surface- rough

Stem<sup>20</sup>

Colour – light grey to greenish

Odour- characteristics

Taste- characteristics

Shape- tall, erect, branched

Size- 1-10 meters

Surface- woody

Flower<sup>29</sup>

Colour - corolla- bright orange

Petals- white

Odour- sweet aromatic

Taste- bitter

Size- corolla- 0.9 - 1 cm in long

Petals- 1.4 - 1.5 cm in long

Fruit<sup>28</sup>

Size- 2 cm in diameter

Shape- heart cordate

Colour- brown

## **Macroscopic character:**

## Stem and bark:

The outer layer of the bark appears as a dusky shade and has an uneven surface characterized by the peeling of circular strips. The trunk is wide and bears four-sided branches<sup>14</sup>.

## Leaves:

The outer layer of the bark presents a dull grey shade and is characterized by a coarse consistency marked with indentations caused by the shedding of circular pieces. The leaves attached to the stem are supported by a stalk presenting a single blade that is accompanied by small appendages at its base. Positioned opposite to one another these leaves measure around 2.5 to 6.3 cm in width and 5 to 10 cm in length with an ovate or pointed shape. The surface facing upwards exhibits a dark green coloration dotted with glandular formations. On the other hand, the underside reveals a delicate fuzzy appearance displaying a pale green hue. The blades possess either a sharp or egg-shaped tip and may be smooth or serrated at the edges. Characterized by a mesh-like pattern and a single primary vein with an average of 12 smaller veins branching out from it the venation adds to the overall aesthetic of the leaf. The stalks supporting these leaves range from 5 to 7.7 to 10 mm in length and exhibit a concave shape on the upper side<sup>10</sup>.

### Flowers:

The petite blossoms of Nycantanthes arbor-tristis are endowed with a fragrant aroma. They possess 3-5 slender bracts 4 angular peduncles and elongated auxiliary and solitary terminal chymes that branch out in a dichotomous pattern. The bracts are either round or broadly ovate measuring 6-10 mm in length with a pointed tip and a fuzzy texture. The calyx measuring 6-8 mm is shaped like a narrow bell and covered in hairs on the outside while the inside remains smooth. Its corolla about 13 mm long is hairless and comprises a 6-8 mm long tube. The stamen bears striking orange hues and has white lobes of unequal size adding to its visual appeal<sup>10</sup>.

### Fruits:

Fruit of approximately two centimeters diameter possess a cordate heart shape. Its size is diminutive and flat with a capsule that is rounded. Each of the two cells in the fruit opens horizontally from the top and each cell holds a solitary seed<sup>14</sup>.

#### Seeds:

The kernel is rich in proteins compacted and with a thick protective layer. It is covered with sizable translucent cells and highly vascularized<sup>18</sup>.

## Microscopic character:

#### Bark:

The outer layer of bark consists of a vast periderm and an inner phelloderm area containing a wide layer of phellem. The exterior of the phellem is unevenly textured and contains many cracks made up of slender vertically arranged layers of phellod cells along with elongated suberized cells in a tangential direction. The cell walls are delicate and have a cubic or rectangular shape arranged in radiating patterns.

The inner bark comprises secondary tissues of phloem divided into two distinct regions:

- 1. Collapsed secondary phloem regions: The external layer of the inner bark composed of bulky phloem sclereids bears a slanted line of compressed sieve tubes and expanded axial parenchyma cells. The phloem rays are comprised of elongated and delicate cells while the thick-walled sclereids are interspersed with parenchymatous tissue.
- **2. Non collapsed secondary phloem regions:** The phloem is a component of the vascular tissue located adjacent to the cambial layer. It consists of intact sieve elements companion cells axial parenchyma cells and slim non-enlarged rays. In the inner region the cells are neatly aligned in radial rows whereas in the more mature areas their arrangement is more sporadic with scattered radial patterns<sup>19</sup>.

## Stem:

The form of this structure is rectangular comprised of a single outer layer with numerous complex strands. The inner layer contains several cells situated beneath the outer layer. The transport system is made up of irregular secondary tissues like phloem (comprised of sieve tubes and companion cells) as well as primary tissues such as cambium (a continuous layer one to three cells thick between phloem and xylem) xylem (secondary and primary) and pith  $^{20}$ .

## Leaves:

The foliage of N. arbor-tristis showcases an arrangement of outer layers including a stretched epidermis with striated nail-like structures known as fingernails on both surfaces. The inner mesophyll is divided into two or three tiers of upright cells as well as five to seven layers of organized mostly equal-sized pliable parenchyma which contains calcium oxalate. The underside of the leaf boasts a limited amount of stomata. The midrib is composed of a solitary layer of epidermis collenchyma on both sides and four to five layers of parenchyma with chloroplasts. In the center of the leaf there are vascular bundles consisting of xylem on the ventral side and phloem on the dorsal side. The petiole is connected to the ventral groove and has a thick epidermis layer. The cortex layer shields the epidermal cells while the hypodermis layer contains collenchyma and spherical parenchyma along with crystals of calcium oxalate<sup>21</sup>.

ISSN: 1001-4055 Vol. 45 No. 3 (2024)

#### Roots:

The cross-sectional layer of the root consists of anywhere from one to seven layers of small cork cells as well as a few larger layered cells diametric parenchyma cells a phellogen layer a sieve tube and companion cells. Additionally, the secondary xylem secondary phloem and xylem fibers can also be found within this section<sup>22</sup>.

#### Flowers:

The flower part is distinguished between two parts.

- **1. Petel:** The outer epidermis is covered with thick cuticle. It is separated by anomocytic stomata. The mesophyll region is thin layer and covered by three layer of parenchyma cell with intracellular space and immature vascular bundles at intervals. The inner epidermis is consisted of cuticle and also presence of anomocytic stomata.
- **2. Corolla tube:** the outer epidermis is made up of thick cuticle. The mesophyll region consists of polygona to polyherbal parenchymatous cell and presence of prismatic calcium oxalate crystals, tannis, and oil globules. The inner epidermis is covered by cuticle. The outer side of corolla is smooth and inner side is rough because of unicellular trichomes<sup>23</sup>.

*Fruits:* The transverse section of fruits is covered by polygonal cells with anti-clinical walls by a thin cuticle having 1-3 layers of collenchyma, spongy parenchymatous tissue, selerchymatous fibres and oil glands. Mesocarp is made up of thin wall, oval shaped parenchymatous cell and pericyclic fibres<sup>24</sup>.

### Powder microscopy:

*Stem:* the powder of stem is creamish brown in colour, having characteristic odour, bitter in taste. Fragment of cork cell, stone cell, starch grains, calcium oxalate crystals and tannib filled cell is presence with the treatment of chloral hydrate solution with 1% saffarin for 5-10 minutes and mounted in 50% glycerine<sup>25</sup>.

### Bark:

Powder of bark is composed of sclerids and periderm. Sclerids is isodiametric, rectangular shape. It has thick wall with canal like simple piths. Periderm is square or rectangular in shape, thin wall<sup>19</sup>.

### Leaves:

The powder microscopy of leaves shows starch grains, palisade cells, trichomes, phloem, xylem vessels<sup>26</sup>.

#### Flowers:

The flower powder microscopy is shown non glandular, unicellular trichomes, tannin filled cells, anomocyctic stomata, oil globules, pollen grains and amber colour pigments<sup>23</sup>.

### Fruits:

It is having multi-layered rapheal bundles, lignified xylem, endothelial tissue, endosperm tissue, oil glands, testa, sclerenchymatous fibre with narrow lumen<sup>24</sup>.

## **Extraction procedure:**

### Fruit:

The fruit is dried on sun dry for 5 days and grinded into powder. The powder is extracted using Soxhlet extractor by petroleum ether and methanol for 72 hours. The extract is filtered. Filtrate is evaporated by rotary evaporator<sup>30</sup>.

#### Leaf:

The leaf is dried at room temperature for 25 days and pulverised into corase fine powder. Then powder is macerated with ethanol (99.9% v/v) for 72 hours at 28 - 30°c temperature. Then filtered and evaporated by dehydration at 30-35°c temperature<sup>31</sup>.

#### Flowers:

Flower is dried in shade and powdered by mixer grinder. The powder is extracted by 95% ethanol in sonicator. Then the extract is evaporated by using rotary evaporator<sup>32</sup>.

#### Stem bark:

The stem bark is dried in room temperature and pulverized into coarse powder by mechanical grinder. The powder is extracted with 1 litre of petroleum ether by Soxhlet extraction until powder becomes fully exhausted. Then defatted extract is extracted with 1 litre by Soxhlet extraction. Then it is filtered and stored in dessicator<sup>33</sup>.

## Quantitative microscopy:

The characteristic of leaf constants like

- a) Stomatal number: It is the average number of stomata per square nm of leaf epidermis.
- b) Stomatal index: stomatal index is the ratio of the number of epidermal cells with each stomata counting as one cell.

The following equation can be used to calculate the stomatal index:

$$I = \frac{s}{E+s} \times 100$$

I= stomatal index

S= no of stomata per unit area

E= no of epidermal cells in the same unit area

- c) Vein- islet number: vein islet is a green tissue with small area covered by veinlets. The average number of vein islet number. The number of expressed as the vein islet number. The number of vein islet in a 4 sq.mm. Area of the central part of the leaf between midrib and the margin is counted.
- d) Veinlet termination number: The number of veinlet terminations per square nm of leaf square, midway between the leaf's midrib and its margin, is defined veinlet termination number<sup>34</sup>.

Sl.no	Parameters		Value
1	Palisade ratio	Upper epidermis	3-5
		Lower epidermis	3-4
2	Vein islet no		14-16
3	Vein termination no		11-14
4	Stomatal index	Upper epidermis	Stomata absent
		Lower epidermis	5.5-6.5

Table III. Quantitative microscopy of leaf surface

# Physicochemical screening

Physicochemical screening parameters like ash value, loss on drying, extractive values are investigated by cold, hot and successive extractions. For the extraction procedure, coarse powder is taken and sieved, then dissolved in various solvent such as petroleum ether, ethyl acetate, chloroform, acetone and methanol<sup>35</sup>.

## Loss on drying

Without pre-drying, the powder drug sample is taken and put in a tarred evaporating dish and dried in a required temperature for few hours. Then the powder weight is taken. The drying process was repeated until two subsequent readings matched or the differences between two successive weighing after drying for few minutes in a desiccator revealed a difference of no more than  $0.01g^{36}$ .

#### Ash value

Ash is the residue which is remaining portion of incineration of crude drug. The residue part is the natural inorganic salts, found in plant drug. Determination of ash value is based on identity and cleanliness of any drug. The advantage of determination process of ash value is to help the test purity and quality of drug.

#### a) Estimation of total ash value

In a tarred silica crucible, take few gm of dry powdered drug and burned progressively increasing the temperature until it is dull red hot and carbon free. It was cooled and weighed again for a constant value. The total ash value was then calculated in percentage using air dried medication as a reference<sup>37</sup>.

## b) Determination of acid insoluble ash

The obtained ash was heated in required amount of dilute hydrochloric acid for five minutes and kept the insoluble matter in a whatman 41 filter paper. Then the residue was washed with water which is previously hot. The acid insoluble ash was calculated in percentages using air dried<sup>38</sup>.

The %acid insoluble ash

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= \frac{\textit{wt of acid insoluble residue}}{\textit{wt of sample taken}} \times 100
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#### c) Determination of water-soluble ash

The obtained ash was diluted in few ml of dilute hydrochloric acid and made up the volume at 50ml with distilled water and heated. The mixture was kept for filtration using conical flask with help of filter paper and the residue was kept in a silica crucible which was weighed and placed on a muffle furnance for specify hours at a temperature. The crucible was removed and weighed after cooling at room temperature<sup>38</sup>. The water-soluble ash was calculated in percentages using air dried.

The percentage of water-soluble ash

= (wt of water-soluble residue)/ (wt of sample taken)  $\times 100$ 

### d) Determination of sulphated ash

The ash was dissolved with conc. Sulphuric acid. Then it was decomposed and occurs oxidation of the organic matter into formation of inorganic sulphates residue. In a large silica crucible, few gm of substance was taken and dissolved into sulfuric acid to maintain moisturizer. Then, it was heated slowly first, then increase the heat gradually to remove volatile substance. It was then ignited again to remove the cool carbon, moistened with sulphuric acid and re-ignited to achieve weight stability. The sulphated ash was calculated in percentages using air dried<sup>39</sup>.

The percentages of sulphated ash

= (wt of extract $\times$ 4)/ (wt of sample taken)  $\times$ 100

#### **Extractive value**

### Water soluble extractive value

Plant material which is air dried, extracted through maceration with 100ml quantity of solvents for required hours, occasional shaking. It was kept for specific hours and then filtered. After filtration, filtrate was removed and put in a tarred flat-bottomed flask. Then the solvent was evaporated for drying at a temperature for required hours. The extractive value was calculated in mg per g of air-dried material<sup>40</sup>.

## Alcohol soluble extractive value

Specific amount of gm of coarsely powdered was extracted through maceration with 100ml of alcohol in a air tight flask for few hours, shake occasionally. It was allowed to stand for specified hours and filtered, transferred exact amount of filtrate to a tarred flat bottomed shallow dish and evaporate to dryness at required to achieve weight stability. The extractive value of alcohol soluble of plant material was calculated in reference of air-dried drug<sup>41</sup>.

### Fluorescence analysis

For the determination of fluorescence analysis, powder drug material of different part was screened under visible light, short ultra violet light, long ultra violet light after treated with different types of organic or inorganic reagent like HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, HCL, and NaOH<sup>42</sup>.

## Proximate analysis

## pH estimation

In a beaker, specified amount of powder plant material was taken and dissolved in purified water. It is gone through filtration and the filtrate pH was checked by a standardized glass electrode<sup>43, 44</sup>.

#### **Detection of total fat content**

By Soxhlet extraction method, few gm of leaves powder drug was extracted using organic solvent such as petroleum ether for few hours. Then, the extract was kept in a conical flask previously weighed, rinsed with ether and evaporated the traces of solvent on an oven at a temperature<sup>45, 46</sup>. The total fat content of leaf is recorded.

### **Determination of fibre content**

Specified quantity of gm of dried powder sample was kept in a beaker and add required amount of hot sulphuric acid. The mixture was simmered for few minutes, but the volume was maintained by adding water at regular intervals. Finally, a muslin cloth was used to filter the mixture. The residue was washed away with hot water until it was no longer there. After that, the data was transferred to the exact amount of mL boiling in the same beaker sodium hydroxide was introduced. After few minutes of boiling (while maintaining the same volume as before) the mixture. A muslin cloth was used to filter the water. The leftovers were washed in hot water until it was no longer stained alkali, followed by some alcohol washing and ether. It was then moved to a critical, dried location and weighed overnight at a temperature (We). The crucial was heated for few hours at a temperature in a muffle furnace, then cooled and weighed again (Wa)<sup>47</sup>. The fibre is calculated by difference in weights We-Wa. The fibre content of N.arbor-tristis of leaf is found.

## **Estimation of resin content**

In a reflux condenser, take weighed drug sample with acetone for required of hours until the solution perfectly cleared. The remaining solvent was evaporated by distillation unit. The residue was then suspended in distilled water and transferred to a separating funnel, where it was extracted with ether several times to completely remove all of the resin content<sup>46</sup>. Excess ether was removed by heating on a water bath after the ether extract was cooled and dried over anhydrous sodium sulphate. The final weight was recorded after it was transferred to a weighed beaker. The leaves are having resin value.

## **Detection of foaming index**

In a conical flask take few gm of accurately weighed coarsely powder plant substance and dissolve in 100ml of boiled water. It was steamed into medium heat for specific mins. Then it was filtered. The filtrate was kept in a 100ml volumetric flask and make up the volume with distilled water. Into 10 stoppered tubes, take serial dilution and the final volume was made up to adjust up. The tubes were closed with stoppered and shaken in a longitude for few sec. Then it was allowed to stand for required mins and the foam is formed. The height of foam is measured<sup>34</sup>. The foam height of leaf is observed less than 1cm in every test tube and so the calculated foaming index of plant material is less than 100.

## Estimation of swelling index

In a glass stoppered measuring cylinder, accurately weighed fine powder was dissolved in water and make up the volume up. The sample was shaken continuously for required hr in every 10 mins. It was incubated for 3hrs at room temp. The swelling index was calculated by measured of individual readings with reference of 1gm of drug material<sup>34</sup>. The leaf of N. *arbor-tristis* is having swelling index of less than 100 which indicates the normal range.

#### **Detection of bitterness value**

A comparison can be made between the threshold level of bitterness in an extract and a diluted solution of quinine hydrochloride to determine it. This is typically expressed in a unit equation measuring the intensity of bitterness in a solution. To create the most dilute solution 1 gram of quinine hydrochloride should be added to 2000 millilitres of water. After cleansing the mouth with safe drinking water 10 mL of the diluted solution should be swirled around the mouth focusing on the base of the tongue for a few seconds. If the bitter sensation disappears within 30 seconds the mouth should be rinsed again with the solution and left for a minute to determine if there is any delayed sensitivity. Following this, the mouth should be rinsed with safe drinking water. The next level of concentration should not be tasted for at least some minutes <sup>48</sup>. The threshold for bitterness is the lowest concentration at which a substance still produces a bitter sensation after a few seconds. It is important for all solutions and mouthwash to be at an appropriate temperature for accurate results.

The formula for calculation of bitterness value

$$\frac{2000 \times c}{a \times b}$$

Where a= conc. Of stock solution (mg/ml)

b= vol of the plant drug with threshold bitter conc.

c= quantity of quinine hydrochloride (in mg) in the tube with the threshold bitter conc.

Bitters are medicinal plant materials with a pronounced bitter taste that are used therapeutically, mostly as appealing agents. Their bitterness enhances gastrointestinal production, particularly of gastric juice. Bitterness value of leaves is found.

### **Determination of total carbohydrate content (Anthrone Method)**

The experiment started by immersing the sample in a boiling tube and subjecting it to hydrolysis through exposure to a heated water bath and hydrochloric acid followed by cooling at ambient temperature. The sample was then neutralized with solid sodium carbonate until it produced effervescence and the final volume was determined. After centrifugation and separation of the supernatant a stock solution was prepared using a range of 0.2-1.0 ml from a standard solution. A blank solution was also created using 1ml of water and a specific amount of anthrone reagent. The resulting solution was heated in a water bath for a brief period then promptly cooled and the green to dark green color was measured at a designated wavelength. The total carbohydrate content was then determined by referencing a standard calibration curve<sup>49</sup>.

### **Detection of protein (Modified Lowry's Method)**

The experiment involved the placement of a specimen into a heated tube which underwent chemical breakdown through submersion in a bath of hot water. For the cold percolation technique, the plant extracts were divided into three tubes and left to soak in purified water overnight. A specific quantity of extract along with a standard and blank were placed in individual tubes as duplicates. To this mixture an appropriate amount of alkaline copper reagent was added and allowed to sit briefly. Then a specified amount of phenol reagent was introduced into each tube immediately mixed and left at room temperature until the absorbance at the desired wavelength was measured against a blank. The protein concentration of the sample was then calculated using a standard curve for reference<sup>50</sup>.

#### Standardisation process

## TLC method process

## Bark and leaf<sup>51</sup>

Organic solvent such as methanol was utilized to extract plant material. The extraction process involved boiling the material in a water bath for a brief period. The stationary phase or TLC plates were prepared using silica gel 60F. The sample was then run through a mobile phase or solvent system and developed until it reached a specific

ISSN: 1001-4055 Vol. 45 No. 3 (2024)

distance following conventional methods. To detect the desired compound a heated mixture of vanillin-sulphuric acid was applied for a short duration.

#### Fruit<sup>52</sup>

The plants were subjected to Soxhlet extraction with organic solvents like methanol and petroleum ether for the necessary duration. Aluminium-coated silica gel GF-254 was employed as the fixed phase. The sample was then separated using a solvent system. Finally, the compound was identified at a specific wavelength and treated with anisaldehyde sulphuric acid before being heated to the desired temperature.

### **HPLC** method process

## Leaf and flower<sup>52</sup>

In order to assess the purity of the samples we conducted a purification analysis on N. arbor-tristis flowers and leaves using HPLC. The HPLC procedure employed a WATERS system with a dual- $\lambda$  UV detector binary pump and column containing particles of  $5\mu m$  in size. The experiment utilized mobile phases to run the samples with an isocratic elution method at a constant flow rate<sup>52</sup>. A predetermined amount of the sample was then injected and analyzed using a UV spectrophotometer set at a specific wavelength. The sample and continuous phase were filtered through a PVDF filter while passing through the column.

#### **Chemical constituent:**

#### Leaves:

It has component of astringent, mannitol, ascorbic acid, sugar, colouring agent, tannic acid, amorphous resin, carotene, methyl salicylate and volatile oil. It also contains glucosides of oleic, lignoceric, linoleic, palmitic acid and stearic acid etc.<sup>53,54</sup> Leaves contain benzoic esters of loganin, arborside-A, arborside-B, arborside-C<sup>55</sup>.

### Stem:

Stem contain glycoside of Narigenin-4´-o- $\beta$ -glucopyranosyl- $\alpha$ -xylopyranoside. It is also having oleanolic acid,  $\beta$ -amyrin, nyctantic acid,  $\beta$ -hydroxyloganin. 55

### Flowers:

Flowers are having flavonoids, modified diterpenoid nyctanthin, anthocyanins etc.<sup>56</sup> It also contain renglyolone, essential oil, nyctanthin, tannin, glucose etc<sup>15</sup>.

#### Roots

The roots contain oleanolic acid and beta-sitosterol<sup>57</sup>.

### Traditional uses:

## Leaves:

It is used as laxative, cholagogue, antimicrobial activity. Anti-helminitic activity and diaphoretic. <sup>58,59,60</sup> leaves is juiced and it is used as mild bitter tonic, digestive, antitode <sup>61</sup>. It is also used in the treatment of fungal skin infection, sciatica, arthritis, spleen disease, dry cough etc <sup>62,63</sup>. Juice of leaf is also used in cure of biliary disorders, loss of appetite, diabetes, piles etc. The young leaves are used as wound healing activity, female tonic, gynaecological problems <sup>64,65</sup>.

### Flowers:

During the menstrual cycle this herb is commonly utilized. The heated concoction of its blossoms is effective as a calming agent. It can also aid in relieving mouth sores promoting expectoration and triggering the production of stomach acid. Furthermore its brew can be applied to treat gout as well as act as a digestive aid liver protector eye healer and bowel regulator<sup>67</sup>.

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**Stem and bark**: It is used in rheumatic joint pains. It is also used in antimicrobial activity. Bark used for treatment of bronchitis and snakebite<sup>68</sup>.

#### Seeds:

The seed powder is used in skin diseases, alopecia. It is found to be used as anthelmintic. It is used in the treatment of bilious fevers<sup>68</sup>.

#### **Roots:**

The root extract is found that it is used in the treatment of anthelmintis <sup>69,70</sup>.

## Pharmacological uses

### Anti -anxiety activity

The extract of *N. arbortristis* from hydro-alcohol, is having anxiolytic properties<sup>71</sup>.

### **Analgesic activity**

The leaf extract displays analgesic effects. The ethanolic solution demonstrates greater inhibition rate and superior pain-relieving properties compared to the petroleum-based extract<sup>72, 73</sup>.

#### **Hepato-protective activity**

The anti-hepatototoxic properties of the leaf and seed infusion distilled water was shown against carbon tetrachloride. The extract exhibits hepatoprotective effects by decreasing the levels of serum glutamic acid oxleacetic acid serum bilirubin and serum glutamic pyruvic transaminase<sup>74, 75</sup>.

## Anti -fungal activity

Anti-fungal activity of the stem bark is found against Aspergillus niger and Candida albicans<sup>76</sup>.

## Tranquilizing, Antistaminic and Purgative Activity

The water-soluble element obtained through an alcoholic extraction of this plant's leaves has been thoroughly investigated for its effects on the central nervous system including its potential as a sedative calming agent and pain reliever. The results align with the properties of common tranquilizers leading Ayurvedic practitioners to recommend the plant for these ailments. Medical professionals have also recognized its efficacy in treating these specific conditions<sup>77</sup>.

# **Anti-pyretic activity**

In laboratory rats the herbal solution demonstrated efficacy in reducing fever induced by brewer's yeast. When orally administered for six consecutive days the soluble fraction of an ethanol extract from the leaves was also evaluated for potential antipyretic and ulcer-causing effects<sup>73</sup>.

### Conclusion

Nyctanthes arbor-tristis a flowering plant does not require any special conditions for cultivation and can be easily found in the tropical and subtropical regions of Asia. This article presents an in-depth study on the pharmacognostical properties of the entire plant of N. arbor-tristis. The review also covers the various methods used for standardization purification and authentication of the plant. Apart from being aesthetically pleasing this plant is also rich in chemical components with every part having unique pharmacological characteristics. The article provides insights into the traditional and pharmacological uses of Nyctanthes arbor-tristis highlighting its significance as an ornamental plant in society. Furthermore, it includes details on the proximate analysis and purification process of this versatile plant.

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