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# PHYSICOCHEMICAL STANDARDIZATION AND METAL ANALYSIS OF *NYCTANTHES ARBOR-TRISTIS* LINN

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## ABSTRACT

*Nyctanthes arbor-tristis* Linn is native to India, distributed widely in sub-Himalayan regions. Different parts of *Nyctanthes arbor-tristis* Linn are known to possess various ailments by tribal people of Indian subcontinent with its use in Ayurveda, Sidha and Unani systems of medicines. The flowers are used as stomachic, carminative, astringent to bowel, antibilious, expectorant, hair tonic and in the treatment of piles. The powdered stem bark is given in rheumatic joint pain, in treatment of malaria and also used as an expectorant. The bark is used for the treatment of snakebite and bronchitis. The leaves of *Nyctanthes arbor-tristis* Linn are used extensively in Ayurvedic medicine for the treatment of various diseases such as sciatica, chronic fever, rheumatism, and internal worm infections, and as a laxative, diaphoretic and diuretic. The seeds are used as anthelmintics and in alopecia.

Physicochemical parameters in plants give valuable information and help to access quality of the sample. The ash values, extractive values, loss on drying, moisture content in the leaves samples were determined as per the WHO guidelines. Heavy metals are a matter of concern in the herbal drugs, especially as certain plants have the tendency of storing heavy metals from the soil, polluted water and atmosphere. Micronutrients are very essential for plant growth and regulation. Hence the presence of metals was determined using Inductively Coupled Plasma – Atomic Emission Spectroscopy. The obtained results revealed that the content of heavy metals was within the permissible levels and hence the plant was safe to be utilized in herbal drug formulations.

**KEYWORDS:** *Nyctanthes arbor-tristis* Linn, Physicochemical standardization, Metal content, Inductively Coupled Plasma – Atomic Emission Spectroscopy.

## INTRODUCTION

Nyctanthes arbor-tristis Linn. is popularly known as 'Night Jasmine' (English) or 'Harsinghar' (Hindi) due to the fact that its flowers emit a very strong and pleasant fragrance during the whole night[1,2].

*Nyctanthes arbor-tristis* is a shrub growing upto 10 m tall. The leaves are opposite, simple, long, broad, with an entire margin. The flowers are fragrant, with five- to eight-lobed white corolla with an orange-red centre. They are produced in clusters of two to seven together, with individual flowers opening at dusk and finishing at dawn. The fruit is a flat brown heart-shaped to round capsule, with two sections each containing a single seed.

Most of the plant material is usually put in a quarantine store. During storage, proper ventilation, humidity control, suitable temperature and light conditions should be ensured to maintain their original pharmacological action. However, it is observed that, crude plant materials are analyzed only after processing. Thus, we are not in a position to ascertain about the original characteristics of the plant. To avoid this, the crude drugs should be tested for the following tests as per the Indian Pharmacopoeia (IP), Vol I, 6th Ed. Ghaziabad: Government of India, Ministry of Health and Family Welfare.

Heavy metals are a matter of concern in the herbal drugs, especially as certain plants have the tendency of storing heavy metals from the soil, polluted water and atmosphere [3.4.5]. The Heavy metals cause metabolic disturbance and the excess produce serious consequences on human health. It's widely acceptable that the metals may react directly with DNA and produces cross links between the DNA strands as was observed after exposure [6]. Humans, animals and plants through air, water and food take up these metals from the environment. Roots and rhizomes of certain plants or semiaquatic vascular plants absorb heavy metals from contaminated water, for example Eichhornia crassipes, Hydrocotyl umbellata, Lemna minor, Azolla pinnata take up metals from polluted water. Calotropis procera and Ricinus communis can accumulate toxicants from soil, whereas Rawolfia serpentina can tolerate Cd, Agrostis tennis can tolerate Cu and Anthoxanthum odoratum can tolerate Zn. Argemone mexicana and Lantana camera also accumulate toxic metals from soil. Pb, Cd, Hg, Ti and As have been shown to be the contaminants of certain herbal ingredients.

The consumption of Ayurvedic medicines and other botanicals in the West has increased manifold in recent years. However, since 1978 more than 80 cases of lead poisoning

associated with Ayurvedic medicine use have been reported worldwide[7]. Of 70 Ayurvedic medicines manufactured in South Asia and sold in Boston, Massachusetts, stores in 2003, it was found that 20% contained lead, mercury, and/or arsenic[8]. Estimated daily lead, mercury, and arsenic intakes for these products were all higher than regulatory limits[9].

## MATERIALS AND METHOD

### COLLECTION OF PLANT

Leaves of *Nyctanthes arbor-tristis* were collected from Kanakeshwar, around 10 km away from Alibag, Maharashtra, India in the month of June. The plant was authenticated and voucher specimen no. PMP-1 was deposited in The Botanical Survey of India, Pune, India.

### PREPARATION OF PLANT MATERIAL

The leaves were washed thoroughly with tap water. The leaves were dried initially using paper to remove excess of water and later were air dried thoroughly under shade at room temperature to avoid direct loss of phytoconstituents from sunlight. The shade dried material was powdered using grinder and sieved through an ASTM 80 mesh. It was then homogenized to fine powder and stored in an air-tight container for further analysis.

### PROXIMATE ANALYSIS

Various physiochemical parameters were checked for the plant powder by the methods recommended by the World Health Organization.

### DETERMINATION OF TOTAL ASH

Two grams powder of leaves of *Nyctanthes arbor-tristis*, was weighed separately in previously weighed silica crucible accurately and incinerated completely in a muffle furnace at 600°C for around 3 hours until the ash became white in colour, indicating free of carbon. The crucible along with ash was cooled in a desiccator and weighed. Total ash content was calculated in percentage.

### ACID-INSOLUBLE ASH

The total ash content was transferred to a beaker and twenty five ml of 2M hydrochloric acid was added to the content. It was covered with a watch-glass and boiled gently for 5 minutes. The watch-glass was rinsed with 5 ml of hot water and the portion was added to the beaker. It was allowed to cool, and filtered through Whatman filter paper no. 41. The residue was then washed with hot water till washings were free from chloride (no white precipitate with

AgNO3 solution). The filter paper containing the insoluble matter was transferred to the original crucible, ignited by gradually increasing the temperature not exceeding than 450°C in a muffle furnace for 3 hours. The residue was allowed to cool in a desiccator for 30 minutes, and then weighed without delay. The percentage of acid insoluble ash was calculated with reference to the air dried sample.

**WATER-SOLUBLE ASH**

Twenty five ml of distilled water was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 minutes. Insoluble matter was collected on Whatman filter paper no. 41. The residue was washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding than 450°C in a muffle furnace. The residue was allowed to cool in a desiccator for 30 minutes and weighed. The water-soluble ash was calculated by difference in weight of this residue and that of the total ash. Finally, the percentage of water soluble ash was calculated with reference to the air-dried sample.

**DETERMINATION OF WATER AND ETHANOL EXTRACTABLE MATTER**

Four grams of coarsely powdered air-dried material accurately weighed in a glass-stoppered conical flask, macerated with 100 ml of distilled water and ethanol separately for 6 hours, frequently shaken and allowed to stand for 18 hours. It was then filtered rapidly and transferred 25 ml of the filtrate to a tarred flat-bottomed dish and evaporated to dryness on a water-bath, dried at 105°C for 6 hours, cooled in a desiccator for 30 minutes and weighed without delay. The content of extractable matter was calculated in percentage.

**LOSS ON DRYING**

Two grams of *Nyctanthes arbor-tristis* powder was weighed in a wide mouth stoppered weighing bottle. The bottle was placed (with lid open) in hot air oven maintained at 100-105°C for 2 hours. The bottle was then transferred to desiccators. The bottle was cooled to room temperature and weighed. Loss of weight was calculated in percentage.

**MOISTURE CONTENT**

Karl-Fisher titrimetric method was used to determine the moisture content in *Nyctanthes arbor-tristis* plant powder. Reaction vessel was rinsed thoroughly with methanol; magnetic stirring rotor was inserted in the vessel and placed in a proper position. The large rubber cork was removed and some Karl-Fisher grade methanol was added through funnel just enough to submerge the metal wires of sensors in the reaction vessel. The cork was replaced

immediately. The Karl-Fisher reagent and methanol bottles were placed in position. The instrument was turned on and the speed of the magnetic stirrer was adjusted. Methanol in the reaction vessel was neutralized and the titer factor was determined by calibrating the Karl-Fisher reagent. This was done by adding 10 µl of distilled water with the help of a micropipette in the reaction vessel and completing the titration. The calibration of the reagent was done in triplicate. The readings were noted and the titer factor was calculated. This titer factor was used to calculate the moisture content.

Titer factor = mg. of water added (wt.) / reading in ml (vol.)

100 mg of the plant powder was weighed and transferred to the titration vessel and the titration was allowed to go for completion. Percentage moisture was calculated using the formula;

Moisture percentage = [(titer factor × reading) / weight of sample (in mg)] ×100

**METAL ANALYSIS**

Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP- AES) is an emission spectrophotometric technique, exploiting the fact that excited electrons emit energy at a given wavelength as they return to ground state after excitation by high temperature Argon Plasma. Although each element emits energy at multiple wavelengths, in the ICP-AES technique it is most common to select a single wavelength (or a very few) for a given element. The wavelengths used in AES ranges from the upper part of the vacuum ultraviolet (160 nm) to the limit of visible light (800 nm). As borosilicate glass absorbs light below 310 nm and oxygen in air absorbs light below 200 nm, optical lenses and prisms are generally fabricated from quartz glass and optical paths are evacuated or filled by a non-absorbing gas such as Argon.

Analysis of the metals in selected plant samples were performed on IIT Bombay, Sophisticated Analytical Instrumentation Facility's ICP – AES, Spectro Analytical Instruments, ARCOS Simultaneous ICP Spectrometer. Initially a complete qualitative scan of the sample was done. Based on the results obtained, for each of the selected metals a standard linear calibration curve of various concentrations was analysed. Method parameters used for the analysis of metals are summarized. The instrument parameters used were as follows:

plasma power was set to 1400 W, pump speed to 30 RPM, Coolant flow was set to 12.00 l/min, Auxiliary flow to 1.00 l/min, and nebulizer flow to 0.80 l/min.

## SAMPLE PREPARATION

Hundred miligram air dried and fine powder leaves of *Nyctanthes arbor-tristis* was placed in a beaker. Four ml of concentrated nitric acid and one ml of perchloric acid, both of AR grade were added to it and kept overnight. The solution was evaporated to dryness on sand bath and allowed to cool. Distilled water was added and filtered through Whatman filter paper no. 41 into a volumetric flask and made up the volume to 25 ml with distilled water.

## RESULTS AND DISCUSSION

## PHYSICOCHEMICAL INVESTIGATIONS

The powdered leaves of *Nyctanthes arbor-tristis* were subjected to evaluate its total ash, acid-insoluble ash, water-soluble ash value, water and ethanol soluble extractive values, loss on drying and moisture content. Total ash content in leaves was 24.50 ± 0.22%, which indicates that the leaves are relatively rich in mineral elements. Acid insoluble ash was found to be 10.81 ± 0.76 %. Water soluble ash was determined to be 2.32 ± 0.51 %. Water and ethanol soluble extractive value of leaved was 13.49 ± 0.23 % and 10.40 ± 0.49 % respectively. Loss on drying in leaves was 15.85 ± 0.42 %. The air dried sample of leaves contains 10.42 ± 0.67 % moisture. The low moisture content of the leaf would hinder the growth of microorganism and storage life would be high.[10]

**Table no. 1. Physicochemical analysis of *Nyctanthes arbor-tristis***

| Parameters | Percent Content |
|---|---|
| Total Ash | 24.50 ± 0.22%, |
| Acid Insoluble Ash | 10.81 ± 0.76 %. |
| Water Soluble Ash | 2.32 ± 0.51 % |
| Water Soluble Extractive | 13.49 ± 0.23 % |
| Ethanol Soluble Extractive | 10.40 ± 0.49 % |
| Loss on Drying | 15.85 ± 0.42 % |
| Moisture Content | 10.42 ± 0.67 % |

## METAL ANALYSIS

The ICP-AES parameters were optimized by considering the wavelength, fuel gas as well supporting gas flow. The full qualitative scan showed presence of following elements Al, B, Ca, Cl, Cr, Cu, Fe, K, Mg, Mn, Na, P, S, Sc, Si, Sr, Ti, Y, Zn. The calibration curves were constructed by plotting the response against the concentration. A linear relationship was

obtained for each compound. The heavy metals; chromium, titanium, zinc; macronutrients like calcium, magnesium; micronutrients; copper, iron and manganese and other elements like scandium, strontium and yttrium were analysed at their particular wavelengths for their quantification. Heavy metal toxicity is frequently the result of long term low level exposure to pollutants common in our environment. Although chromium is found to be a stimulant for plant growth, several investigators reported its toxic effect. Concentration of Cr, Ti and Zn was 0.028, 0.786 and 0.303 respectively. Concentration of micronutrients; Cu, Fe and Mn in leaves was found to be 0.098 ppm, 12.488 ppm and 0.372 ppm respectively. Copper, iron and manganese are very essential for plant growth and regulation. Copper is necessary for carbohydrate and nitrogen metabolism and, inadequate copper results in stunting of plants. Iron is involved in the production of chlorophyll. Iron is also a component of many enzymes associated with energy transfer, nitrogen reduction and fixation, and lignin formation. Manganese is necessary in photosynthesis, nitrogen metabolism and to form other compounds required for plant metabolism. Magnesium helps in uptake of phosphorus and regulates uptake of other nutrients. Zinc is responsible for formation of growth hormones and chlorophyll.

**Table 2: Concentration of Metal in *Nyctanthes arbor-tristis***

| Metals | Wavelength (nm) | Concentration (ppm) |
|---|---|---|
| **Manganese** | **257.611** | **0.372** |
| **Strontium** | **407.771** | **0.419** |
| **Zinc** | **213.856** | **0.303** |
| **Titanium** | **334.941** | **0.786** |
| **Yttrium** | **371.030** | **0.01** |
| **Scandium** | **361.384** | **0.491** |
| **Calcium** | **422.673** | **199.98** |
| **Chromium** | **267.716** | **0.028** |
| **Iron** | **259.941** | **12.448** |
| **Magnesium** | **279.553** | **39.454** |
| **Copper** | **324.754** | **0.098** |

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