

Phytochemical, Antibacterial and in Vitro Antioxidant Analysis of Nyctanthes Arbor-Tristis Leaves

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ABSTRACT: The aim of this study to examined the preliminary phytochemical analysis, Antioxidant and antibacterial activity of Nyctanthes arbor-tristis methanol leaves extract against the non-pathogenic and pathogenic bacteria. The phytochemical analysis on methanol leaves extract of Nyctanthes arbor-tristis shown the presence of carbohydrates, glycosides, flavonoids, alkaloids and phenolics. The methanol leaves extract of Nyctanthes arbor-tristis showed highest zone of inhibition against Pseudomonas aeruginosa (20mm) and Salmonella typhi (11mm) shown lowest inhibition. DPPH percentage of scavenging activity of Nyctanthes arbor-tristis leaves was (48.6%) recorded. The strong reducing power and high phenolics and flavonoids contents could be responsible for the antioxidant activity.

KEYWORDS: Carbohydrates, DPPH, Antioxidant, Scavenging, Antibacterial.

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I. INTRODUCTION

Nyctanthes arbor-tristis Linn. (Night Jasmine) is a valuable therapeutic and attractive tree which belongs to family Oleaceae with fragrant white flowers which bloom during night and starts falling to the ground after midnight which makes the plant look dull during the day. The name 'Nyctanthes' is derived from two Greek words 'Nykhta' (Night) and 'anthos' (flower) (Vats M, et al 2009; Meshram MM, 2012). Night jasmine is extensively cultivated all over India (Anonymous, 1988). It tolerates modest shade and is frequently found in dry deciduous forests (Rout et al., 2007). Its flowers, seeds and leaves are used for several diseases like skin diseases, asthma, grayness of hair, chronic fever, bronchitis, constipation, and baldness (Kirtikar and Basu, 1981). The unpleasant leaves of night jasmine are used in Ayurvedic system of medicine for treatment of sciatica, rheumatism, diuretic and intestinal worms. The powdered seeds are used to treat scurvy (Kirtikar and Basu, 1988). The flower contains nyctanthin a coloring agent used for coloring silk and suitable for printing purposes (Das and Mondol, 2012). Seed coat contains phenolic compounds causing the death of seedlings resulting in low germination rates (Anonymous, 1988), (Bhattacharya et al., 1999). The germination rate is increased either by eliminating both the coverings or by using antioxidants like polyvinylpyrrolidone (PVPP) and polyvinylpyrrolidone (PVP) before germination (Rout et al., 2008; Bansal S, et al 2013; Sah AK et al 2012).

The flowers of Nyctanthes are used to provoke menstruation in India, Malaysia and Indonesia. Some elderly Sri Lankan Buddhist monks used the hot infusion of inflorescence as a sedative. Scabies and other skin diseases can be treated using flowers. They also help in reducing mouth ulcers (Sasmal D, et al 2007). Oral intake of flower decoction deals with improvement of gastric secretions and helps to remove mucus from the lungs (Suresh V, et al 2011), and in the treatment of gout (Kirtikar KR, et al 1993). Juice made from flowers is used in preventing baldness and graying of hair (Tuntiwachwuttiku P, et al 2003).

The leaves are used as diuretic, laxative, diaphoretic and chologogue. Piles, chronic fever, malarial fever, liver disorders, biliary disorders, obstinate sciatica, rheumatism and loss of appetite in children due to roundworms and threadworms are treated by leaf juice. A leaf decoction is mostly used in Ayurveda to treat malaria and arthritis. The leaves are used as female tonic and in alleviating gynecological problems in dry cough, fungal skin infection (Nawaz AHMM, et al 2009). The seed powder is used for scurvy. The bark is used to treat snakebite, bronchitis and roots are used as anthelmintics (Narendhirakannan RT, et al 2010) (Rathod N, et al 2010). Cough, pyrexia, high blood pressure and diabetes are treated by mixing honey in leaf succulent (Nawaz AHMM, et al 2009). Leaves are used in the spleen enlargement, digestives, antidote to reptile venoms, mild bitter tonic, laxative, diaphoretic and diuretic. (Vats M, et al 2009; Suresh V, et al 2010). Anorexia, liver disorders, intestinal worms, obstinate sciatica, biliary disorders, rheumatism, hemorrhoid, chronic pyrexia and pyrexia with rigors are treated by Nyctanthes leaf succulent (Nair R, et al 2005).

II. MATERIAL AND METHODOLOGY

Collection of Plant material

Nyctanthes arbor-tristis plant leaves were collected and washed 2-3 times with tap water and dried in shade at room temperature. The dried plant material was made into fine powder using a clean motor and pestle stored in sterile air tight container in a dry place until analysis

Preparation of Plant Extract

30 g of dried leaves were extracted using 300 ml methanol as solvent in a soxhlet apparatus for 8 h. The extracts obtained were concentrated and stored at 4°C.

Microorganisms

The methanol extract of Nyctanthes arbor-tristis leaves was tested against microorganisms such as Pseudomonas aeruginosa (MTCC), Salmonella typhi (MTCC), Staphylococcus aureus (MTCC), Klebsiella pneumoniae (MTCC), Escheichia coli (MTCC) and Bacillus subtilis (MTCC). The bacterial strains were maintained on nutrient agar slants at 4°C. A loopful of each bacterial strain was inoculated into 50 mL sterile nutrient broth. The flasks were incubated at 37°C for 24 h.

Phytochemical Screening

A variety of phytochemical compounds includes alkaloids, terpenoids, flavonoids, steroids and glycosides have been characterized from leaves of N. arbor-tristis. The preliminary phytochemical screening was performed according to the Brindha (1981).

1. Test for Alkaloids

1ml extract mixed with 3-5 drops of Wagner's reagent and observe for the formation of reddish brown precipitate or coloration.

2. Test for Carbohydrates

1ml extract mixed with 3-5 drops of Molisch's reagent, along with this add 1ml of concentrated H₂SO₄. Then allowed the mixture to stand for 2-3 min. Observed for the formation of red or dull violet color at the interface of the two layers is a positive result.

3. Test for Cardiac Glycosides

1ml extract mixed with 1ml of glacial acetic acid and 2-3 drops of 5% ferric chloride solution and added 0.5ml of conc. H₂SO₄. Observed brown ring at the interface shows the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

4. Test for Flavonoids

1ml extract and added 3-5 drops of 20% NaOH solution. Observed for the formation of intense yellow colour, which becomes colourless on addition of 0.5ml dilute HCl indicates the presence of flavonoids.

5. Test for Phenols

1ml extract and added 5-6 drops of 5% aqueous ferric chloride solution and observed for the formation of deep blue or black colour.

6. Test for Amino acid and Proteins

1ml extract and added 2-5 drops of Ninhydrin solution and kept it in a boiling water bath for 1-2 min and observed formation of purple colour.

8. Test for Tannins

1ml extract and added 1ml of 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour.

9. Test for Terpenoids

1ml extract and added 0.5 ml chloroform along with 3-5 drops of conc. H₂SO₄. Observed reddish brown precipitate produced immediately.

Antibacterial activity

The antibacterial activity of Nyctanthes arbor-tristis leaves methanol extract was investigated by agar well diffusion method. 24 hours broth cultures of test organisms were used for assay. 100µl Cultures were spreaded in the Muller-Hinton agar. The plates were prepared in Petri dishes with 22 mL of agar medium. Wells were punched inoculated agar surfaces and filled with 30µl of methanol extract. All plates were incubated at 37°C for 24 h. The antimicrobial activity was measured the radius of the zone of inhibition (mm). All tests were performed in triplicate. Streptomycin (30 µg) was used as positive controls. Methanol was used as a negative control.

Total Phenolic Content

The total phenolic content was investigated by the Folin-Ciocalteu method (Wu et al., 2003). Briefly, 1.5 ml of Folin-Ciocalteu reagent and 1.2 ml sodium carbonate solution (75%) was taken in test tube and 0.3 ml of plant extract of *N. arbor-tristis* leaves was added. The tube was mixed well and allowed to stand for 30 min at room temperature. Absorbance was recorded at 765 nm. Results were expressed as milligram of gallic acid equivalent per gram of extract weight.

DPPH radical Scavenging Activity

DPPH free radical scavenging assay using 2, 2, diphenyl-1-picrylhydrazyl (DPPH) was performed spectrophotometrically using Kumar et al., (2008) described method. DPPH stock solution, 2.366 mg of DPPH was dissolved in 100 ml of absolute ethanol (60 μ M). Ascorbic acid in 1 mg ml⁻¹ concentration used as a positive control. Leaves methanol extract of *N. arbor-tristis* (500 μ l) was mixed with the same volume of DPPH solution and allowed to stand for 1.5 hours at room temperature in dark condition. The absorbance was observed at 517 nm. The test was performed in triplicates. Scavenging activity was calculated by following formula-

$$\% \text{ antioxidant activity for DPPH} = (A - A_x) / A \times 100$$

Where,

A- Absorbance of DPPH solution with ethanol,

A_x- Absorbance of DPPH solution with test solution.

III. RESULTS AND DISCUSSION

Phytochemical Screening

Preliminary phytochemical analysis shown that the occurrence of bioactive compounds such as alkaloids, flavonoids, carbohydrates, triterpenoids, glycosides, saponins, tannins and anthraquinonines in the leaves methanol extract of *N. arbor-tristis*. (Table 1)

Table 1. Phytochemical Screening of Leaves Methanol Extract of *N. arbor-tristis*

Test	Leaves extract of <i>Nyctanthes arbor-tristis</i>
Alkaloids	+ve
Phenols	+ve
Flavonoids	+ve
Tannins	-ve
Glycosides	+ve
Terpenoids	-ve
Carbohydrates	+ve

Antibacterial Activity

The antibacterial activity of the methanol leaves extracts of *N. arbor-tristis* was observed against gram positive and gram negative bacteria. *mirabilis* and *Staphylococcus aureus* were observed. Results were shown on Table 2.

Table 2. Antibacterial Activity of *N. arbor-tristis* Leaves.

Bacteria Name	Zone of Inhibition (mm)
<i>Pseudomonas aeruginosa</i>	20
<i>Salmonella typhi</i>	11
<i>Staphylococcus aureus</i>	15
<i>Klebsiella pneumoniae</i>	17
<i>Escheichia coli</i>	19
<i>Bacillus subtilis</i>	16

Total Phenol Content

The total phenolic content the methanol leaves extracts of *N. arbor-tristis*. The results were expressed as gallic acid equivalent. The phenolic content of methanolic extracts was observed 260.3 \pm 12.2 mg equivalent gallic acid.

Antioxidant Activity

The DPPH free radical scavenging activity of leaves methanol extracts of *N. arbor-tristis* is presented in Table 3. The methanol extract showed well-mannered antioxidant activities with methanolic extract of *N. arbor-tristis* leaves showed percentage scavenging activity (48.6%).

Table 3. Total Phenol Content and DPPH Scavenging Activity of Methanol Extracts of *N. arbor-tristis* Leaves.

	Total Phenol Content mg GAE/G	DPPH Free Radical Scavenging Activity (%)
Methanol Leaves Extract	260.3 ± 12.2	48.6%

IV. CONCLUSION

The current study recommended that the methanol extract of *Nyctanthes arbor-tristis* leaves possess various bioactive compounds having antioxidant and antibacterial capacity which may be used as a oxidative stress related agents and further investigation for isolation and characterization may show the way to newer chemical agents for clinical or pharmaceutical.

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