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# Phytochemical screening and determination of minerals and heavy metals in the flowers of *Nyctanthes arbor-tristis* L.

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## Abstract

Dried powder of the flowers of *Nyctanthes arbor-tristis* L. was analyzed for phytochemical screening, proximate compositions, mineral constituents and heavy metals analysis. The phytochemical screening indicated the presence of carbohydrates, flavonoids, glycosides, cardiac glycosides, reducing sugar, saponins, steroids, tannins and terpenoids. Alkaloids, anthraquinones and phlobatannins were absent. The proximate compositions was found to be high in moisture content (92.27 ± 0.09 %), the ash content was found to be (0.53 ± 0.02 %) while the protein content was 0.97 ± 0.05 % by fresh weight basis. The air dried flowers sample revealed highest in oxygen content (50.16 %) and lowest in sulfur content (0.10%). A total of six anions were analyzed. The sample was found rich in fluoride (94.87 ± 2.501 mg/Kg) and sulfate (165.24 ± 5.14 mg/Kg) content. A total of fifteen metals were analyzed. Heavy metals such as Pb, Cd, Hg, Cr and As were found in trace amounts, which were within the acceptable limits according to WHO and FAO.

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## Introduction

The use of plant and plant products as a therapeutic agent is as old as human civilization. From the ancient time different plant species were used as herbal medicine for various diseases (Annan *et al*., 2010). In the present days, the interest in the use of medicinal plants has been increased because of their less toxicity especially when compared with synthetic drugs. Today, natural products are responsible for about half of the approved drugs that are currently available (Vlietinck and Apers, 2001). The World Health organization (WHO) also supports the use of medicinal plants (WHO, 2005) for therapeutic use. Thus, medicinal plants are far more intriguing in the field of drug discovery. The medicinal values of different plants lie in its bioactive chemical constituents that create a definite physiological action in human body (Edeoga *et al*., 2005). The most important bioactive chemical constituents are alkaloids, tannins, flavonoids, steroids, terpenoids, carbohydrates and phenolic compounds (Hill, 1952; Pascaline *et al.,* 2011). Many medicinal plants have been consumed for medicinal purposes as well as for food (Jacob and Shenbagaraman, 2011). The mineral contents of medicinal plants play a vital role in human health (Annan *et al*., 2010). Moreover excess consumption of minerals higher than their estimated safe daily intake, may create toxicity in human body (Annan *et al*., 2013). Hazardous toxic metals such as As, Al, Pb, Cd, Cr, and Hg can be present in medicinal plants (Yap *et al*., 2010). In recent days, people have more awareness about the risks associated with the presence of hazardous metals in medicinal plants and their implications (Chong *et al*., 2007).

Bangladesh is a good land source of medicinal plants. *Nyctanthes arbor-tristis* L. (Bengali: Sheuli phul, Shefali phul) is an important medicinal plant, which belongs to the genus *Nyctanthes* and the family Oleaceae. This plant normally grows in tropical and subtropical regions of the country (Rani *et al*., 2012). Herbal industries are using this plant is for their herbal preparations and formulations. The present study was designed to evaluate the phytochemical

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constituents and the concentration of selected heavy metals and minerals in the flowers of the mentioned plant (Jain and Pandey, 2016; Anuradha *et al.,* 2017). The preliminary study revealed interesting findings which will draw attention to the need for further investigation of the active ingredients identified in the reported species.

## Materials and methods

*Collection and Identification of plant materials*

The flowers of *Nyctanthes arbor-tristis* L. were collected from BCSIR campus, Dhaka, Bangladesh. The plant (specimen # DACB 38734) was identified from Bangladesh National Herbarium (BNH), Dhaka by their Taxonomist. The collected flowers were air dried at 25-30 ˚C in the absence of sunlight. The dried flowers were powdered by a grinder machine. Then they were weighed and stored in an air tight container in dark until use.

*Phytochemical screening*

Specific chemical tests were carried out for specific phytochemical constituents. Standard procedures were followed to identify the constituents as described by Sofowara (1993), Trease and Evance (1996), Harborne (1998), Ghani (2003), Mukharjee (2002).

*Determination of proximate composition*

The moisture, ash and crude protein contents were determined using the standard methods of the Association of Official Analytical Chemists (AOAC, 1990). The average weight of flowers was determined by weighing fresh flowers under ambient condition. For the determination of Loss on air drying, the fresh flowers were kept in room temperature for 4 to 5 days in absence of sunlight. The average weight loss was calculated by difference. The moisture content was determined by heating the fresh flower samples in a temperature controlled electric oven at 105 °C until constant weight was achieved (ca. 6 to 10 hours). The moisture content was calculated in percentage (m/m). For determination of ash content, previously weighed fresh flower sample was taken. Then the sample was made moisture free and the moisture free sample was incinerated at 600 °C about 6-12 hours in a temperature controlled muffle furnace until ash becomes almost white or grayish white in color. Crude protein was calculated by using following formula.

Protein content (%) = nitrogen content (%) × 6.25

*CHNS/O analysis*

Powdered flowers samples were analyzed for elements such as carbon, hydrogen, nitrogen, sulfur and oxygen by using a CHNS/O Elemental Analyzer (Vario Micro Cube, Elementar, Germany). The results for carbon, hydrogen, nitrogen, sulfur content percentage were calculated directly and the percentage of oxygen was calculated indirectly by using the following formula:

Oxygen content (%) = 100 - (the sum of carbon, hydrogen, nitrogen, sulfur content percentage)

*Determination of anion content*

The sequential determination of fluoride, chloride, nitrite, bromide, nitrate, phosphate and sulfate ions were performed by chemically suppressed ion chromatography.

An aliquot of sample solution was injected into an ion chromatography instrument. The sample was pumped through two columns and a suppressor device into a conductivity detector. The analytical column and the guard column were packed with low-capacity anion exchanger. Ions are separated based on their affinity for the exchange sites of the resin. Anions are identified based on their retention times compared to known standards. Quantization was accomplished by measuring the peak height or area and comparing it to a calibration curve generated from known standards.

*Determination of metal composition*

The analysis of minerals and metal compositions were carried out on the sample using the standard methods of the Association of Official Analytical Chemists (AOAC, 1990).

*Instrumentation*

K and Na were analyzed by a Flame Photometer (JENWAY, PFP 7, UK). Mn, Fe, Zn, Cu, Mg, Ca, As and Hg were analyzed by using Varian AA -240 FS, Australia, while Pb, Cd, Cr, Ni, and Co were analyzed by a Varian Spectra AA 240 Z, Australia, AAS instruments.

*Preparation of sample*

The dried powdered sample was used for metal analysis. For analysis of Mn, Pb, Zn, Cd, Fe, Ca, Cu, Co, Cr, As, Na, K, Mg and Ni the plant samples were incinerated at 550 °C. About 0.1-1.0 g ash of the sample was dissolved in 5 mL of 1 M nitric acid ($HNO_3$) by warming on a hot plate for 2-3 minutes. The solution was taken in a 50 mL volumetric flask and 2 additional portions of 1 M $HNO_3$ were added and diluted to 50 mL with 1 M $HNO_3$. The solution was filtered through 0.45 μ filter paper. The filtrate was used for further metal analysis. For analysis of Mercury (Hg), the moisture free powdered sample was heated for 30 min with 20 mL conc. nitric acid. The solution was allowed to cool at room temperature and 10 mL perchloric acid was added to it. The solution was heated on a hot plate until the reaction with $HClO_4$ had completed (after completion of reaction the solution was colorless or yellow). Finally the solution was cooled and filtered in a 50 mL volumetric flask and the volume was made up to 50 mL with de-ionized water. A blank sample was also prepared without adding plant sample.

*Standards*

NIST traceable standards were used for individual metal analysis. The standards were collected from Scharlau, Sentmenat, Spain and the concentration of each individual stock standard for testing elements was 1000 mg/L. Working standards were prepared by necessary dilutions.

*Calibration procedure*

Independent calibration curve was constructed for individual elements from particular working standards. Element concentration in sample was determined from the calibration curve.

## Results and discussion

*Phytochemical screening*

The phytochemical screening for the flowers of *Nyctanthes arbor-tristis* L. showed (Table I) the presence of carbohydrates, flavonoids, glycosides, cardiac glycosides, reducing sugar, saponins, steroids, tannins and terpenoids.

**Table I. Phytochemical screening on the flower of *Nyctanthes arbor-tristis* L.**

| Serial no. | Phytochemicals | Test results |
|---|---|---|
| 1 | Alkaloids | Negative |
| 2 | Carbohydrates | Positive |
| 3 | Reducing sugars | Positive |
| 4 | Anthraquinones | Negative |
| 5 | Glycosides | Positive |
| 6 | Anthraquinone glycosides | Negative |
| 7 | Cardiac glycosides | Positive |
| 8 | Saponins | Positive |
| 9 | Flavonoids | Positive |
| 10 | Steroids | Positive |
| 11 | Tannins | Positive |
| 12 | Condensed tannins or phlobatanins | Negative |
| 13 | Terpenoid | Positive |

The therapeutic ingredients of plant and animal origins have been used from ancient times by a simple process without the isolation of pure compounds (i.e. in the form of crude drugs or the galenicals prepared from those). About 80% of the world's population use medicinal plants for treatment of different diseases (WHO, 2005). Phytochemicals are secondary metabolites of a plant which are formed by normal metabolic processes of plant. The medicinal value of a plant lies in these phytochemicals that have a definite physiological action on human body.

Terpenoids are chemically reactive compounds. The presence of terpenoids in medicinal plants was previously reported (Rahila *et al*., 1994). They are important due to their relationship with essential compounds such as vitamin A and could be of immense medical applications (Ladipo *et al*., 2011). Flavonoids are the key groups of secondary metabolites and bioactive compounds in plants. Flavonoids have been demonstrated to have antibacterial, anti-inflammatory, anti-allergic, antiviral and anti-aging activity (Alan and Miller, 1996; Vasantha *et al*., 2012). Flavonoids are also free radical scavengers that prevent oxidative cell damage, and have strong anticancer activities (Pourmorad *et al*., 2006; Ugwu *et al*., 2013). Medicinal plants containing tannins are used for the treatment of intestinal disorders such as diarrhoea and dysentery (Bajai, 2001). Carbohydrates are essential for both plants and animals (Ebun-Oluwa and Alade, 2007). Along with protein and fat, carbohydrates are one of the main energy sources in food (Usunobun *et al.*, 2015). An important class of phytoconstituents known as cardiac glycosides have an important role in medicine because of their actions on heart and used in cardiac insufficiency (Balch and Balch, 2000). It's actions helps in the treatment of congestive heart failure (Yukari *et al*., 1995). Moreover, glycosides, flavonoids and tannins have hypoglycemic activities (Dhar *et al.*, 1987). Saponins are active constituents with a marked hormonal activity, strong expectorant and aid in the absorption of nutrients (Osabor *et al*., 2015). They have properties of precipitating and coagulating red blood cells, binding cholesterol, formation of foams in aqueous solutions and hemolytic activity (Abidemi, 2013). Steroids fulfill important roles at different stages of mammalian development comprising prenatal development, growth, reproduction, sexual and social behavior (Adam *et al.*, 2002). Steroids have been reported to have antibacterial properties (Raquel *et al.*, 2007). Some plant steroids reduce the amount of cholesterol in the mammal bloodstream (Wisegeek, 2013). The present phytochemical screening indicated the presence of carbohydrates, flavonoids, glycosides, cardiac glycosides, reducing sugar, saponins, steroids, tannins and terpenoids. The present study also revealed that alkaloids, anthraquinones and phlobatannins were absent (Table I).

*Proximate composition*

The proximate composition obtained for the flower of *Nyctanthes arbor-tristis* L. are presented in Table II. There were found to the high moisture content (92.27 ± 0.09%), fresh weight basis. The moisture content gives an indication of water soluble vitamins present in the sample (Adinortey *et al.,* 2012). The ash content was found to contain (0.53 ± 0.02%), fresh weight basis. The ash content gives an indication to the rich source of inorganic minerals element that may be present in the sample (Adinortey *et al.,* 2012). The protein content was found to contain 0.97 ± 0.05% (fresh weight basis). The proximate composition also included lipid analysis in addition to the ash, moisture, and protein content that provides a general overview of the nutritional value of the plant flower (Kirk, 1993). The data of proximate composition for the flower of *Nyctanthes arbor-tristis* L. has been reported for the first time. This study shows that *Nyctanthes arbor-tristis* L. flowers are rich in phytochemicals and that their usage should be strongly recommended for good health.

**Table II. Proximate composition for the flower of *Nyctanthes arbor-tristis* L.**

| Sl. No. | Test parameters | Result (fresh weight basis) |
|---|---|---|
| 1 | Average weight of each flower (g) | 0.14 ± 0.03 (n = 98) |
| 2 | Loss on air drying (%) | 91.51 ± 0.06 (n = 3) |
| 3 | Moisture (%) | 92.27 ± 0.09 (n = 3) |
| 4 | Ash content (%) | 0.53 ± 0.02 (n = 3) |
| 5 | Protein (%) | 0.97 ± 0.05 (n = 3) |

Data are expressed as Mean ± SD

*CHNS/O composition*

The results for CHNS/O composition in the flower of *Nyctanthes arbor-tristis* L. are presented in Table III. Air dried powdered sample were used for this analysis, and five elements such as nitrogen (N), carbon (C), hydrogen (H), sulfur (S) and oxygen (O) were analyzed. The oxygen content (50.16%) was found to be highest in quantity and the sulfur content (0.10%) was found to be the lowest among the five elements. These elements play the principle role in formation of chemical constituents of medicinal plants. So quantification of these elements is most essential for physiological and pharmaceutical aspects. In biological system these minerals play significant role for activation of metabolic processes (Shute, 1964).

**Table III. CHNS/O composition in the flower of *Nyctanthes arbor-tristis* L.**

| Sl. No. | Test parameters | Percent (%) composition |
|---|---|---|
| 1 | Nitrogen (N) | 1.82 |
| 2 | Carbon (C) | 41.32 |
| 3 | Hydrogen (H) | 6.60 |
| 4 | Sulfur (S) | 0.10 |
| 5 | Oxygen (O) | 50.16 |

*Anion content*

A total of six anions were analyzed in the flowers of *Nyctanthes arbor-tristis* L.. The obtained results are presented in Table IV. The concentrations of the anions were calculated on fresh weight basis. The concentrations of anions like fluoride ($F^-$), chloride ($Cl^-$), bromide ($Br^-$), nitrate ($NO_3^-$), nitrite ($NO_2^-$) and sulfate ($SO_4^{2-}$) were found to be 94.87 ± 2.501 mg/Kg, 65.75 ± 1.83 mg/Kg, <DL, 27.44 ± 1.58 mg/Kg, <DL, 165.24 ± 5.14 mg/Kg, respectively.

**Table IV. Anions content in the flower of *Nyctanthes arbor-tristis* L.**

| Sl. No. | Anions | Concentrations (mg/kg) (fresh weight basis) |
|---|---|---|
| 1 | Fluoride ($F^-$) | 94.87 ± 2.50 |
| 2 | Chloride ($Cl^-$) | 65.75 ± 1.83 |
| 3 | Bromide ($Br^-$) | Less than detection limit |
| 4 | Nitrate ($NO_3^-$) | 27.44 ± 1.58 |
| 5 | Nitrite ($NO_2^-$) | Less than detection limit |
| 6 | Sulfate ($SO_4^{2-}$) | 165.24 ± 5.14 |

Data are expressed as mean ± SD (n = 3)

Chloride ($Cl^-$) ion is important in the pH balance of blood and body, and in the formation of hydrochloric acid in the stomach. It is found in intracellular and extracellular fluids. It is present mainly as $Cl^-$, although plants do contain compounds with covalently bound Cl (Engvild, 1986).

Nitrate and nitrite are naturally occurring anions that are part of the nitrogen cycle. The nitrate ion ($NO_3^-$) is the stable form of combined nitrogen for oxygenated systems. Plants have a high demand for nitrogen and developing meristematic tissues provide a very strong sink for the metabolically controlled process of $NO_3^-$ absorption by roots (Rao and

Rains, 1976). Several vegetables and fruits contain 200–2500 mg/kg of nitrate (Duijvenboden and Matthijsen, 1989). The nitrite ion ($NO_2^-$) contains nitrogen in a relatively unstable oxidation state. Chemical and biological processes can further reduce nitrite to various compounds or oxidize it to nitrate (ICAIR Life Systems, 1987). Especially nitrate ($NO_3^-$) has received considerable attention in recent years and is quickly gaining attraction as a health and performance enhancing nutritional supplement. Nitrate ($NO_3^-$) rich plants has also recently gained popularity for proposed anti-cancer and anti-inflammatory properties, and for reduced risk for adverse cardiovascular conditions including stroke, myocardial infarction, systemic and pulmonary hypertension, as well as the formation of gastric ulcers (Detopoulou *et al.*, 2008; Lundberg *et al.*, 2008). It has also been reported that dietary $NO_3^-$, administered, enhances exercise tolerance (Bailey *et al.*, 2010; Larsen *et al.*, 2011), reduces blood pressure (BP) and lowers the oxygen ($O_2$) cost of cardiovascular exercise (Lansley *et al.*, 2011). It is reported that the inorganic cations ($K^+$, $Ca^{2+}$, $Mg^{2+}$, and $Na^+$) were found to be largely balanced by the organic acid anions together with $NO_3^-$, $H_2PO_4^-$, $Cl^-$, and $SO_4^{2-}$ in the plants (Kirkby, 1974; Lorenz, 1976).

*Heavy metals and minerals compositions*

The heavy metals and minerals content in the flower of *Nyctanthes arbor-tristis* L. were determined by Flame Photometer and Atomic Absorption Spectrometer (Flame, Graphite Furnace and Hydride Generation AAS). A total of fifteen elements designated as essential elements and toxic elements were analyzed. The obtained results are presented in Table V and VI. The concentrations of the elements were calculated on fresh weight basis.

**Table V. Essential elements composition in the flower of *Nyctanthes arbor-tristis* L.**

| Serial no. | Essential elements | Unit | Results (fresh weight basis) |
|---|---|---|---|
| 1 | Potassium (K) | mg/kg | 1841.32 ± 6.26 |
| 2 | Sodium (Na) | mg/kg | 85.74 ± 2.26 |
| 3 | Calcium (Ca) | mg/kg | 207.60 ± 1.05 |
| 4 | Magnesium (Mg) | mg/kg | 138.95 ± 0.64 |
| 5 | Iron (Fe) | mg/kg | 20.73 ± 1.34 |
| 6 | Copper (Cu) | mg/kg | 0.97 ± 0.01 |
| 7 | Nickel (Ni) | mg/kg | 0.04 ± 0.001 |
| 8 | Cobalt (Co) | mg/kg | Less than detection limit |
| 9 | Zinc (Zn) | mg/kg | 2.23 ± 0.34 |
| 10 | Manganese (Mn) | mg/kg | 0.63 ± 0.20 |

Data are expressed as Mean ± SD (n = 3)

**Table VI. Toxic elements content in the flowers of *Nyctanthes arbor-tristis* L.**

| Serial no. | Toxic elements | Concentrations (mg/kg) (fresh weight basis) | Maximum recommended level (mg/kg) according to WHO and FAO |
|---|---|---|---|
| 1 | Lead (Pb) | 0.16 ± 0.002 | 10 |
| 2 | Mercury (Hg) | 0.02 ± 0.001 | 0.5 |
| 3 | Chromium (Cr) | 0.05 ± 0.002 | 2.3 |
| 4 | Arsenic (As) | Less than detection limit | 5.0 |
| 5 | Cadmium (Cd) | 0.002 ± 0.001 | 0.3 |

Data are expressed as mean ± SD (n = 3)

The concentrations of essential minerals such as K, Na, Ca, Mg, Fe, Zn, Cu, Ni, Co and Mn were found 1841.32 ±.6.26 mg/kg, 85.74 ± 2.26 mg/kg, 207.60 ± 1.05 mg/kg, 138.95 ± 0.64 mg/kg, 20.73 ± 1.34 mg/kg, 2.23 ± 0.34 mg/kg, 0.97 ± 0.01 mg/kg, 0.04 ± 0.001 mg/kg, <DL and 0.63 ± 0.20 mg/kg, respectively in the flowers of *Nyctanthes arbor-tristis* L.

The toxic elements like Pb, Hg, Cr, As and Cd were found 0.16 ±0.002 mg/kg, 0.02 ± 0.001 mg/kg, 0.05 ± 0.002 mg/kg, <DL and 0.002 ± 0.001 mg/kg, respectively. Those values are within acceptable limits according to WHO (WHO, 2005) and FAO (Codex Alimentarius Commission, 2001).

The minerals, especially Ca, Mg, Fe, Zn, Na and K play a significant role in human metabolism and life process. Calcium (Ca) is an important element because of its role in formations of bones, teeth, heart functions and muscle system (Brody, 1994). Magnesium (Mg) improves insulin sensitivity, protects against diabetes complications (Iwaoka *et al.*, 2011; Saracoglu *et al.*, 2009), reduces blood pressure and also plays a significant role in traumatic brain injury (Cerenak and Vink, 1999). Iron (Fe) is an essential mineral to prevent anemia and cough associated with angiotensin converting enzyme (ACE) inhibitors (Malik *et al.*, 2010; Archana *et al.*, 2011). Zinc (Zn) is necessary for growth and multiplication of cells. Deficiency of zinc may contribute to arrested sexual maturation, growth retardation and hair loss, delayed wound healing and emotional disturbance (Iwaoka *et al.*, 2011). Sodium (Na) is a mineral that human body must have in order to function properly. Sodium is vital to a number of routine body functions. Concentrations of sodium control the distribution of fluids in the body, maintains the osmotic equilibrium in cells, concern with the conduction of nervous impulses, muscle contractility and control heart muscle conduction (Rajurkar and Damame, 1997). Sodium also helps to transport other minerals and substances into, and out of, cells. Manganese (Mn) can help to assist the human body in metabolizing protein and carbohydrates (Fraga, 2005). Potassium (K) plays vital role in many physiological reactions in human body and its deficiency or excess can affect human health (Fraga, 2005).

For normal growth medicinal plants not only need nutrients, but also can selectively uptake and accumulate some elements which are good and may also be toxic for human health if these are not within the limits.

Lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) have no beneficial effects in humans, and there is no known homeostasis mechanism for them (Draghici *et al.*, 2010; Vieira *et al.*, 2011). These elements are generally considered the most toxic to humans and animals, even at low concentrations (Jomova and Valko, 2010; Tokar *et al.*, 2011). Chromium (Cr) is considered as human carcinogen in one form or another or in particular routes of exposure (NTP, 2002).

**Conclusion**

The results from this study showed that the flowers of *Nyctanthes arbor-tristis* L. contain several important phytochemicals and phytonutrients. The flowers have proven to be considered as a major source of potassium, calcium, magnesium, sodium, iron and zinc. The presence of necessary phytoconstituents, safe level of toxic elements and good source of minerals in the flowers of *Nyctanthes arbor-tristis* L. suggest and justify that, it is safe for consumption and also usable for preparations for herbal formulations, products and tonics.

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