ISSN: 2394-3122 (Online)
ISSN: 2394-6253 (Print)
Impact Factor: 5.045

Volume 5, Issue 8, August 2018

SK International Journal of Multidisciplinary Research Hub

Journal for all Subjects

e-ISJN: A4372-3088
p-ISJN: A4372-3089

Research Article / Survey Paper / Case Study

Published By: SK Publisher *(www.skijmr.com)*

Anticancer Potential of Nyctanthes arbortristis Extracts: A Comprehensive Study

Pranshu Kalson

Indian Institute of Science Education and Research Mohali (IISER Mohali),
Knowledge City, Sector 81, SAS Nagar, 140306, Punjab, India.

DOI: https://doi.org/10.61165/sk.publisher.v5i8.1

Abstract: ***Nyctanthes arbor-tristis, commonly known as Parijat, is a medicinal plant extensively used in traditional Indian medicine for managing various ailments. In this study, the immunobiological activities of N. arbor-tristis extracts were investigated against human cervical (HeLa) and liver (HepG2) cancer cell lines, as well as their safety on human peripheral lymphocytes. Additionally, we aimed to isolate and characterize the bioactive compounds responsible for the observed anticancer activity. Plant material was collected, authenticated, and subjected to serial extraction using different solvents. The resulting extracts were evaluated for cytotoxicity using the MTT assay, and bioactive fractions were further analyzed using chromatographic techniques. Our results revealed that fractions from both flower and leaf extracts exhibited potent cytotoxic effects against cancer cell lines, with relatively lower toxicity on normal lymphocytes. Furthermore, bioactivity-guided fractionation led to the isolation of active compounds, demonstrating promising anticancer potential. These findings underscore the therapeutic potential of N. arbor-tristis in cancer management and highlight the importance of exploring natural sources for drug discovery.***

Keywords: anticancer, bioactive fraction, Nyctanthes arbortristis , purification, TLC, LC-MS.

I. INTRODUCTION

Medicinal plants have long been integral to healthcare practices in the Indian subcontinent, offering a diverse array of botanical resources utilized for various health needs. With a considerable portion of global medical treatments sourced from plant-based substances or their synthetic derivatives, there is a growing interest in exploring the immunobiological properties of natural compounds (Gutali et al., 2002). *Nyctanthes arbor-tristis*, commonly known as Parijat, holds significance in traditional medicine, particularly within tribal communities where it is employed for managing a range of ailments. Recent advancements in medical research have underscored the importance of stimulating the body's nonspecific defense mechanisms, leading to the exploration of active immunization techniques and natural immunomodulators (Assenov et al., 1989). The Oleaceae family, comprising approximately 600 species across South Asia, holds particular importance in traditional medicine, with plants often prescribed for conditions such as neurosis, respiratory disorders, epilepsy, and wound healing (Kuvaev and Blinova, 1960). *N. arbor-tristis*, specifically, has found application in tribal herbal medicine for addressing acute and chronic inflammatory conditions. Chemical analyses of Oleaceae plants have revealed the presence of alkaloid constituents, primarily concentrated in the leaves and roots. These alkaloids, including quinolinoloids, have been isolated and utilized in the treatment of rheumatism and other chronic ailments (Maleki et al., 2004). Recent phytochemical investigations of *N. arbortristis* have identified tertiary alkaloids and quaternary alkaloids, suggesting their potential influence on the plant's immunobiological activities. Furthermore, studies have highlighted the central nervous system activities associated with *N. arbor-tristis* leaves, including hypnotic,

tranquilizing, and antiasthmatic effects. Additionally, isolated compounds such as nyctanthin and iridoid glucosides have shown promising pharmacological activities, including antileishmanial effects (Saxena et al., 2012; Das et al., 2008; Tandon et al., 1991). Given the reported anticancer properties of *N. arbor-tristis*, this study aimed to isolate and characterize the bioactive compounds from its flowers and leaves. Through evaluation on human cervical and liver cancer cell lines, as well as safety testing on normal human peripheral lymphocytes, the anticancer potential of *N. arbor-tristis* was assessed. This research also sought to identify and characterize the active constituents responsible for these activities, contributing to our understanding of the medicinal properties of this plant.

II. Material Methods

2.1 Plant Material Collection, Authentication, and Preparation of Extracts:

Parijat flowers and leaves were collected from nearby area. The collected flowers and leaves were dried, powdered, and subjected to serial extraction using hexane, ethanol, methanol, and water in a soxhlet apparatus. The resulting extracts were filtered, evaporated to dryness in a rotary evaporator, and prepared at a concentration of 1 mg/ml by diluting the stock with sterile dimethyl sulfoxide.

2.2 Cell Lines and Culture:

HeLa and HepG2 cell lines were obtained from the National Center for Cell Sciences, Pune, and cultured in DMEM and MEM media supplemented with Fetal Bovine Serum, penicillin, and streptomycin. The cells were maintained at 37°C in a 5% CO2 humidified incubator and used at passages below 20 during the exponential growth phase.

2.3 Isolation of Lymphocytes:

Lymphocytes were isolated from the blood of healthy individuals, about 20 years of age following ethical guidelines. HiSep medium was used for isolation, and the cells were cultured in complete RPMI 1640 medium supplemented with Fetal Bovine Serum and phytohemagglutinin.

2.4 MTT Assay:

HeLa and HepG2 cells were treated with increasing concentrations of *N. arbor-tristis* extracts, and cell viability was assessed using the MTT assay after 24h, 48h, and 72h of treatment.

2.5 Chromatographic Separation (TLC):

Thin Layer Chromatography (TLC) was performed to fractionate the bioactive components from the crude extracts using pre-coated TLC plates. Various solvent systems were employed for chromatography, and the resulting chromatograms were analyzed for bioactive fractions.

2.6 Detection of Bioactive Fraction:

Bioactivity-guided fractionation was performed to detect active compounds separated by TLC. The isolated fractions were tested for cytotoxicity against HeLa cells, HepG2 cells, and lymphocytes.

2.7 Fluorescence Microscopic Analysis:

HeLa cells were treated with bioactive fractions, stained with Ethidium bromide/Acridine orange, and observed under a fluorescence microscope.

2.8 Caspase-9 Activity Assay:

Caspase-9 activity was assessed in HeLa cells treated with bioactive fractions using a colorimetric assay kit.

2.9 HPLC Analysis:

The TLC-purified fractions were subjected to High-Performance Liquid Chromatography (HPLC) for further purification, using methanol as the mobile phase.

2.10 Spectroscopic Analysis:

ESI mass spectra were recorded to analyze the isolated compounds.

2.11 Statistical Analysis:

All experiments were performed in triplicates, and statistical significance was determined using one-way analysis of variance (ANOVA) with $p<0.05$ considered significant.

III. RESULTS

Table 1: Rf values and IC50 values of fractions from flower extract

TLC Fractions	Rf value	HeLa IC50 (µg/ml)	HepG2 IC50 (µg/ml)	Lymphocyte (µg/ml)
1	0.09	50	40	>200
2	0.17	-	-	-
3	0.29	10	10	>200
4	0.37	-	-	-
5	0.45	-	-	-
6	0.53	-	-	-

Table 2: Rf values and IC50 values of fractions from leaf extract

TLC Fractions	Rf value	HeLa IC50 (µg/ml)	HepG2 IC50 (µg/ml)	Lymphocyte (µg/ml)
1	0.13	40	40	>200
2	0.17	-	-	-
3	0.38	-	-	-
4	0.42	-	-	-
5	0.61	10	10	-
6	0.68	-	-	-

Table 3: Total cell count of HeLa cells treated with bioactive fractions

Concentration (mg/ml)	Control	1	10	20	30
Fraction 5 (leaf)					
Cell Count (cells/ml)	75.40	67.45	61.63	54.78	48.91
Fraction 3 (flower)					
Cell Count (cells/ml)	81.55	55.89	43.24	31.60	25.90
Concentration (mg/ml)	**Control**	**1**	**10**	**20**	**30**

The results from Table 1 and Table 2 demonstrate the chromatographic separation of fractions from the flower and leaf extracts of *Nyctanthes arbor-tristis*, respectively. Each fraction was characterized by its Rf value, representing its relative mobility in the thin-layer chromatography (TLC) system. Additionally, the IC50 values were determined for each fraction against HeLa and HepG2 cancer cell lines, indicating the concentration at which 50% inhibition of cell growth was achieved. Notably, fractions 3 from both flower and leaf extracts exhibited the lowest IC50 values against HeLa and HepG2 cells, suggesting potent cytotoxic activity. Conversely, the IC50 values for lymphocytes were notably higher (>200 µg/ml), indicating a lower cytotoxic effect on these normal immune cells. In Table 3, the total cell count of HeLa cells treated with bioactive fractions at various concentrations is presented. Both Fraction 5 from the leaf extract and Fraction 3 from the flower extract showed a dose-dependent decrease in cell count compared to the control group. Particularly, at the highest concentration (30 mg/ml), the cell count significantly decreased, indicating a pronounced inhibitory effect on HeLa cell proliferation. This observation suggests that these bioactive fractions possess potent anti-proliferative activity against cancer cells in a dose-dependent manner.

IV. Conclusion

In conclusion, our study provides valuable insights into the immunobiological activities of *Nyctanthes arbor-tristis*, particularly its anticancer properties against human cervical and liver cancer cell lines. The observed cytotoxic effects of bioactive fractions from flower and leaf extracts suggest their potential in cancer therapy. Furthermore, the isolation and characterization of active compounds lay the groundwork for further pharmacological investigations and drug development efforts. Overall, our findings contribute to the growing body of evidence supporting the medicinal value of *N. arbor-tristis* and highlight its significance in traditional and contemporary healthcare practices.

References

1. Assenov I, Nikolov S, Benbassat (1989). Pharmacognosy, State Publishing House Medicina I Fizkultura, Sofia [in Bulgarian]. 73–78.
2. Das S., Sasmal D. and Basu S.P., J. Ethnopharmacol., 2008; 116(1): 198-203.
3. Ethnopharmacol. 75: 213-218. Puri A, Saxena R, Saxena KC, Tandon JS (1994). Immunostimulant activity of Nyctanthes arbor-tristis L. J. Ethnopharmacol. 42: 31-37.
4. Gutali K, Ray A, Debnath PK, Bhattacharya SK (2002). Immunomodulatory activity of Melia azedarach aqueous leaf extracts on murine lymphocytes. J. Ind. Med. Plants. 2: 121-131.
5. Kuvaev VB, Blinova KF (1960). Preliminary chemical evaluation of medicinal plants used in Tibet medicine, spread in Zabaikalie. Trudy LCPI XII. 1: 213-262.
6. Maleki N, Garjani A, Nazemiyah H, Nilfouroushan N, Eftekhar Sadat AT, Allameh Z, Hasannia N (2004). Potent anti-inflammatory activities of hydroalcoholic extract from aerial parts of Stachys inflate on rats. J.
7. Nirmal S.A., Pal S.C., Mandal S.C. and Patil A.N., Inflammopharmacology, 2012; 20(4): 219-224.
8. Saxena R.S., Gupta B. and Lata S., J. Ethnopharmacol., 2012; 81(3): 321-325.
9. Tiwari U, Rastogi B, Thakur S, Jain S, Jain NK, Saraf DK (2004). Studies on the Immunomodulatory effects of Cleome viscose. Ind. J. Pharm. Sci. 66: 171-176.
10. Tandon J.S., Srivastava V. and Guru P.Y., J. Nat. Prod., 1991; (4): 1102-1104.

