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RESEARCH ARTICLE

Phytochemical Screening, Antimicrobial and Antioxidant Potential of *Nyctanthes arbor-tristis* L. Floral Extracts

Dilla Jose, S. Pandiammal and P. Senthilkumaar*
School of Enzymology and Environmental Toxicology, P.G. and Research Department of Zoology, Sir Theagaraya College, Chennai, India
drsenthilkumaar@yahoo.co.in*

Abstract

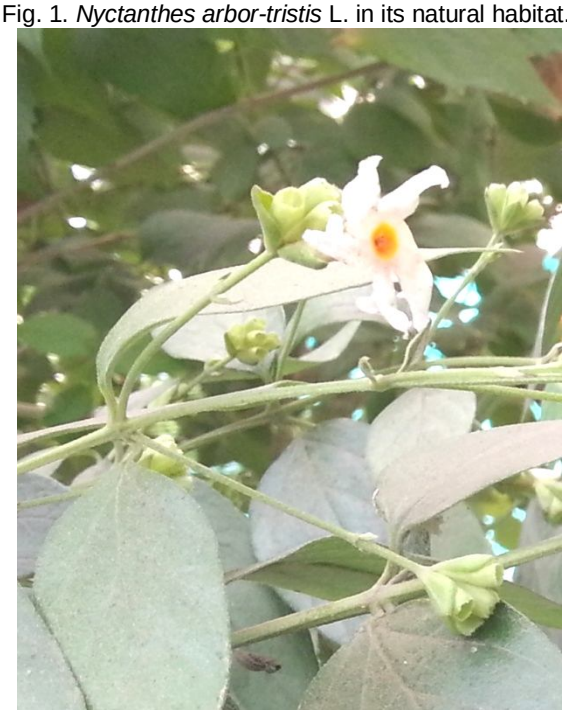
Methanolic extracts of *Nyctanthes arbor-tristis* L. flower showed the presence of flavonoid, saponins, alkaloids and coumarins in qualitative phytochemical analysis. The extract showed total flavonoid and saponins content as 50.46 mg quercetin equivalent/gram of extract and 232 mg of saponins (dry weight)/20g of extract, respectively. Methanolic extract of *N. arbor-tristis* showed less inhibition against *Escherichia coli* (11 mm/5 mg) and the maximum inhibition was found against *Bacillus subtilis* (15 mm/5 mg) and *Klebsiella pneumoniae* (16 mm/5 mg). Antioxidant activity of methanolic extract exhibited a significant dose dependent inhibition of DPPH, ABTS and nitric oxide activity. The 50% of Inhibition (IC_{50}) was found at a concentration of 1000, 1200 and 400 µg/mL for DPPH, ABTS and nitric acid respectively.

Keywords: *Nyctanthes arbor-tristis* L., methanolic extract, phytochemicals, antimicrobial, antioxidant.

Introduction

Therapeutic properties of medicinal plants have been used traditionally to treat human diseases. Growing populations of developing countries use plant derived medicines to be a normal part of primary healthcare. Hundreds to thousands of diverse secondary metabolites with different biological activities were found recorded in higher plants (Hamburger and Hostettmann, 1991). In recent years, multiple drug resistance (MDR) human pathogenic microorganisms has developed due to indiscriminate use of synthetic antimicrobial drugs commonly used in the treatment of infectious diseases (Singh *et al*., 2013). Natural antibiotics may have meager side effects and easy to treat human pathogenic microorganisms (Mitscher *et al*., 1987). Only 5-15% of 250k higher plants have been studied for a potential therapeutic value in the world and a large number remains to be investigated (Kinghorn, 1992). *Nictanthes arbor-tristis* L. (Night Jasmine) belongs to the family Oleaceae is found on rocky ground dry hills side and as under growth in dry deciduous forests. It is native of South Asia, Nepal through Northern India to Southeast Thailand. Flowering usually occurs from July to October. *Nictanthes arbor-tristis* L. stem bark of this plant is taken to cure dysentery, ulcer of palate and internal injuries (Ray and Gupta, 1980). The researchers used the juice of the leaves to treat chronic and bilious fever, rheumatism, as a laxative, diaphoretic and diuretic and the plant has been reported to be effective anti-leishmanial, antiviral and anti-amoebic infections (Saxena *et al*., 1980; Puri *et al*., 1994; Khatune *et al*., 2001). Use of *N. arbor-tristis* has long been known Ayurvedic system of medicine for the cure of snake bite, bites of wild animals, cancer, sores, ulcers, dysentery, menorrhagia (Purushothaman *et al*., 1985).

Fig. 1. *Nyctanthes arbor-tristis* L. in its natural habitat.



Nictanthes arbor-tristis flowers have shown promising results as potential sources for the isolation of bioactive compounds with anticancer activities (Timsina and Nadumane, 2016). Only very few reports have been published in *N. arbor-tristis* flower extracts and no systematic work has been done on the phytochemical and antioxidant potential, hence the present study has been taken with the aim of extraction, phytochemical characterization and evaluation of antimicrobial and antioxidant potential.

Materials and methods

Plant collection: Fresh and disease free *Nictanthes arbor-tristis* L. floral parts were collected from the healthy plants grown in the premises of Jayagen Biologics, Kottur, Chennai, TN, India in the month of Sep 2015 (Fig. 1). The sample was identified and authenticated by Dr. I.V.S. Nimal Christhudas, Senior Scientist, Jayagen Biologics, Kottur, Chennai.

Extraction of N. arbor-tristis flower: The whole flower was shade dried and powdered. The powder (20 g) was extracted three times by cold percolation method with methanol at room temperature for 72 h. The filtrates were concentrated under reduced pressure at 40°C and stored in a refrigerator at 2-8°C for use in subsequent experiments.

Phytochemical analysis: Phytochemical screening of methanol extract of *Nictanthes arbor-tristis* L. floral (MeNaF) was carried out to detect the phytoconstituents using standard conventional protocols (Harborne, 1998).

Test for carbohydrates (Molisch's test): Few drops of Molisch's reagent were added to 2 mL of the methanolic floral extract. This was followed by addition of 2 mL of conc. H_2SO_4 down the side of the test tube. The mixture was then allowed to stand for 2-3 min without shaking. Formation of a red or dull violet colour at the interphase of the two layers was a positive test.

Test for proteins: One mL of methanolic extract was mixed with 2 mL of millon's reagent. Appearance of white precipitate which turned red upon gentle heating confirms the presence of protein.

Test for alkaloids: A fraction of extract was treated with 3-5 drops of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 mL of water) and observed for the formation of reddish brown precipitate.

Test for flavonoids: Extract was dissolved in diluted NaOH and HCl. A yellow solution that turns colourless indicates the presence of flavonoids.

Test for terpenoids: Extract was treated with 2 mL of chloroform and filtered. The filtrate was treated with few drops of conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicated the presence of terpenoids.

Test for phlobatannins: Deposition of a red precipitate when 2 mL of extract was boiled with 1 mL of 2% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Test for phenolic compounds: To 1 mL of the extract, few drops of 0.5% ferric chloride solution were added. Formation of bluish black colour indicated the presence of phenolic compounds.

Test for saponins: One mL aliquot of floral extract was combined with 5 mL distilled water at 60°C, shaken for 2 min, as saponins are known to possess frothing activity, the volume of froth produced in this experiments was observed and recorded every 10 min for a period of 30 min.

Test for tannins: Two ml of the extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

Tests for glycosides: To 2 mL of the extract, added 3 ml of $CHCl_3$ and 10% ammonia solution. Formation of pink colour indicates the presence of glycosides.

Test for cardiac glycosides: About 5 mL of floral extract was treated with 2 mL of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully under layered with 1 mL conc. sulphuric acid. A brown ring at the interface indicated the presence of de-oxy sugar characteristic of cardenolides.

Test for coumarin: To 2 mL of floral extract, few drops of alcoholic sodium hydroxide were mixed well and the formation of yellow colour indicates the presence of coumarin.

Determination of total flavonoids: The method is based on the formation of the flavonoids-aluminium complex which has an absorptivity maximum at 415 nm. About 100 µL of the floral extract in methanol (10 mg/mL) was mixed with 100 µL of 20% aluminium tri-chloride in methanol and a drop of acetic acid, and then diluted with methanol to 5 mL. The absorption at 415 nm was read after 40 min. Blank samples were prepared from 1.0 mL of floral extract and a drop of acetic acid, and then diluted to 5 mL with methanol. The absorption of standard quercetin solution (0.5 mg/mL) in methanol was measured under the same conditions. All determinations were carried out in triplicates.

Determination of total saponins: The samples were ground and 20 g of each were put into a conical flask and 100 mL of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 mL of 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90°C. The concentrate was transferred into a 250 mL separatory funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. About 60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath.

After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated.

Antimicrobial activity: The antibacterial and antifungal activity of methanolic floral extract was carried out by agar well diffusion method. The following Gram negative pathogenic bacteria *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424), *Bacillus subtilis* (MTCC 441), *Klebsiella pneumoniae* (clinical sample) and Gram positive bacteria such as and *Staphylococcus aureus* (MTCC 96) were used in this study. The pathogenic fungi such as *Candida albicans* (MTCC 227), *Aspergillus flavus* and *A. niger* were used for the antifungal study. Nutrient agar plates were inoculated with test organisms as spread plate. Then wells were made in the plates with a cork borer. Each well was loaded with 3 different concentrations of crude extract (1.25, 2.5, 5.0 mg/100 µL/well). The well that received similar volume of respective carrier solvent (Dimethyl sulphoxide) served as negative control and Chloramphenicol was used as positive control. The plates were incubated for 48 h at 37°C. The development of zone of inhibition around the well was measured and recorded.

ABTS radical assay: The total antioxidant activity of the samples was measured by ABTS radical cat ion decolorization assay according to the method of Re *et al*. (1999). ABTS was produced by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium per sulphate in the dark for 12-16 h at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30°C to give an absorbance at 734 nm of 0.700±0.02. The stock solution of the sample extracts were diluted such that after introduction of 10 µL aliquots into the assay, they produced between 20% and 80% inhibition of the blank absorbance. After the addition of 1 mL of diluted ABTS solution to 10 µL of sample or ascorbic acid standards, absorbance was measured at 30°C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of the standard and the percentage inhibition was calculated using the blank absorbance at 734 nm.

DPPH radical scavenging activity: A methanol DPPH solution (0.15%) was mixed with serial dilutions (200-1,000 µg/mL) of the MeNaF and after 10 min, the absorbance was read at 515 nm. The antiradical activity was expressed as IC_{50} (µg/mL). Vitamin C was used as standard. The ability to scavenge the DPPH radical was calculated by the following formula:

DPPH radical scavenging activity (%) = $(A_0 - A_1)/A_0 \times 100$

Where A_0 is the absorbance of the control at 30 min and A_1 is the absorbance of the sample at 30 min. Methanolic extract was analyzed in triplicate.

Nitric oxide scavenging activity: Sodium nitroprusside in aqueous solution at physiologic pH spontaneously generates nitric oxide; it interacts with oxygen to produce nitrite ions, which can be estimated by the use of Griess-Ilosvoy reaction. In the present investigation, Griess-Ilosvoy reagent was modified using naphthyl ethylene diamine dihydrochloride (NEDD-0.1% w/v) instead of 1-naphthylamine (5%). The reaction mixture (3 mL) containing sodium nitroprusside (10 mM, 2 mL), phosphate buffer saline (0.5 mL) and different concentrations of MeNaF (200-1,000 µg/mL) or standard solution (0.5 mL) was incubated at 25°C for 150 min. After incubation, 0.5 mL of the reaction mixture containing nitrite was pipette out and mixed with 1 mL of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 mL of naphthyl ethylene diamine dihydrochloride (1%) was added and mixed well then allowed to stand for 30 min. A pink colour chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank. Ascorbic acid was used as standard.

Statistical analysis: The data for biochemical and physiologic parameters were analyzed and expressed as mean±SD. The IC_{50} values were calculated from linear regression analysis. Results were processed by Microsoft Excel 2007.

Results

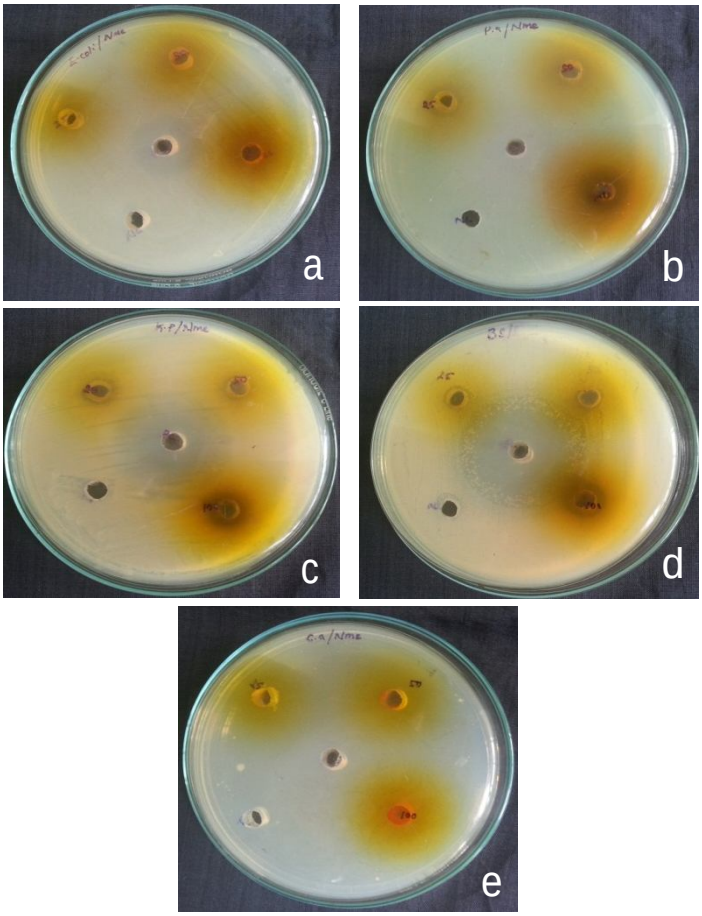
The methanolic floral extracts of *Nictanthes arbor-tristis* showed the presence of flavonoid, saponins, alkaloids and coumrins in qualitative phytochemical analysis (Table 1). In quantitative phytochemical analysis of crude methanolic extract, the total flavonoid and saponins content of the extract was found to be 50.46 mg quercetin equivalent/g of extract, 232 mg of saponins (dry wt.)/20 g of extract respectively (data not shown).

Table 1. Qualitative phytochemical analysis of methanlolic floral extract of *N. arbor-tristis.*

Phytochemicals	Results
Carbohydrates	++
Proteins	+
Alkaloids	++
Flavonoid	+++
Terpenoid	-
Phlobatannins	-
Phenols	-
Saponins	+++
Tannins	-
Coumarins	++
Cardiac glycosides	++

(+++)-Predominantly present; (++)- moderate;
(+)- slightly present; (-) absent.

Fig. 2. Antimicrobial activity of methanolic floral extract of *N. arbor-tristis.*



a) *E. coli*; b) *P. aeruginosa*; c) *K. pneumoniae*; d) *B. subtilis*; e) *C. albicans.*

Table 2. Antimicrobial activity of methanolic floral extract of *N. arbor-tristis.*

Pathogens	Zone of inhibition (mm)			
	Sample conc. (mg/mL)			Control (30 µg/mL)
	1.25	2.5	5	
Klebsiella pneumoniae	11	12	14	27*
Escherichia coli	10	10	11	19*
Bacillus subtilis	11	11	13	27*
Pseudomonas aeruginosa	10	12	15	22*
Candida albicans	-	-	-	15$

*Streptomycin; $Clotrimazole.

The antibacterial and antifungal activity of MeNaF exhibited increasing antibacterial activity. Table 2 shows the antibacterial activity of MeNaF. Comparatively MeNaF inhibited the growth of gram positive and gram negative bacterial pathogens. The less inhibition was found against *E. coli* (11 mm/5 mg) and the maximum inhibition was found against *Bacillus subtilis* (15 mm/5 mg), *Klebsiella pneumoniae* (16 mm/5 mg) (Fig. 2). In antifungal activity, comparatively less inhibition was found than the antibacterial activity. Methanolic floral extract of *N. arbor-tristis* did not inhibit the growth of *Candida albicans*, *Aspergillus niger* and *A. flavus*.

Fig. 3. DPPH scavenging activity of methanolic floral extract of *N. arbor-tristis.*

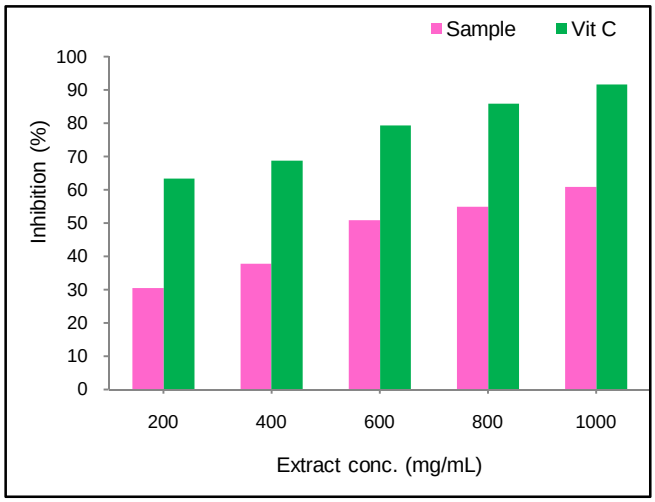


Fig. 4. ABTS radical scavenging ability of methanolic floral extract of *N. arbor-tristis.*

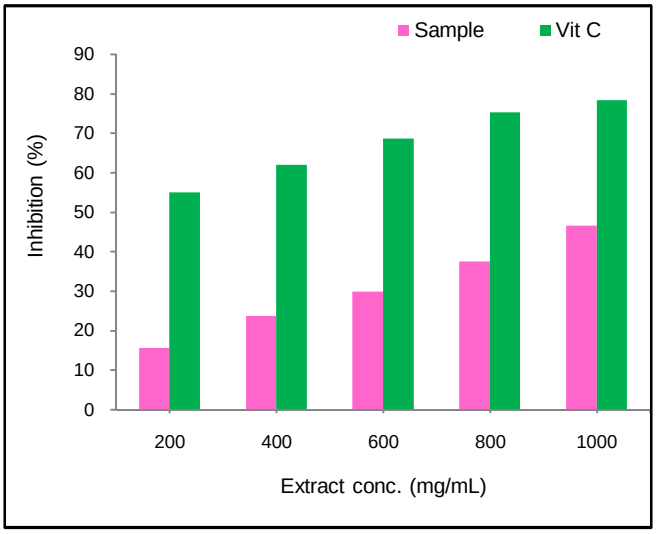
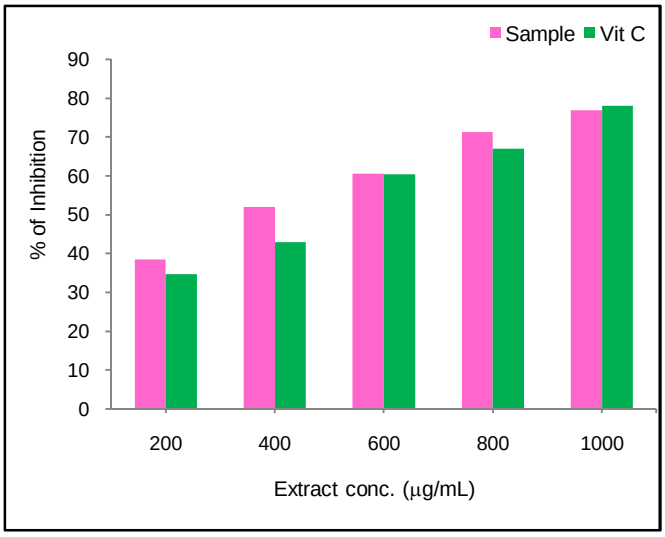


Fig. 5. DPPH scavenging activity of methanolic floral extract of *N. arbor-tristis.*



In antioxidant activity, MeNaF exhibited a significant dose dependent inhibition of DPPH activity. The 50% of inhibition (IC_{50}) was found at a concentration of 1000 µg/mL in the DPPH radical scavenging activity (Fig. 3). IC_{50} value of the vitamin C was 200 µg/mL. In ABTS assay for the determination of antiradical and antioxidant activities, the obtained results revealed that, 1 mg of methanolic floral extract showed 46.62% radical scavenging effect. While ascorbic acid showed 78.32% in free radical scavenging activity, against ABTS radicals (Fig. 4). The scavenging of nitric oxide by methanolic floral extract increased in a dose-dependent manner as illustrated in Fig. 5. At a concentration of 400 µg/mL of extract, 50% of NO generated by incubation was scavenged.

Discussion

Nictanthes arbor-tristis L. is one of important traditional medicinal plants. Leaves, roots and stem bark of *N. arbor-tristis* are used to treat many infectious diseases. Ethanolic extracts of leaves and root rich in phenols, steroids, alkaloids, saponins, tannins and flavonoids were reported by Ramachandran *et al*. (2014). Meanwhile fewer reports are available for floral extracts of *N. arbor-tristis* L. In the present study, methanolic floral extract of *N. arbor-tristis* exhibited high flavonoid content and absence of alkaloids, tannins and phenols. Flavonoids have been demonstrated to have antibacterial, anti-inflammatory, anti-allergic, anti-viral activity (Alan and Miller, 1996). Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Iwu *et al*., 1999). In the present study, antimicrobial activities of floral extract form *N. arbor-tristis* significantly inhibited in the tested pathogens. Maximum inhibition was recorded against *E. coli*. Antioxidants play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxide. In our study, the antioxidant properties of *N. arbor-tristis* floral extracts were evaluated with varying parameters. MeNaF had the ability to reduce the stable radical DPPH to the yellow-coloured diphenylpicryl hydrazine. This method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by the reaction (Brand-Williams *et al*., 1995; Nitin *et al*., 2010). Nitric oxide plays an important role in various types of inflammatory processes in the animal body. Nitric oxide radical inhibition study showed that the extract was a potent scavenger of nitric oxide. The extract also inhibited nitrite formation by competing with oxygen to react with nitric oxide directly and also to inhibit its synthesis. DPPH test is usually used as the substrate to evaluate antioxidants (Oyaizu, 1986). Scavengers of nitric oxide competed with oxygen leading to reduced production of nitric oxide (Marcocci *et al*., 1994).

Conclusion

The present study suggested that the methanol extract of *N. arbor-tristis* flower possess various phytochemical compounds having antimicrobial and antioxidant potential which may be used for the oxidative stress related conditions and further investigation related to the active principle isolation and characterization may lead to newer chemical entities for clinical use or combinatorial synthesis.

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