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Phytochemical and Antibacterial Studies on Methanolic Flower Extracts of *Nyctanthes arbor-tristis* L

Aparna Bhardwaj[1] and Somendra Sharma[2]*

1. Department of Chemistry, Mithibai College, Vile Parle (W), Mumbai, **INDIA**
2. Department of Botany, M.D. College, Parel, Mumbai, **INDIA**

Email: dr.somendra@rediffmail.com, dr.aparna73@rediffmail.com

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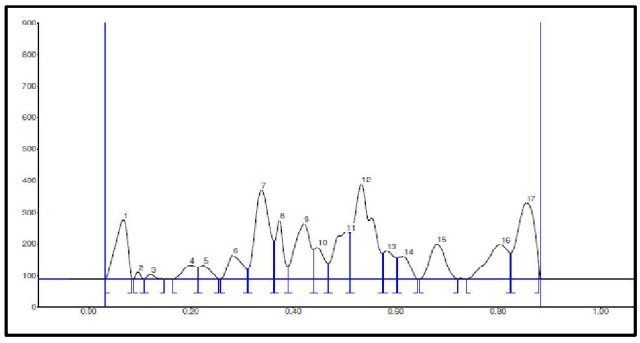
ABSTRACT

The present study was aimed to investigate the preliminary phytochemical analysis, HPTLC profiling and the antibacterial activity of Nyctanthes arbor-tristis methanolic flower extracts against the bacteria. The phytochemical studies on methanolic flower extract of Nyctanthes arbor-tristis revealed the presence of glycosides, flavonoids, phenolics, saponins, catechin and alkaloids. The HPTLC separation was achieved using toluene: chloroform: ethanol (4:4:2 v/v/v) as the mobile phase. The methanolic extract of Nyctanthes arbor-tristis showed four different Rf values 0.51, 0.65 and 0.74 which indicated various alkaloids, flavonoids and glycosides present in the flower extract. The methanolic extract of Nyctanthes arbor-tristis showed the maximum zone of inhibition against Proteus mirabilis followed by Salmonella typhi.

Graphical Abstract



HPTLC analysis of *Nyctanthes arbor-tristis* flower extract.

Keywords: *Nyctanthes arbor-tristis*, Phytochemical, HPTLC, Antibacterial activity.

INTRODUCTION

Medicinal plants are the most exclusive sources of life saving drugs for the majority of the world's population [1]. The medicinal actions of plants are unique to a particular plant species, consistent with

the concept that the combination of secondary metabolites in a particular plant is taxonomically distinct [2]. Natural products have traditionally played a pivotal role in drug discovery that make the compounds of interest in the development of active anti-hypertensive pharmaceutical agents [3]. Aqueous extracts of *Moringa oleifera* showed significant antiinflammatory effect [4]. *Nyctanthes arbor-tristis* commonly known as Night jasmine or coral jasmine, characterized by the presence of phenyl ethanoid derivatives and iridoid glucosides. Earlier studies on this plant have resulted in isolation of a number of glycosides from leaves, seeds and flowers. Leaves contain tannic acid, methyl salicylate, amorphous glucosides, mannitol, resin, ascorbic acid, carotene and traces of volatile oil [5] along with ß-amyrin, ß-sitosterol, nyctanthic acid and iridoid glucosides, arborside D and E (Minor iridoid arbortristosides). The iridoid arbortristoside A has been reported to have anti - proliferative activity [6].

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 [7, 8, 9]. 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 [10]. *Nictanthes arbor-tristis* flowers have shown promising results as potential sources for the isolation of bioactive compounds with anticancer activities [11].

The study was set out to investigate the antibacterial activity of *Nyctanthes arbor-tristis* flower extracts against the Gram positive and Gram negative bacteria isolated from human infections. In addition, the preliminary phytochemical analysis and HPTLC profiling was carried out to confirm the presence of various secondary metabolites constituents and trace the probable compounds responsible'.

MATERIALS AND METHODS

Collection and methanolic extract: Fresh flowers of *N. arbor tristis* were plucked from the tree and shade dried for a week at room temperature. 50 g of dried flowers were extracted using 300 mL methanol as solvent in a soxhlet apparatus for 8 h. The extract obtained was concentrated and stored at 4°C. The preliminary phytochemical screening was performed according to the Brindha *et. al,* method [12].

HPTLC: HPTLC studies were carried out following Harborne [13] and Wagner *et al* [14]. For the present study CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 with 12bit CCD camera for photo documentation, controlled by WinCATS-4 software were used. All the solvents used for HPTLC analysis were obtained from MERCK. The 100 mg extract was dissolved in 5 mL of Methanol (96%) and the solution was centrifuged at 3000 rpm for 5 min and used for HPTLC analysis as test solution. The samples (5 µL) were spotted in the form of bands of width 5mm with a Camagmicrolitre syringe on precoated silica gel glass plate 60F-254 (20cm x 10cm with 250 µm thickness (E. Merck, Darmstadt, Germany) using a Camag Linomat IV (Switzerland). The plates were pre-washed by methanol and activated at 60°C for 5 min prior to chromatography**.** The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapor) with respective mobile phase and the plate was developed in the respective mobile phase up to 90 mm. The Toluene-Chloroform-Ethanol was employed as mobile phase. Linear ascending development was carried out in 20 cm x 10 cm twin trough glass chamber (Camag, Mutenz, Switzerland) saturated with the mobile phase and the chromatoplate development for two times with the same mobile phase to get good resolution of phytochemical contents. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25 ±2°C). The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images under White light, UV 254 nm and UV 366 nm. The developed plate was sprayed with Antimony (III) chloride as spray reagent and dried at 100-120°C in hot air oven for 5-10 min. The plate was photo-documented

at UV 366 nm and daylight using Photo documentation (CAMAG REPROSTAR 3) chamber. Finally, the plate was fixed in scanner stage and scanning was done at 366 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag). The source of radiation utilized was deuterium lamp emitting a continues UV spectrum between 190 and 400 nm.

Microorganisms: The methanolic extracts of *Nyctanthes arbor-tristis* were tested against a panel of microorganisms including *Salmonella typhi*, *Staphylococcus aureus*, *Proteus mirabilis* and *Bacillus subtilis*.

Agar well diffusion method: The antibacterial activity of *Nyctanthes arbor-tristis* was evaluated by their methanolic extracts through agar well diffusion method. 24 h broth cultures of test organisms were used for assay. Cultures were spreaded in the Muller-Hinton agar surface. The agar plates were prepared in 90 mm Petri dishes with 22 mL of agar medium giving a final depth of 3 mm. Cylinders (diameter 5.5 mm) were placed on the inoculated agar surfaces and filled with 50, 75, 100 and 125 µg mL^{-1} of methanol extract. All plates were aerobically incubated at 37°C for 18-24 h. The antimicrobial activity was estimated by measuring the radius of the zone of inhibition (mm). Each test was performed in triplicate and repeated thrice. Streptomycin (30 µg) and Tetracycline (30 µg) were used as positive controls. Methanol was used as a negative control.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis revealed that the presence of secondary metabolites like alkaloids, carbohydrates, glycosides, saponins, triterpenes, fats and oils, tannins, , flavonoids, photobatalin and anthraqunonines in the flowers of *N. arbor-tristis* (Table 1). Methanolic flower extracts of *N. arbor-tristis* were examined for the antibacterial activity against the isolated human pathogens and the results are depicted in the (Table 2). The antibacterial activity of the methanolic flower extracts of *N. arbor-tristis* was observed in all the tested bacteria with varied degree activity. The maximum zone of inhibition 15 mm for *Salmonella typhi*, 14 mm for *Bacillus subtilis, Proteus mirabilis* and *Staphylococcus aureus* were observed (Table 2).

Table 1. Phytochemical screening of flower extracts of *N. arbor-tristis*

Test	Flower extract of *N. arbor-tristis*
Flavonoids	+++
Alkaloids	++
Glycoides	++
Carbohydrates	++
Proteins	+
Terpenoids	-
Phenols	+
Saponins	+
Tannins	-
Catechin	+

Table 2. Antibacterial activity of Methanolic flower extract of *N arbor-tristis*

Pathogens	Zone of inhibition (mm)					
	Methanolic extract in (µg mL^{-1})				Streptomycin	Tetracycline
	50	75	100	125		
S. aureus	8	10	11	14	5	6
P. mirabilis	6	11	13	14	4	5
B. subtilis	9	11	13	14	4	5
S. typhi	6	11	13	15	5	4

Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. The desired aim was achieved using Toluene-Chloroform-Ethanol (4:4:2 v/v/v) as the mobile phase (Table 3, Fig. 1). The methanolic extract of *N. arbor-tristis* showed 17 peaks having different Rf values, among them 0.31, 0.51, 0.65 and 0.74 which indicated various glycoside, alkaloid, flavonoid present in the flower extract (Fig. 2). Blue colored fluorescent zone at UV 366 nm appeared in both tracks flower extracts (Fig. 2). It confirms the presence of glycosides in the flower extracts of *N. arbor-tristis*.

Table 3. HPTLC analysis of methanolic flower extract of N. arbor-tristis

Peak	Start RF	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
1	0.03	0.6	0.07	186.3	8.50	0.09	3.2	4289.2	7.36	Unknown
2	0.09	1.9	0.10	22.1	1.01	0.11	0.7	192.1	0.33	Unknown
3	0.11	1.2	0.12	14.5	0.66	0.15	0.8	214.9	0.37	Unknown
4	0.17	0.2	0.20	45.1	2.06	0.21	38.5	1130.2	1.94	Unknown
5	0.21	38.5	0.23	45.2	2.06	0.25	0.8	952.8	1.64	Unknown
6	0.26	0.1	0.28	74.5	3.40	0.31	30.7	2019.0	3.47	Unknown
7	0.31	31.5	0.34	280.7	12.80	0.36	119.3	7501.3	12.88	**Glycoside**
8	0.36	119.8	0.37	185.6	8.46	0.39	40.6	2541.9	4.36	Unknown
9	0.39	41.9	0.42	174.9	7.97	0.44	91.9	4963.0	8.52	Unknown
10	0.44	92.5	0.45	99.8	4.55	0.47	51.1	1833.7	3.15	Unknown
11	0.47	51.2	0.50	149.4	6.81	0.51	144.5	4101.9	7.04	Unknown
12	0.51	144.6	0.53	298.7	13.62	0.58	80.0	10032.8	17.22	**Alkaloid**
13	0.50	81.0	0.50	87.1	3.97	0.60	67.2	1800.5	3.09	Unknown
14	0.60	67.5	0.62	70.8	3.23	0.65	0.1	1533.7	2.63	Unknown
15	0.65	0.2	0.68	110.0	5.02	0.72	0.8	3118.5	5.36	**Flavonoid**
16	0.74	0.2	0.81	109.1	4.97	0.83	80.0	4333.1	7.44	**Glycoside**
17	0.83	80.7	0.86	239.7	10.93	0.88	60.3	7689.6	13.20	Unknown

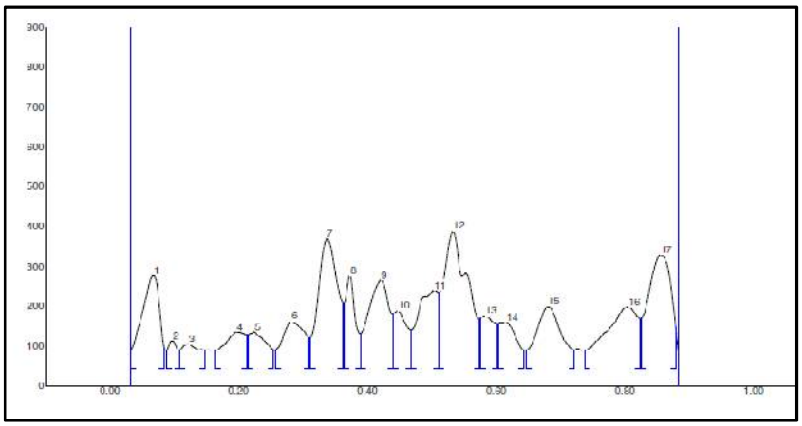


Figure 1. Track 1 HPTLC analysis of *N arbor-tristis* flower extract.

Phytochemical constituents such as tannins, saponins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and other herbivores [15]. The zone of inhibition for every bacterial strain was found to be increased as the extract concentrations were increased. The demonstration of antimicrobial activity against both Gram-positive and Gram-negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds and coincided with Cichewicz and Thorpe [16] and Srinivasan *et al* [17]. The secondary metabolites presence confirms the demonstration of antimicrobial activity by the flower extracts of *N. arbor-tristis*. The HPTLC method was validated by determining linearity, peak purity, limit of detection, repeatability, percentage recovery of glycosides from flower extracts of *N. arbor-tristis*. Similar to the present study Tiwari *et*

al [18] observed six spots in the alcohol extract of Helicteresisora root at 366 nm. Yamunadevi *et al* [19] reported glycosides presence in different parts of A. lanata. The HPTLC profile can be used as a tool for the taxonomical identification. Yamunadevi *et al* [19] also used the HPTLC profile for the identification of *A. lanata* from the adulterant.

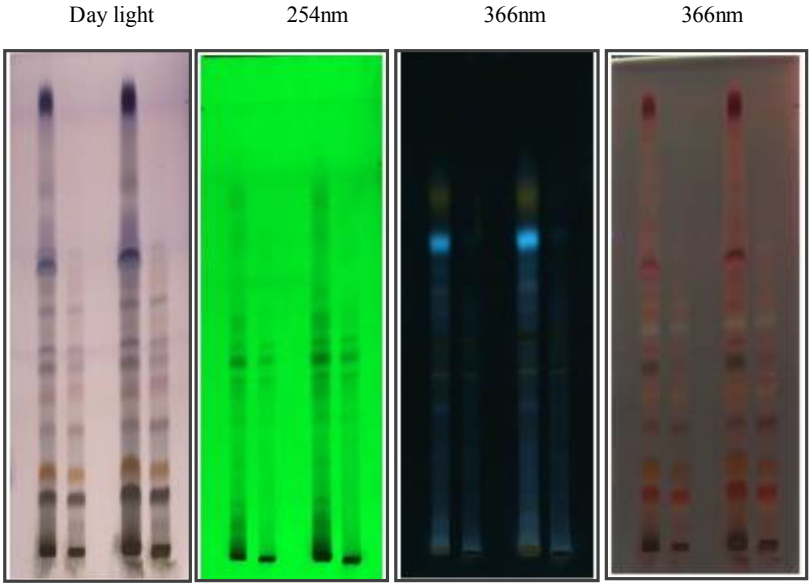


Figure 2. HPTLC analysis of methanol flower extracts of *N arbor-tristis.*

Natural product substances have historically served as the most significant the most significant source of new leads for pharmaceutical development [20]. These products as drugs play a significant role in the pharmaceutical care. The terrestrial plants have played a vital therapeutic role in the treatment of human ailments from time immemorial. Various active compounds from bark, leaves, root, flower and other parts of plants are found in a new guise in the existing treatments or may be used as basis for design of novel medicinal agents [19]. I n the present study also we observed the different types of glycosides presence in the methanolic flower extracts of *N. arbor-tristis* Glycosides comprise a very wide range of compounds that are of common and ubiquitous occurrence in almost all plants. Glycosides play important roles in our lives. Many plants store medicinally important chemicals in the form of inactive glycosides. The non-sugar portion contains the biochemically active properties of medical interest. Once the glycoside is split into its two components (sugar and non-sugar parts), the non-sugar component is now free to exert its chemical effects on the body. For example, digitalis is a glycoside that when ingested, causes the heart to contract (pump) more forcefully. This is useful in medicine, where heart failure is present. A considerable number of glycosides are of great medicinal value, all of them are of natural origin. These pharmaceutically valuable glycosides contribute to almost every therapeutic class, cardiac drugs, laxatives, counter irritants, analgesics, renal disinfectants, anti-rheumatics, anti-inflammatory, anti-tuberculosis, expectorant and antispasmodic action. In the present study we observed a number of glycoside from the flowers of *N. arbor-tristis*, thus the present study authenticated the traditional medical practice and previous pharmacological observations and supplement to treat other health problems such as cardiac disorder, rheumatism, tuberculosis etc. By isolating and identifying these bioactive compound new drugs can be formulated to treat various diseases.

APPLICATION

Nyctanthes arbor-tristis has medicinal properties, so it can be used in different pharmaceutical formulations by lowering the cost of production and adding more medicinal value to the respective drugs.

CONCLUSIONS

The present study suggested that the methanol extract of *Nyctanthes arbor-tristis* flower possess various phytochemical compounds having antibacterial 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. It may provide nature friendly and cost effective drugs.

REFERENCES

[1]. S. Sagwan; D.V. Rao, R. A .Sharma, *J. Chem. Pharm*. Res., **2010**, 2 (6), 46-50.
[2]. Anu Kumar, P. K. Chauhan, V. S. Bhardwaj, Ramesh Kumar, Ankur Tyagi, *J. Pharm. Chem. Res.*, **2011**, 3 (4), 166-171.
[3]. N. S. Dighe, S. R. Pattan D. S. Musmade P. A. Chavan S. K. Tambe, M. S. Sanap, V. M. Gaware, *J. Chem. Pharm. Res*., **2009** , 1, 53-61.
[4]. K. S. Chandarasekar, A. Thakur, K. S. Prasanna, *J. Chem. Pharm. Res*., **2010**, 2 (3), 179-181.
[5]. S. H Zaheer, B. Prasad, R. N. Chopra, H. Santapau, M. S. Krishnan, *The Wealth of India Vol.7. Publications and Information Directorate*, CSIR, New Delhi, **1966**, 69-70.
[6]. I. Siddique, M. Anis, A. A. Jahan, *World J. Agri. Sci*., **2006**, 2, 188-192.
[7]. R. S. Saxena, B. Gupta, K. K. Saxena, V. K. Srivastava, D. N. Prasad, Analgesic, antipyretic and ulcerogenic activity of Nyctanthes arbortristis leaf extract. *J. Ethnopharmacol*. **1987**, 19, 193-200.
[8]. A. Puri, R. Saxena, R. P. Saxena, K. C. Saxena, V. Srivastava, J. S. Tandon, Immunostimulant activity of Nyctanthes arbortristis L. *J. Ethnopharmacol*. **1994**, 42, 31-37.
[9]. N. A. Khatune, M. A. Mosaddik, M. E. Haque, Antibacterial activity and cytotoxicity of Nyctanthes arbortristis flowers. *Fitoterap*, **2001**, 72, 412-414.
[10]. K. K. Purushothaman, M. Venkatanarasimhan, A. Sarada, A. Arbortristoside, Two iridoid glucosides from Nyctanthes arbortristis, *Phytochem*, **1985**, 24, 773-776.
[11]. B. Timsina, V. K. Nadumane, Purification and evaluation of bioactive fractions for anti-cancer potentials from the flowers and leaves of Nyctanthes arbortristis L. *Chiang Mai J. Sci.,* **2016**, 43(1), 100-111.
[12]. P. Brindha, B. Sasikala, K. K. Purushothaman, Pharmacological studies on Merugankizhangu. *Bulletin of Medico-EthnoBotanicalResearch*, **1981**, 3(1), 84-96.
[13]. J. B. Harborne, *Phytochemcial methods.* 3rd ed. London: Chapman and Hall, **1998**.
[14]. H. Wagner, S. Baldt, E. M. Zgainski, Plant Drug Analaysis, Berlin: Springer, **1996**.
[15]. G. H. S. Bonjar, A. K. Nik, S. Aghighi, Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of south east regions of Iran, *J. Biolog Sci*., **2004**, 4(3), 405- 412.
[16]. R. H. Cichewicz, P. A. Thorpe, The antimicrobial properties of Chile peppers (Capsicum species) and their uses in Mayan medicine*, J. Ethnopharm*, **1996**, .52(2), 61- 70.
[17]. D. Srinivasan, L. P. Perumalsamy, S. Nathan, T. Sures,. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *J. Ethnopharm*, **2001**, 49, 217- 222.
[18]. V. Tiwari, A. Tiwari, V. Madhavan, Preliminary Phytochemical Analysis, HPTLC Studies and Antipyretic Activity of Alcohol and Aqueous Extract of HelicteresIsoraL .Root. International *J. Pharmacy and Pharma. Sci*., **2010**, 2(2), 74 -79.
[19]. M. Yamunadevi, E. G. Wesely, M. Johnson, A Chromatographic Study on the Glycosides of Aervalanata L. *Chinese Journal of Natural Medicines*, **2011**, 9(3), 210-214.
[20]. M. S. Butler, Natural products derived compounds in clinical trials. *Nat. Prod, Rep*., **2005**, 22, 162-195.