



Original Research Article

Exploring the medicinal potential of *Nyctanthes arbor-tristis* leaves: Phytochemical profiling and immunological effects in the avian system

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Abstract

Antioxidants are vital compounds that play a key role in safeguarding the body against damage induced by oxidative stress. Since ancient times, a variety of ailments have been managed by modulating the immune system through the use of medicinal plants. The poultry industry is particularly vulnerable to both biotic and abiotic stresses, which often lead to substantial economic losses. Enhancing the immune system of poultry, or immunomodulation, is one strategy employed to combat poultry diseases. In light of this, the present study investigated the immunomodulatory and antioxidant potential of *Nyctanthes arbor-tristis* (NAT) in a chicken lymphocyte culture model. The aqueous NAT leaf extract (NAE) was prepared and assessed for the presence of various phytochemical constituents. The antioxidant activity of NAE was evaluated using the DPPH assay. The non-cytotoxic concentration of NAE was established in the lymphocyte culture system to be 300 µg/mL. NAE induced significant T cell proliferation in cultures stimulated with PHA and Con A, and also led to an increase in B cell proliferation in LPS-stimulated cultures compared to the control. A notable reduction in lipid peroxidation percentage was observed following treatment with NAE. Furthermore, levels of reduced glutathione (GSH), superoxide dismutase (SOD), and catalase were elevated in NAE-treated cells relative to untreated controls. NAE demonstrated clear antioxidant properties and showed immunomodulatory effects in chicken lymphocytes, indicating its potential application in developing herbal immunomodulatory preparations for poultry. Nonetheless, further in-depth research is warranted to identify the specific phytochemicals responsible for NAE's immunostimulatory effects, employing advanced biochemical and molecular biology methodologies.

Keywords: Antioxidants, DPPH, Immunomodulation, Medicinal plants, Phytochemicals.

Introduction

Ancient Indian texts highlight that every plant on earth serves a purpose for humans, animals, and other plants. According to the World Health Organization, around 80% of the global population still depends on traditional medicine for their mental and physical well-being. Pharmaceutical companies are increasingly investigating plant-based materials, particularly those from rainforests, for their potential therapeutic applications. Historically, medicinal plants have played a significant role in treating diseases by influencing the immune system (Kumari et al., 2022). In recent decades, poultry production has outpaced crop production, making disease prevention in poultry through immune modulation a key focus. A well-functioning immune system depends on a balance between immune stimulation and suppression. Secondary metabolites in medicinal plants have shown

positive effects on immune responses (Suresh et al., 2018). To evaluate the effects of medicinal plants on animals, *in-vitro* methods are commonly used (Ambwani et al., 2025). One such plant is *Nyctanthes arbor-tristis*, also known as parijat, coral jasmine, harsingar, or queen of the night. Belonging to the Oleaceae family, it has a long history of use in traditional medicine. Various parts of the plant — including leaves, seeds, flowers, bark, and fruit — are known for their therapeutic properties. Traditionally, it has been used to manage ailments such as fever, pain, hemorrhoids, irregular menstruation, asthma, and cough (Sharma et al., 2023; Dewi et al., 2022). *N. arbor-tristis* is widely utilized in Ayurvedic and Siddha practices, as well as by the herbal pharmaceutical industry. Scientific studies have confirmed a broad spectrum of its medicinal effects, including antipyretic, anti-inflammatory, anthelmintic,

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sedative, laxative, expectorant, and treatments for rheumatism (Parekh & Soni, 2020). The plant also shows effectiveness against several bacterial and viral infections. Its medicinal capabilities are largely due to its diverse secondary metabolites (Haque et al., 2019). Phytochemical studies have identified various active compounds such as phenolics, flavonoids, glycosides, alkaloids, phytosterols, tannins, saponins, carbohydrates, and terpenoids (Figure 1). Notably, compounds such as gallic acid, caffeic acid, and rutin contribute to its antioxidant and anti-inflammatory functions (Rajani et al., 2021; Thapa & Kumar, 2024). The plant is especially recognized for iridoids and secoiridoids, which possess potent pharmacological effects. The plant's pharmacological profile includes anti-inflammatory, analgesic, antidiabetic, antioxidant, hepatoprotective, antitussive, antimalarial, immunomodulatory, amoebicidal, antifilarial, antileishmanial, and larvicidal activities, as well as cytotoxic potential against some cancer cell lines (Sharma et al., 2023). Extracts from the whole plant and specific parts like seeds, stem, and leaves have shown both acute and sub-acute anti-inflammatory effects in animal models. In particular, inflammatory responses were reduced by agents such as carrageenan, histamine, and formalin, and also in chronic inflammation models like granuloma and cotton pellet tests. Additionally, the plant inhibited immune-induced inflammation in models such as Freund's adjuvant arthritis (Rawat et al., 2021). The aqueous leaf extract of *N. arbor-tristis* has demonstrated strong immunomodulatory effects in both humoral and cell-mediated immune responses. Disruptions in antioxidant defence systems, including enzymes like SOD, CAT, GPx, and GR, alongside compromised glutathione metabolism, can be mitigated by the plant's antioxidant properties. These antioxidants play a crucial role in protecting cells from damage caused by reactive oxygen species (ROS), which can lead to lipid peroxidation, protein degradation, and DNA damage (Rathod et al., 2010). Furthermore, the flowers of the plant have shown immunostimulatory effects by activating immune cells. Keeping in view of the above, the present study was designed to evaluate the *in-vitro* antioxidant and antinflammatory potential of *N. arbor-tristis* in chicken splenocytes culture system along with its qualitative and quantitative phytochemical analysis.

Materials and Methods

Collection of plant material

The leaves of *N. arbor-tristis* were sourced from the Medicinal Plant Research and Development Centre (MRDC) at GBPUA&T, Pantnagar, Uttarakhand, India. Their identification was confirmed by Dr. D.S. Rawat, Assistant Professor in the Department of Biological

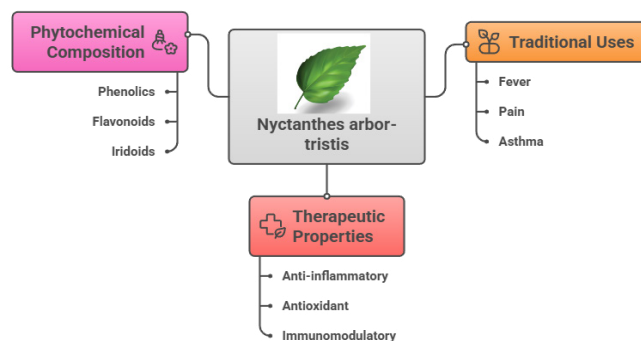


Figure 1: Functional attributes of plant contributing to health

Sciences at the College of Basic Sciences and Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar. Subsequently, an aqueous extract of the plant was prepared. The study utilized chemicals of cell culture grade.

Extraction procedure

The aqueous leaf extract of *N. arbor-tristis* (NAE) was prepared following established methods (Singh et al., 2021; Chand et al., 2024; Ambwani et al., 2024). A total of 100 g of shade-dried leaves powder was immersed in 1000 mL of double-distilled water. This mixture was incubated and continuously agitated in a shaker at 37°C for 48 hours. The resulting solution was first filtered using a muslin cloth, followed by filtration through Whatman No. 1 filter paper. The filtrate was concentrated at 45°C using a rotary evaporator to eliminate excess solvent, followed by lyophilization. The process involves freezing at -60°C; the cycle typically lasts around 24-30 hours. The dried extract was weighed and stored at -20°C until further use (Figure 2).

Qualitative phytochemical screening

Phytochemical tests were performed to identify the presence of bioactive compounds, using standard

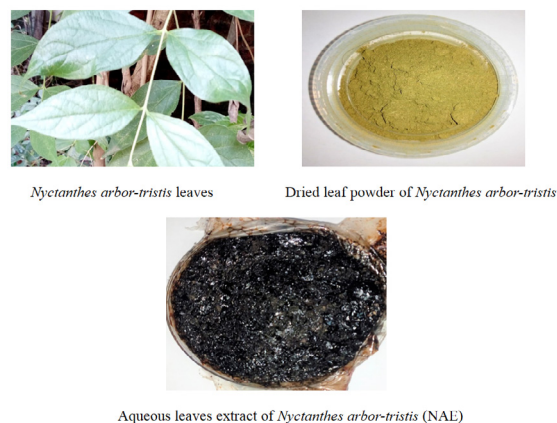


Figure 2: Preparation of the aqueous leaves extract of *Nyctanthes arbor-tristis* (NAE)

protocols as outlined by Mir et al. (2013); Shabbir et al. (2013).

Tannins

About 50 mg of plant extract was boiled in 20 mL of distilled water and filtered. To the filtrate, 0.1% ferric chloride solution was added. The formation of a brownish-green or blue-black colour indicated the presence of tannins.

Flavonoids

Over 50 mg of plant extract was dissolved in 100 mL of distilled water and subsequently filtered. A 10 mL portion of the resulting filtrate was combined with 5 mL of dilute ammonia solution (1%). The development of a yellow colour upon the addition of a few drops of concentrated sulfuric acid signified the presence of flavonoids.

Terpenoids

In 5 mL of the aqueous extract was mixed with 2 mL of chloroform, followed by the careful addition of 3 mL concentrated sulfuric acid. A reddish-brown interface indicated the presence of terpenoids.

Phenols

About 500 mg of the extract was dissolved in 5 mL of distilled water. The addition of a few drops of neutral 5% ferric chloride solution resulted in a dark green colour, indicating the presence of phenolic compounds.

Phlobatannins

An aqueous extract of the dried leaves was boiled with 1% hydrochloric acid. The formation of a red precipitate confirmed the presence of phlobatannins.

Quantitative phytochemical analysis

Total phenolic content (TPC)

TPC was measured using the Folin-Ciocalteu method with slight modifications from Truong et al. (2019). In this

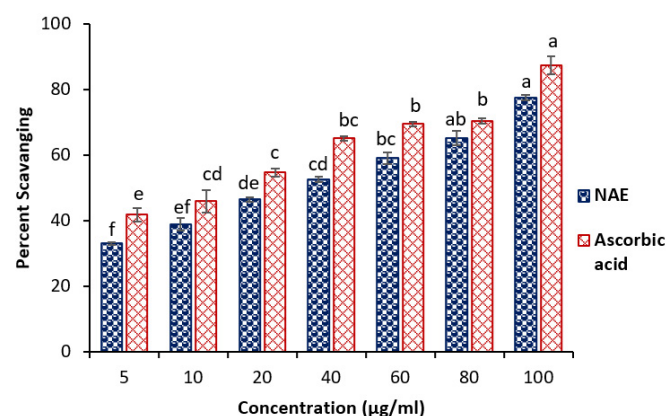


Figure 3: Antioxidant activity of NAE with reference to Ascorbic acid as standard in DPPH radical scavenging. Significant differences ($p < 0.05$) are indicated by different letters

method, 5 mL of 10% Folin-Ciocalteu reagent was added to 1-mL of plant extract (1-mg/mL). After 5 minutes, 4 mL of 7.5% sodium carbonate was added, and the mixture was incubated at room temperature for 30 minutes. A series of gallic acid standards (10–100 µg/mL) were prepared similarly. Absorbance was read at 760 nm using a UV/Vis spectrophotometer. TPC was calculated using the calibration curve and expressed in milligrams of gallic acid equivalents per gram of dried crude extract (mg GAE/g DCE). All measurements were performed in triplicate.

Total flavonoid content (TFC)

The aluminium chloride colorimetric assay, modified from Gonfa et al. (2020), was used to determine TFC. For this, 1-mL of plant extract (1-mg/mL) was placed in a 10 mL volumetric flask. To it, 5 mL of distilled water and 0.3 mL of 5% NaNO₂ (Sodium nitrite) were added. After 5 minutes, 0.5 mL of 5% AlCl₃ (aluminium chloride) was added, followed by 2 mL of 1 M NaOH after another 5 minutes. The solution was then diluted to volume with distilled water and mixed thoroughly. Absorbance was recorded at 415 nm. A standard curve was generated using rutin (10–100 µg/mL), and TFC was expressed as milligrams of rutin equivalents per gram of dried crude extract (mg rutin/g DCE). All tests were conducted in triplicate.

Assessment of antioxidant activity via DPPH assay

The antioxidant potential of the aqueous leaf extract of *N. arbor-tristis* was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. In a dark environment, 1-mL of the extract at varying concentrations (10–100 µg/mL) was mixed with 3 mL of DPPH solution and incubated. For comparison, 3 mL of methanol served as the blank, while the control consisted of 1-mL DPPH solution and 2 mL methanol. After a 30-minute incubation, the absorbance was measured at 517 nm using a UV-visible spectrophotometer. Ascorbic acid was used as a reference standard (Brahma & Baruah, 2023).

The scavenging activity was calculated using the formula:

$$\% \text{ Inhibition} = \left[\frac{\text{Absorbance of Control} - \text{Absorbance of Sample or Standard}}{\text{Absorbance of Control}} \right] \times 100$$

The IC₅₀ (Inhibitory concentration) value, indicating the concentration required to inhibit 50% of the DPPH radicals, was determined using linear regression. A lower IC₅₀ value denotes higher antioxidant efficiency.

Determination of maximum non-cytotoxic dose (MNCD) of NAE in chicken lymphocytes

Chicken lymphocytes were isolated from spleens collected from healthy birds, under laminar air flow as per standard

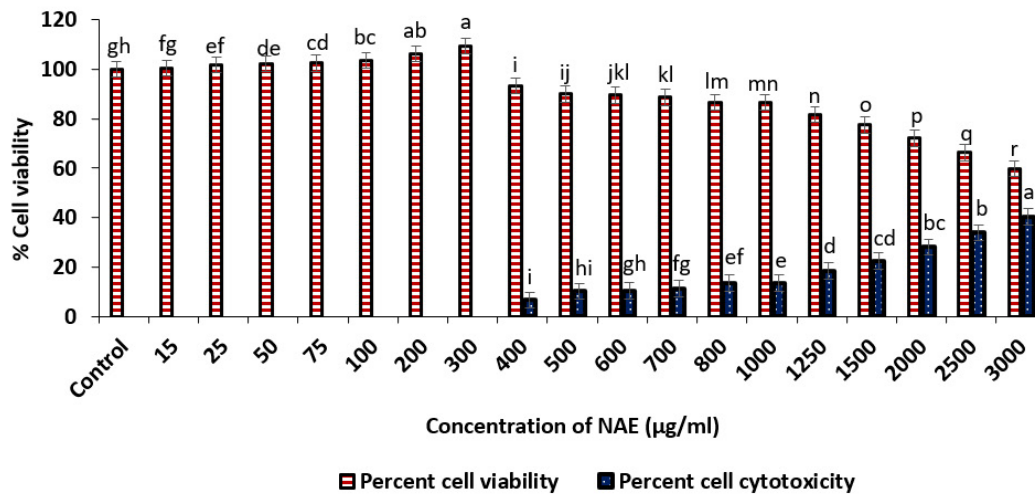


Figure 4: Percent viability of chicken lymphocytes in the presence of different concentrations of NAE. Significant differences ($p < 0.05$) are indicated by different letters.

procedure (Ambwani et al., 2023), and lymphocytes were isolated using density gradient centrifugation, as per the procedure of Lightbody (1976). Cell viability was evaluated *via* the 0.1% trypan blue exclusion method using a hemocytometer (Janossy & Greaves, 1971), and the final cell concentration was adjusted to 10^7 cells/mL in RPMI-1640 medium supplemented with 0.1% antibiotics antimycotic solution (HiMedia antibiotic antimycotic liquid 100x), and 10% fetal bovine serum (FBS). Various concentrations of NAE (diluted in RPMI-1640) were tested in triplicates in 96-well tissue culture plates. The maximum non-cytotoxic dose (MNCD) was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] reduction assay. The highest concentration of extract that maintained 100% cell viability compared to untreated controls was selected as the MNCD for further studies.

Lymphocyte proliferation assay

To assess the immunomodulatory potential of NAE, a lymphocyte proliferation assay (B- and T-cell blastogenesis) was conducted using mitogen stimulation (Ambwani et al., 2023; 2024). Mitogens included Concanavalin A (ConA), Phytohaemagglutinin-M (PHA-M), and *Escherichia coli*-derived Lipopolysaccharide (LPS), each at a concentration of 5 µg/mL in RPMI-1640. A 200 µL suspension containing 1×10^6 lymphocytes/mL was seeded into 96-well flat-bottom tissue culture plates. Cells were treated with the MNCD of NAE along with each mitogen in triplicate. The response was measured by MTT assay, evaluating lymphocyte proliferation as an indicator of immunomodulatory activity (Lee et al., 2009).

Evaluation of antioxidant status in lymphocytes

Lymphocytes were treated with the MNCD of NAE and incubated overnight in a CO₂ incubator. After incubation,

the cells were harvested, lysed, and the lysates were stored at -80°C for analysis of antioxidant biomarkers (Ambwani et al., 2024; 2025).

Lipid peroxidation (LPO)

LPO was determined by measuring malondialdehyde (MDA), a secondary product of lipid degradation, which reacts with thiobarbituric acid (TBA) to form a colored complex. Absorbance was read at 532 nm and results were expressed as nmol MDA/mg protein (Li et al., 2014). Briefly, cell lysates from control, FEE-treated, and ascorbic acid-treated cells were mixed with sodium phosphate buffer (pH 7.4) and incubated at 37°C for 1-hour. After adding 5% TCA (Trichloroacetic acid), the mixtures were centrifuged, and the supernatants were collected. Each was then combined with 1% TBA, heated in a boiling water bath for 15 minutes, cooled, diluted with distilled water, and the absorbance was measured at 535 nm. A blank was prepared using the same steps without cell lysate.

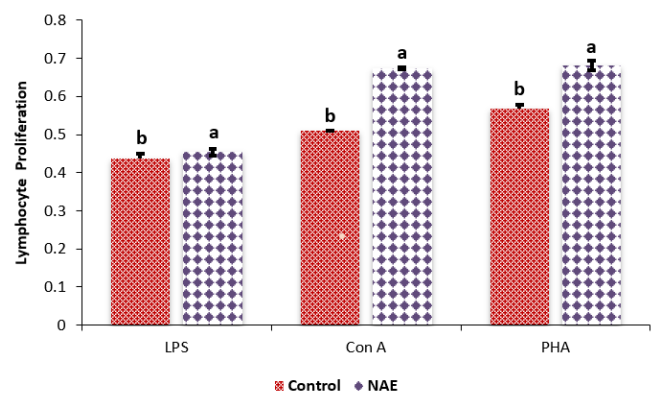


Figure 5: Effect of NAE on lymphocyte proliferation upon mitogenic stimulation. Significant differences ($p < 0.05$) are indicated by different letters.

Superoxide dismutase (SOD) activity

SOD activity was assessed by the enzyme's ability to inhibit pyrogallol auto-oxidation, measured through its suppression of MTT reduction to formazan. The reaction was stopped with DMSO and absorbance measured at 570 nm (Madesh & Balasubramanian, 1998). The reaction mixture was prepared by adding specific reagents to the control, sample, and blank tubes. For the control, 0.65 mL of phosphate buffer saline (PBS) and 0.65 mL of MTT solution were added, followed by 30 μ L of cell lysate and 75 μ L of dimethyl sulfoxide (DMSO). For the sample and blank, 0.65 mL of PBS and 30 μ L of MTT were added, followed by 10 μ L of cell lysate and 75 μ L of pyrogallol. The reaction mixtures were incubated at room temperature for 5 minutes. After incubation, 0.75 mL of DMSO was added to the sample and blank, while 10 μ L of cell lysate was added to the control. The absorbance of each reaction mixture was measured at 570 nm using distilled water as the blank.

Reduced glutathione (GSH)

GSH levels were estimated using Ellman's reagent (DTNB), which reacts with GSH to form a yellow chromogen. The absorbance was recorded at 412 nm (Nanto-Hara et al., 2021). Cell lysates were prepared from untreated control cells, as well as cells treated with FEE and ascorbic acid. Each lysate sample (0.1 mL) was mixed with 0.9 mL of 5% TCA and centrifuged at 2300 $\times$ g for 15 minutes at 4°C. A 0.5 mL portion of the resulting supernatant was then reacted with 1.5 mL of 0.01% DTNB solution, and the absorbance was recorded at 412 nm.

Catalase (CAT) activity

Catalase activity was evaluated using UV spectrophotometry by tracking the decomposition of hydrogen peroxide (H_2O_2) at 240 nm. Caution was observed as high concentrations of H_2O_2 may inhibit catalase activity through structural modification of the active site (Aebi, 1984). A fresh 30 mM H_2O_2 solution was prepared by diluting 30% H_2O_2 with phosphate buffer. For the assay, 20 μ L of cell lysate was mixed with 2 mL of phosphate buffer in a quartz cuvette, followed by the addition of 1-mL of H_2O_2 to start the reaction. Absorbance was measured at 240 nm every 30 seconds for 2 minutes using a UV-visible spectrophotometer. Catalase activity was calculated based on the rate of H_2O_2 decomposition, using a molar extinction coefficient of 36, and expressed as mM H_2O_2 consumed per minute per mg of cell lysate protein.

Statistical Analysis

All experiments were performed in triplicate, and data were expressed as mean $\pm$ standard error (SE). Statistical significance between treatment and control groups was assessed using one-way analysis of variance (ANOVA). A *p*-value of less than 0.05 was considered statistically significant. Origin Software was used for data analysis and graph generation.

Results

Plant extract

The percentage yield of the plant extract (NAE) was found to be 10.69% (Table 1).

Qualitative analysis

The present investigation on *N. arbor-tristis* uncovered the presence of therapeutically active constituents. The phytochemically active compounds of *N. arbor-tristis* were qualitatively investigated in the leaves, and the results are presented in Table 2.

Quantitative analysis of plant extract

Total phenolic content

Phenolic compounds are the key molecules for the antioxidative potential of plant products. Due to their redox properties, hydrogen donors and singlet oxygen quenchers, phenolics are potent antioxidant molecules. Total phenolic content in NAE was calculated from the gallic acid standard curve ($y = 0.0181x$, $R^2 = 0.9476$) and represented as mg gallic acid equivalent (GAE) per g of extract. The total phenolic content of *N. arbor-tristis* leaf extract was found to be 180.55 mg GAE/g extract.

Total flavonoid content

In this study, total flavonoid content of the plant extract was calculated from the rutin standard curve ($y = 0.0018x$, $R^2 = 0.9655$) and represented as mg rutin equivalent (RE) per g of extract. The total flavonoid content of *N. arbor-tristis* was found to be 125 mg rutin equivalents/g extract.

Assessment of antioxidant activity via DPPH assay

NAE showed a significant antioxidant activity in the DPPH free radical scavenging assay. Dose dependent relationship was observed in DPPH assay *i.e.*, the scavenging activity increases with increase in the concentration (Figure 3). The IC_{50} value for NAE was found to be 50.65 μ g/mL and for ascorbic acid, IC_{50} value was determined to be 75.10 μ g/mL.

Table 1: Percentage yield of the plant extract

S. No.	Plant name	Dry weight of leaves (g)	Weight of the extract obtained (g)	Percent yield
1.	<i>Nyctanthes arbor tristis</i>	100	21.38	10.69%

Table 2: Qualitative phytochemical analysis in the plant extract

S. No.	Phytochemical analysis	NAE
1.	Phenolics	+
2.	Flavonoids	+
3.	Tannins	+
4.	Terpenoids	-
5.	Phlobatannins	-

Table 3: Effect of NAE on LPO, SOD, GSH and CAT activity in chicken lymphocytes

S. No.	Treatment groups	MDA (nm/mL)	GSH (mM/mL)	SOD (units/mg of protein)	CAT (H ₂ O ₂ utilized mM/min/mg of protein)
1.	Control	3.8461 ± 0.0322	0.4461 ± 0.0307	3.469 ± 0.0215	50.7782 ± 0.1581
2.	NAE	2.5641 ± 0.0641*	0.4769 ± 0.0320*	3.573 ± 0.065*	56.7162 ± 0.0902*
3.	Ascorbic acid	1.282 ± 0.0074*	0.5384 ± 0.0444*	4.079 ± 0.008*	57.6857 ± 0.1775*
SE(m)		0.042	0.014	0.04	0.142

*Significant at $p < 0.05$

Determination of maximum non-cytotoxic dose (MNCD) of NAE in chicken lymphocytes

To determine MNCD, chicken lymphocytes were exposed to various concentrations of NAE percent viability of chicken lymphocytes in presence of different concentrations of NAE is presented in Figure 4. Microscopic observations of treated cells were conducted to cross check the MNCD of NAE. The cells were in healthy condition up to 300 µg/mL and subsequent dose showed increase in granularity, clump formation and detachment of cells. NAE showed cytotoxicity from 400 µg/mL onwards. Thus, MNCD of NAE was determined to be 300 µg/mL which was further used for exploring immunomodulatory and antioxidative potential of NAE.

Lymphocyte proliferation assay

The study investigated the effect of NAE on lymphocyte proliferation using specific mitogens. T-lymphocyte proliferation was assessed with phytohaemagglutinin (PHA) and concanavalin A (Con A), while B lymphocyte proliferation was evaluated using lipopolysaccharide (LPS). Treatment with NAE significantly enhanced T cell proliferation, showing a 19.94% increase with PHA and a 32.24% increase with Con A compared to untreated controls. Similarly, B cell proliferation increased by 11.09% following NAE treatment in the presence of LPS. These findings suggest that NAE positively influences both T and B cell proliferation (Figure 5).

Evaluation of antioxidant status in lymphocytes

Membrane lipid peroxidation, glutathione reductase, superoxide dismutase, and catalase assays were conducted to evaluate the antioxidant status in chicken lymphocytes following exposure to the plant extract (Table 3). Ascorbic acid was used as a positive control in each assay.

Lipid peroxidation (LPO)

Membrane lipid peroxidation in chicken lymphocytes was resolved after exposure to NAE at dose level of 300 µg/mL. The amount of the thiobarbituric acid-reactive substances (TBARS) determined the degree of lipid peroxidation. MNCD of NAE in chicken lymphocytes was tested for the inhibition of LPO, which revealed a

decrease in lipid peroxidation, which is indicative of the antioxidative potential of NAE. Cells treated with ascorbic acid were taken as a positive control, and untreated cells were taken as a normal control.

Reduced glutathione (GSH)

GSH has significant role in maintaining intracellular antioxidative status in cells. Reduced GSH is one of the most important biomolecule because it protect cells from chemically induced cytotoxicity, hydrogen peroxide reduction and free radicals by direct quenching these highly reactive molecules. Reduced glutathione in chicken lymphocytes cells was resolved after exposure to NAE with dose level of 300 µg/mL. MNCD of NAE in chicken lymphocytes was tested for the determination of GSH level, which revealed an increase in GSH content. This indicated that NAE has antioxidant activity.

Superoxide dismutase (SOD)

Superoxide dismutase plays one of the key enzymes in defence against oxidative stress. Decrease in metalloenzyme SOD activity results in enhancement of oxidative radicals and these cause oxidative stress. NAE treated chicken lymphocytes exhibited higher SOD activity in comparison to NAE untreated cells. Cells treated with ascorbic acid and untreated cells were taken as positive control and as normal control.

Catalase

Catalase is an antioxidative enzyme that protects tissues from highly reactive hydroxyl radicals. *In-vitro* treatment of MNCD of NAE increased catalase activity in chicken lymphocytes. Cells treated with ascorbic acid and untreated cells were taken as positive controls and normal controls, respectively.

Discussion

The medicinal value of plants primarily stems from the phytochemicals they contain. These naturally occurring compounds play a key role in the plant's defence mechanisms, protecting them from various diseases (Pandey and Ambwani, 2022; Ambwani et al., 2025). This study conducted a phytochemical screening to identify compounds such as terpenoids, flavonoids, phenolics, and tannins in the aqueous leaf extract of NAT. Among

these, phenolics and flavonoids were found in the highest concentrations. Similar findings were also observed in methanolic leaf extract, as reported by Chouhan et al. (2014), who investigated the antimicrobial activity of NAT and identified *Colletotrichum gloeosporioides*, an endophyte. Hassan et al. (2016) found the presence of flavonoids, phenolic compounds, and saponins in the plant, indicating potential antioxidant and antitussive (cough-relieving) properties. Sah et al. (2012) highlighted the presence of terpenoids in the methanolic-aqueous extract, suggesting its possible use in treating viral fevers. Antioxidants are vital compounds that mitigate oxidative stress caused by excess free radicals in the body stress that can lead to damage in lipids, proteins, and DNA, thereby contributing to various diseases (Dewi et al., 2022). A separate study revealed that both ethanol and aqueous extracts from the seeds and leaves of NAT showed dose-dependent free radical scavenging activity in a DPPH assay. The antioxidant effects were stronger in leaf extracts compared to seeds (Karan et al., 2019). Akki et al. (2009) also demonstrated the concentration-dependent antioxidant activity of various NAT leaf extracts. The current study also evaluated the immunostimulatory effects of the in a chicken lymphocyte culture, revealing enhanced cellular and humoral immunity in mice—thus identifying NAE as a promising immunomodulator (Chatterjee & Bhattacharjee, 2007). Bharshiv et al. (2016) reported that a 50 µg/mL dose of aqueous flower extract from NAT increased IL-2 and IL-6 cytokine levels in mice. These immunostimulatory effects could be attributed to active compounds in the extract, such as nyctanthine, flavonoids, and iridoid glycosides (Sana et al., 2024). Additionally, the current study observed a significant reduction in lipid peroxidation following extract treatment, alongside an increase in antioxidant markers like GSH, SOD, and catalase when compared to untreated control cells. Vajravijayan et al. (2013) found that ethanolic extract of NAT could inhibit lipid peroxidation by 50% at a concentration of 399.09 ± 1.56 µg/mL. Similarly, Hussain and Ramteke (2012) observed a 1.22-fold increase in GSH levels in lymphocytes treated with NAT flower extract compared to controls. Phanindhra et al. (2014) also reported that NAE helps counter oxidative stress by preserving catalase activity. Overall, the findings underscore the pharmacological potential of NAT, which has been extensively explored for its phytochemical and biological activities. The plant exhibits a wide range of therapeutic properties, including antioxidant, anti-inflammatory, analgesic, antidiabetic, antihyperlipidemic, anticancer, antimicrobial, antigenotoxic, antimalarial, antitussive, hepatoprotective, and anti-*Malassezia* effects. These diverse actions are likely due to the presence of numerous secondary metabolites such as steroids, terpenes, flavonoids, iridoid glycosides, and alkaloids.

Conclusion

To summarize, the aqueous extract of *N. arbor-tristis* has shown strong antioxidant and immune-boosting effects. Containing a high concentration of phenolic compounds and flavonoids, NAE effectively reduced oxidative stress, as demonstrated by its performance in DPPH assays and its impact on biomarkers such as LPO, GSH, SOD, and catalase in cultured chicken lymphocytes. These results indicate that NAE has considerable potential as a natural agent for enhancing immune function and combating oxidative damage. Nonetheless, further research, including comprehensive *in-vitro* and *in-vivo* studies, is necessary to fully understand its pharmacological benefits. While studies have explored the therapeutic potential of *N. arbor-tristis*, further research is needed to investigate other bioactive molecules and their implications for treating severe diseases like cancer and infections. More detailed studies are also required to identify and characterize the specific bioactive compounds responsible for these activities, and elucidate their molecular mechanisms before human use.

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Conflict of Interest

Authors declare no conflict of interest.

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