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Phytochemical characterization and *in vitro* evaluation of antioxidant and anti-inflammatory potential of hydro-methanolic leaf extract of *nyctanthes arbor-tristis*

Vipin Kumar Pandey * and Praveen Kumar Soni

School of Pharmacy, Sangam University, NH-79, Atoon, Bhilwara, 311001, Rajasthan, India.

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Abstract

Introduction: Natural plant sources are recognized for their abundance of bioactive constituents with therapeutic relevance. The present work evaluates the phytochemical profile and biological activities of the hydro-methanolic extract of *Nyctanthes arbor-tristis* leaves.

Material and Methods: The plant material was collected, authenticated, and subjected to maceration to obtain the extract, yielding 8.8% dried residue. Qualitative and Quantitative phytochemical screening was performed by the Hydro-methanolic extract of the NAT leaf. The oxidative property of NAT leaf was assessed using DPPH and FRAP assays, and Anti-inflammatory activity was evaluated by the egg albumin denaturation method.

Result: Preliminary phytochemical screening revealed the presence of flavonoids, phenolic compounds, carbohydrates, anthraquinones, anthocyanins, cardiac glycosides, proteins, and amino acids. Quantitative analysis revealed total flavonoid content of 196.63 mg quercetin equivalents/g dry weight and total phenolic content of 38.45 mg gallic acid equivalents/g dry weight. The extract exhibited significant radical scavenging activity (RSA) with an IC_{50} value of 63.83 µg/mL and notable ferric reducing antioxidant power. Anti-inflammatory activity was evaluated by the egg albumin denaturation method, showing dose dependent response with maximum inhibition of 95.94% at100 µg/ml comparable to standard drugs.

Conclusion: These findings suggest that *Nyctanthes arbor-tristis* leaf extract possesses considerable antioxidant and anti-inflammatory properties, likely due to its rich phytochemical constituents. Future studies should focus on isolating and characterizing, the specific biologically active compounds that show pharmacological effects.

Keywords: *Nyctanthes arbor-tristis* (NAT); Hydro-methanolic extract (HME); Antioxidant activity; Anti-inflammatory activity; Total phenolic content; Flavonoids

1. Introduction

For centuries, plants have served as a primary source of both raw materials and completed medicinal formulations(1). Systems such as Ayurveda and Siddha represent India's long-standing medical traditions(2). Given the undeniable therapeutic significance of these traditional practices, scientific interest has increasingly turned toward isolating and characterizing their bioactive constituents, accompanied by rigorous investigation—both in vivo and in vitro—into the underlying mechanisms of their pharmacological effects.

Inflammation is typically defined by a consistent set of clinical manifestations, including erythema, edema, elevated temperature, dolor, and, in specific instances, the development of exudative processes and impairment of

* Corresponding author: Vipin Kumar Pandey.

physiological function(3). Conditions in which inflammatory responses contribute significantly to pathological damage include asthma, rheumatoid arthritis, vasculitis, and glomerulonephritis(4, 5).

Inflammation due to oxidative stress can be managed by both natural and synthetic antioxidants. An antioxidant reduces oxidation, and the phytochemicals obtained from natural sources have the capacity to neutralize the oxidative stress produced in our body. Some of the common examples of antioxidants include curcumin, alkaloids, ascorbic acid, uric acid, glutathione, lipoic acid, β-carotene, selenium, etc(6).

The harmful effects of steroidal and NSAIDs can be reduced by using drugs from natural sources. This led to an interest among the people in the use of herbal medicines to reduce pain and inflammation(7).

Nyctanthes arbor-tristis has long been recognized for its application in traditional medicine. Nyctanthes is often known as "Harshingar," which translates to "night-flowering jasmine" in English, and "Parijat/Vishnu Parijat" in Sanskrit **(Figure 1)**. Fresh leaves of *Nyctanthes arbor-tristis* are frequently used to treat rheumatism, malaria, chronic fever, liver issues, and constipation in youngsters(8, 9). Herbal medicine makes use of the secondary metabolites of abundant plant sources, such as flavonoids, glycosides, polyphenols, phenolic compounds, and alkaloids. Hence, NAT leaf extract has been used-up as model to study against the Inflammation & oxidative properties (10-12).

Figure 1 Nyctanthes arbor-tristis plant

2. Material and Methods

2.1. Collection and Authentication of the Plant

The raw leaf of *N. arbor-tristis* was gathered from the Central University of Rajasthan (India) from September to October. The samples were authenticated and deposited at the CSIR, Delhi, India, and the authentication number is NIScPR/RHMD/Consult/2023/4641-42

2.2. Preparation of the extract

NAT leaves extraction performed according to method established by(13). Briefly, the powdered leaves of NAT (500g) are dissolved in 2000ml of 70% hydromethanolic solvant and kept in a maceration process for 3 to 4 days. Extraction was evident by the mixture's colour change from light green to brown. A Whatmann No. 1 filter was used to remove any undissolved elements from the extract. The resulting solution is then kept in sealed containers in the refrigerator for future research purposes.

2.3. Preliminary phytochemical screening analysis

The purpose of the extract was to qualitatively analyses the presence of several phytochemical elements, including phenolic compounds, proteins and amino acids, cardiac glycosides, anthraquinones, anthocyanins, alkaloids, flavonoids, carbohydrates, and reducing sugar(14).

2.4. Determination of total flavonoid content (TFC)

TFC were quantified using a colorimetric assay. A 50 µL aliquot of the HME extract was combined with 40 µL of purified water and 60 µL of a 5% sodium nitrite ($NaNO_2$) solution, followed by a 5-minute incubation period at ambient temperature. Subsequently, 120 µL of a 10% $AlCl_3$ solution was introduced to the mixture. Thereafter, 700 µL of 1M sodium hydroxide was added to achieve a final reaction volume of 1mL. The samples were then subjected to additional 15-minute incubation under the same temperature conditions. Measurements of absorbance were carried out at 510 nm with the help of UV–Visible spectroscopy (15, 16).

2.5. Determination of TPC

The sum of soluble phenolic content across varying concentrations of leaf extract was quantified by the modification of the Folin–Ciocalteu procedure(17). Each extract or fraction (10 mg) was dissolved in 10 mL of dimethyl sulfoxide. From the resulting stock solution, 50 µL was incorporated with 200 µL of Folin–Ciocalteu reagent and incubated for 10 minutes in darkness. Following this incubation, 600 µL of a 20% Na_2CO_3 solution was introduced, and the final volume was alter to 1 mL distilled water. The reaction mixture was then incubated in the dark for 2 hours prior to absorbance measurement at 765 nm via UV-Visible spectrophotometry, with a blank sample serving as reference(15).

2.6. *In-vitro* Antioxidant assay (DPPH Assay)

The percentage % RSA of plant extracts was calculated by using a modified method of Brand-Williams et al., 1994(18). Stock solution of the hydro-methanolic plant extract was prepared by dissolving 100 mg of dried extract in 100 mL of methanol. Before UV measurements, 1 ml of DPPH solution (7.89 mg of DPPH dissolved in 100 ml of ethanol) was stirred with various concentrations (62.5, 125, 250, 500, 1000 µl) of plant extract solution in disposable micro cuvettes with a 1 cm path length. As a reference standard, similar concentrations of ascorbic acid were employed (62.5, 125, 250, 500, 1000 µl). On a UV-visible light spectrometer, the color variations (light yellow color from deep violet) were measured at 517 nm.

2.7. *In vitro* FRAP assay

The FRAP assessment operates along the principle of reducing the ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) to its ferrous form (Fe^{2+}-TPTZ), which exhibits an intense blue color, under acidic conditions via electron transfer from antioxidant compounds(19). To perform the assay, combine 900 µL of FRAP reagent with 70 µL of DW and 30 µL of plant extract sample, previously prepared in DMSO at varying concentrations. A blank control is constituted solely of FRAP reagent and distilled water, devoid of the test sample. Absorbance readings are recorded at a wavelength of 593 nm to quantify the formation of the reduced Fe^{2+}-TPTZ complex(20, 21).

2.8. *In-vitro* anti-inflammatory study

NAT extract was assessed by evaluating its inhibitory effect on egg albumin denaturation. The total reaction volume of 5 mL included egg albumin (0.2 mL), PBS (2.8 mL, pH 6.4) and 2 mL of extract at different concentrations (10–100 µg/mL). A control was prepared using an equivalent volume of distilled water. The substances were located in incubator at 37.2°C for 15 minutes, after that heating at 70°C for 5 minutes to induce protein denaturation. Subsequently, the absorbance of each sample was measured at 660 nm. Acetylsalicylic acid (ASA), used as a reference standard, was tested under identical conditions at the same concentration range (10–100 µg/ml) and analyzed at the same wavelength for comparative evaluation(22, 23).

The inhibition of protein denaturation is determined using the following equation:

% inhibition of egg albumin denaturation = [(Vc − Vt) / Vc] × 100,

Where Vt = absorbance of the test sample and Vc = absorbance of the control.

3. Results and Discussion

3.1. Percentage yield of the extract of the *NAT* plant

The 500 g dry coarse powder was macerated with hydro-methanolic solvent, then the solvent was evaporated, and a solid residue was dried in an oven, and then the weight obtained 44 gm of extracted residue material. The resulting residue of the NAT leaves % yield was 8.8.

3.2. Phytochemical screening:

Active constituents (Flavonoids, carbohydrates, anthraquinones, anthocyanins, cardiac glycosides, Proteins and amino acids, phenolic compounds) in the HME of NAT plant, obtained through maceration methods, were subjected to phytochemical screening.

Table 1 Phytochemical screening Hydro-methanolic extract of NAT

S. No.	Phytochemical Constituents	Hydro-methanolic extract of NAT
1.	Alkaloids	-
2.	Flavonoids	+
3.	Carbohydrates	+
4.	Anthraquinones	+
5.	Anthocyanins	+
6.	Cardiac glycosides	+
7.	Detection of reducing sugars	-
8.	Proteins and amino acids	+
9.	Phenolic compounds	+

'-' indicates absence, '+' indicates present

3.3. Analysis of TPC and TFC

The estimation of phenolic content was performed with slight modifications. The leave residue of NAT was analysed for total phenolic content, expressed as GA equivalent, in the range of various concentrations (10-100) µg/ml (**Figure 2**). The phenolic contents were determined in the NAT leaf extract using Gallic acid as a standard. The results showed the NAT leaves had a hydro-methanolic extract of 38.455 ± 0.012. For flavonoid contents, the hydro-methanolic extract of 196.637 ± 0.006, Quercetin hydrate served as the standard for comparison (**Figure 3**).

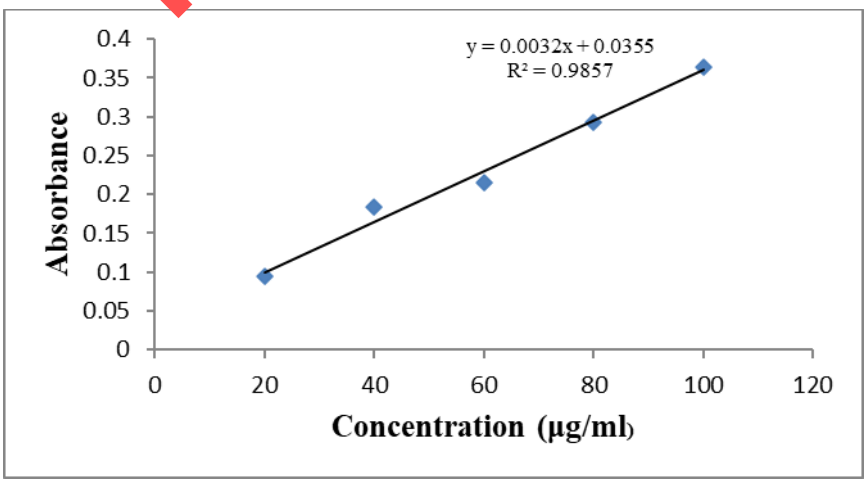

Figure 2 Calibration curve of Gallic acid

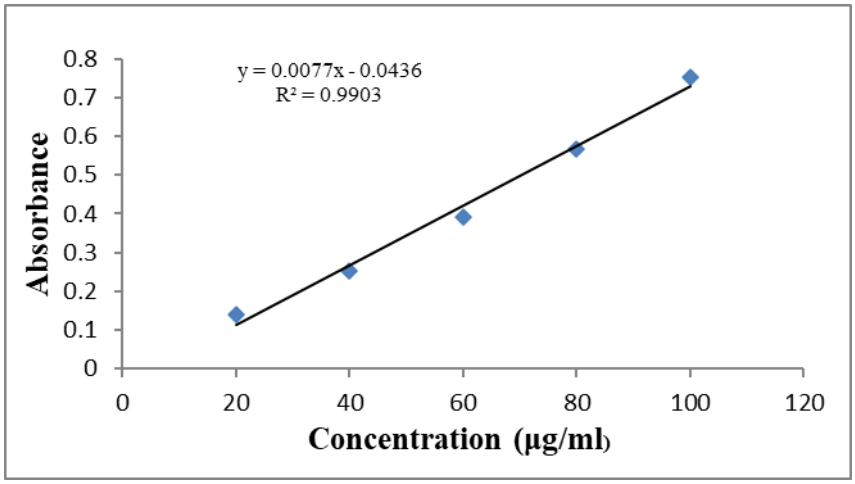

Figure 3 Calibration curve of QE hydrate

3.4. DPPH assay

Various concentrations (10-100 µg/ml) of the percentage scavenging effect of DPPH radicals versus the concentration of extract and standards. Plant extracts were found to have a lower level of free radical-scavenging activity than ascorbic acid, a well-known antioxidant. Ascorbic acid had an IC_{50} of 84.184 µg/ml, and leaf residue contains IC_{50} of 63.83µg/ml against DPPH free radicals (**Figure 4**).

Table 2 Percentage inhibition of plant extract against ascorbic acid in the DPPH assay

S. No.	Concentrations (µg/ml)	HME	STD
1.	10	26.95	27.36
2.	20	36.809	38.298
3.	40	45.319	50.638
4.	60	57.376	61.915
5	80	60.284	67.731
6.	100	63.83	84.184

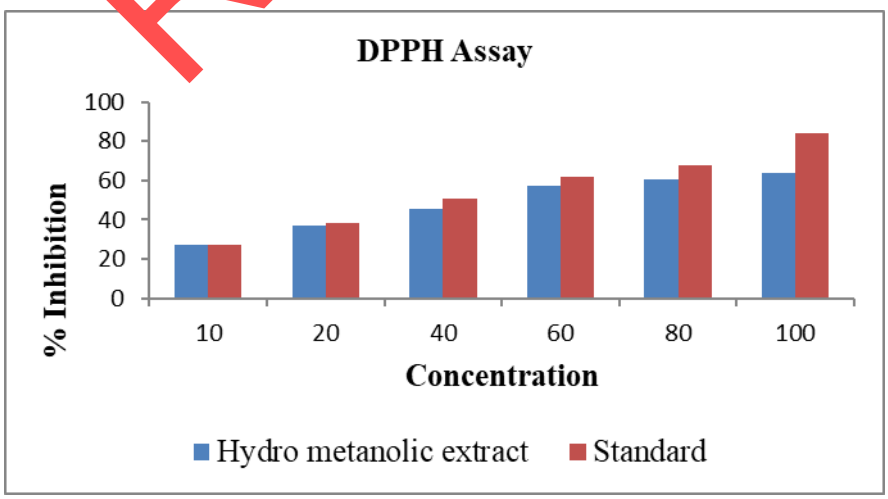

Figure 4 DPPH radical scavenging activity of *N. arbor-tristis* leaf extract

3.5. FRAP Assay

The FRAP assay was employed to assess the antioxidant potential of the hydro-methanolic extract (HME) of *Nyctanthes arbor-tristis*. The HME shows 38.9161 µg/ml FRAP value, and standard ferrous sulphate shows 59.82153 µg/ml FRAP value (**Figure 5**). The higher activity of the hydro-methanolic extract may be attributed to its ability to extract more phenolic and flavonoid compounds, which are known for their redox properties.

Table 3 FRAP percentage inhibition of HME of *N. arbor-tristis*

S. No.	Concentrations (µg/ml)	HME	STD
1.	10	6.899554	26.15083
2.	20	12.90674	33.05039
3.	40	19.75188	39.66699
4.	60	26.55349	45.38035
5.	80	33.33333	48.54718
6.	100	38.9161	59.82153

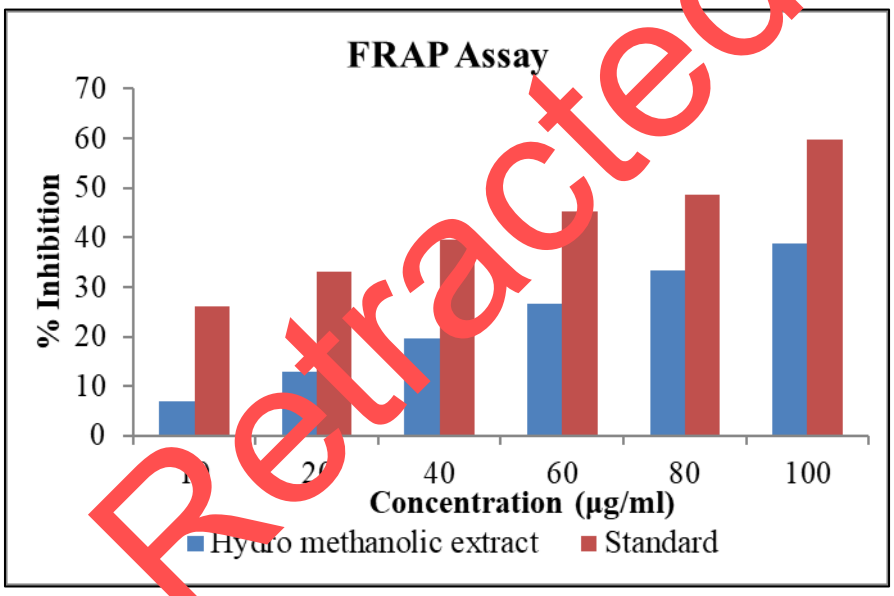

Figure 5 FRAP % inhibition activity of *N. arbor-tristis* leaf extract

3.6. In-vitro Anti-Inflammatory activity by egg albumin protein denaturation method

The anti-inflammation capacity assess at concentrations of 10-100 µg/ml showed in (**table 4 and figure 6**) inhibition of Egg Albumin denaturation whereas, standard diclofenac at 10-100 µg/ml which showed in (**table 4 and figure 6**) inhibition of Egg Albumin denaturation.

Table 4 Percentage inhibition of the Egg albumin protein denaturation method

S. No.	Concentrations (µg/ml)	HME	STD
1.	10	62.51513	54.50375
2.	20	67.15007	64.24655
3.	40	76.17327	77.47105
4.	60	77.69416	84.64186

5.	80	89.1291	94.46007
6.	100	95.94953	98.3487

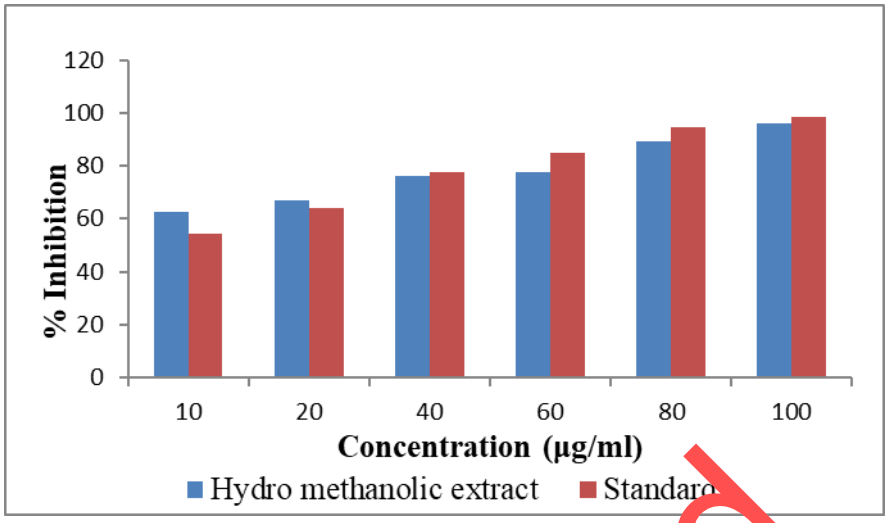

Figure 6 Percentage inhibition of the egg albumin protein denaturation method

4. Conclusion

The aim & objective of the study to investigate the extraction process and phytochemical composition of the NAT plant, with a focus on evaluating its oxidative stress and inflammatory properties. Following the preparation of a hydro-methanolic extract (HME), qualitative identification tests confirmed the presence of various bioactive constituents, including flavonoids and phenolic compounds. Quantitative analysis revealed TFC and TPC values of 196.63 and 38.45 mg/g equivalent per dry weight of NAT leaves, respectively. Antioxidant potential was employed through biological assays: the DPPH radical scavenging assay yielded an IC_{50} value of 63.83 µg/ml, referenced against ascorbic acid, while the FRAP assay demonstrated an IC_{50} of 38.91 µg/ml relative to ferrous sulphate. The in vitro anti-inflammatory study demonstrated that HME inhibited protein (albumin) denaturation in a dose-responsive manner. Despite these findings, Future studies should focus on isolating and characterizing, the specific biologically active compounds that show pharmacological effects in NAT leaf extract.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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