Journal of Advanced Zoology
ISSN: 0253-7214
Volume 43 Issue 1 Year 2022 Page 1218 -1229

Nyctanthes arbortristis: In Vitro Efficacy Of Anti-Oxidant Potential And Cytotoxicity Study Of Bark Extracts On Sw982 Rheumatoid Arthritis Cell Line Followed By Phytochemical Profiling By GCMS

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Abstract

Nyctanthes arbortristis, commonly known as the night-jasmine, is a plant rich in bioactive compounds with potential therapeutic benefits. This study aimed to evaluate the antioxidant properties and cytotoxic effects of *Nyctanthes arbortristis* bark extracts on SW982 rheumatoid arthritis cell line in vitro. Sequential solvent extraction method was employed to obtain extracts with varying polarities. The antioxidant potential of the extracts was assessed using DPPH, ABTS, NO, and H_2O_2 radical scavenging assays and total phenolic and flavonoid content determination. Cytotoxicity was evaluated using MTT assay on SW982 cells. Results revealed that the bark extracts exhibited significant antioxidant activity, with the methanol extract showing the highest radical scavenging activity, total phenolic content and total flavonoids contents. Furthermore, the bark extracts demonstrated concentration-dependent cytotoxic effects on SW982 cells, with the ethyl acetate extract exhibiting the most potent cytotoxicity. These findings suggest that *Nyctanthes arbortristis* bark extracts possess substantial antioxidant potential and cytotoxic effects against SW982 rheumatoid arthritis cells, indicating their potential as therapeutic agents in the management of rheumatoid arthritis. Further investigations are warranted to elucidate the underlying mechanisms and evaluate their efficacy in vivo.

Keywords: Nyctanthes arbor-tristis, antioxidant activity, phenolics, flavonoids, cytotoxicity, GCMS.

Introduction

Nyctanthes arbor-tristis, commonly known as the "Night-flowering Jasmine" or "Parijat," is a well-known medicinal plant in traditional medicine systems across Southeast Asia, particularly in Ayurveda. The plant has been utilized for its broad spectrum of therapeutic properties, including anti-inflammatory, antimicrobial, antioxidant, and hepatoprotective effects (Agarwal et al., 2018; Dutta et al., 2020). Recent research has increasingly focused on exploring the pharmacological potential of various extracts derived from different parts of the plant, including its leaves, flowers, and bark, which are rich in bioactive compounds (Chatterjee & Bhattacharya, 2019). The growing interest in *Nyctanthes arbor-tristis* is driven by the need to identify alternative therapeutic agents for chronic inflammatory diseases such as rheumatoid arthritis (RA). RA is a debilitating autoimmune condition characterized by persistent joint inflammation, which leads to severe pain,

swelling, and eventually joint destruction (Smolen et al., 2016). Conventional treatment options often involve nonsteroidal anti-inflammatory drugs (NSAIDs) and disease-modifying anti-rheumatic drugs (DMARDs), which can have significant side effects and may not be effective in all patients (Scott et al., 2010). As a result, there is a critical need for the development of novel therapeutic agents with better efficacy and safety profiles. In this context, the bark of *Nyctanthes arbor-tristis* has attracted attention due to its potential as a source of bioactive compounds with anti-inflammatory and antioxidant properties (Kumar and Bajpai, 2017). Preliminary studies have indicated that extracts from the bark may exhibit cytotoxic effects on certain cell lines, suggesting potential applications in cancer therapy as well (Singh et al., 2020). However, the specific mechanisms underlying these effects, and the identification of the active phytochemicals involved, require further investigation. The present study aims to evaluate the in vitro efficacy of *Nyctanthes arbor-tristis* bark extracts on the SW982 cell line, a model for rheumatoid arthritis, with a particular focus on their antioxidant and cytotoxic potential. The study employs various biochemical assays to determine the extracts' IC50 values, providing insights into their potency. Additionally, the phytochemical profile of the most potent extracts is characterized using Gas Chromatography-Mass Spectrometry (GC-MS), which allows for the identification of the key bioactive compounds.

This research not only contributes to the understanding of the pharmacological properties of *Nyctanthes arbor-tristis* bark but also paves the way for its potential application in the development of novel therapeutic agents for the treatment of rheumatoid arthritis and other inflammatory conditions.

Materials and Methods:

Collection of plant material and extracts preparation

Fresh Bark of *Nyctanthes arbor-tristis* were collected from Maharshi Dayanand University, Rohtak Campus, and subsequently subjected to washing, shade drying, and pulverization into powder form. The 20 grams of powder dissolved in 5 to 6-time solvent and sequentially extracted in increasing order of solvent polarity starting from ethyl acetate, methanol and water using a Soxhlet apparatus (Parekh & Chanda, 2007). After being concentrated using a rotary evaporator at lower pressure, the extracts were stored at 4°C until additional analysis.

Evaluation of antioxidant capacity

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay:

The antioxidant activity of the test samples was evaluated by assessing their capability to scavenge free radicals, which was quantified by monitoring the change in optical density of DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals. Methanol, ethyl acetate, and aqueous plant Bark extracts were prepared in various concentrations (20, 40, 60, 80, and 100 µg/ml). A stable free radical with a nitrogen centre, DPPH gives methanol solutions a violet-blue colour. Introduce a substrate that can contribute hydrogen atoms into the DPPH solution, and it reduces to a yellow product called diphenyl picryl hydrazine. 95% methanol was used to prepare a 0.5 mmol/L DPPH solution. The test tube added 2 mL of DPPH solution (0.5 mnol/L) and 1 ml of various extract concentrations. The combination was left for half 30 min at room temperature in the dark (Manzocco et al. 1998). Inhibition of DPPH radical (%) = [(A0 - At)/ (A0)] ×100

Where A_0 = control absorbance

At = test samples absorbance

ABTS (2, 2'- azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) assay:

The Re et al. (1999), method was used to ascertain the formulation's ABTS activity. This assay's basis is the decolourisation resulting from reducing the radical cation $ABTS^+$ to ABTS. The radical was produced by the reaction of 2.45 mM potassium persulfate ($K_2O_8S_2$) and a 7 mM solution of ABTS in water (1:1). The solution was kept at 27°C in the dark for 16 hours, long enough to achieve steady absorbance at 734 nm. The radical solution was diluted with water (1 ml of ABTS reagent + 27 ml DW) after the incubation period, until it attained its initial absorbance value of 0.7 ± 0.005 at 734 nm. To assay test samples, 20 µl of the sample or standard was combined with 980 µl of ABTS+ reagent. After six minutes, absorbance was measured at 734 nm. 20 µg/ml to 100 µg/ml of ascorbic acid was employed as a reference. The percentage of the extract's ability to scavenge ABTS+ was calculated using the following equation, and it was compared to ascorbic acid.

%inhibition = [(Abs control – Abs sample)]/ (Abs control)] × 100

Where A_0 = control absorbance

At = test samples absorbance

Hydrogen Peroxide (H_2O_2) assay:
The method by which Ruch et al. (1989), determined the ability of several plant extracts to scavenge hydrogen peroxide. In phosphate buffer (50 mmol/L, pH 7.4), a 40 mmol/L hydrogen peroxide solution was prepared. In distilled water, hydrogen peroxide was mixed with plant extracts at varying concentrations (20, 40, 60, 80, and 100 µg/ml). A blank solution containing phosphate buffer but no hydrogen peroxide was used to test the absorbance at 230 nm after 10 minutes.
Scavenged H_2O_2 (%) = $[(A_0 - At)/A_0] \times 100$
Where A_0 = control absorbance
At = test samples absorbance

Nitric oxide assay:
The Griess-Ilosvoy reaction is a commonly used method to measure the scavenging activity of nitric oxide (NO). In this assay, sodium nitroprusside, a nitric oxide donor, decomposes in an aqueous solution at physiological pH (7.2) to release nitric oxide (NO), which then reacts with oxygen to produce nitrite ions (NO_2^-) in the presence of Griess reagent. Methanol, ethyl acetate, and aqueous extracts of plant Bark were combined at varying concentrations (20-100 µg/ml) with sodium nitroprusside (10 mM) in PBS (Phosphate Buffered Saline). The mixture was left to sit at a temperature of 30°C for a duration of two hours. A control was also prepared, containing the same reaction mixture but with ethanol instead of the extract. After the designated incubation period, 0.5 ml of Griess reagent was added to the reaction mixture. The Griess reagent typically consists of 1% sulfanilamide, 2% phosphoric acid (H_3PO_4), and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride. Following the addition of the Griess reagent, the absorbance of the resulting solution was measured at a wavelength of 550 nm using a spectrophotometer. This measurement allows for the quantification of nitrite ions produced as a result of the Griess-Ilosvoy reaction, providing insight into the nitric oxide scavenging activity of the test sample.
Radical scavenging activity (%) = $[(A_0 - At)/A_0] \times 100$
Where A_0 = control absorbance
At = test samples absorbance

Determination of total phenolic content
The determination of total phenolic content is a common assay used to quantify the concentration of phenolic compounds in a sample, which serves as an indicator of its antioxidant potential and health-promoting properties. One widely used method for this determination is the Folin-Ciocalteu assay. Methanol, aqueous and ethyl acetate extracts were prepared at varying concentrations (20-100 µg/ml). Mix the sample with the Folin-Ciocalteu reagent. Incubate the mixture for a specific period at room temperature, allowing the reaction between the phenolic compounds and the Folin-Ciocalteu reagent to occur. After the incubation period, add a neutralizing agent, such as sodium carbonate (Na_2CO_3), to stop the reaction. Measure the absorbance of the resulting blue-coloured solution at a specific wavelength (typically 725 nm) using a spectrophotometer. Determine the total phenolic content of the sample by comparing its absorbance to a standard curve generated using known concentrations of a phenolic compound (e.g., gallic acid) under the same conditions. Express the results as milligrams of gallic acid equivalents (GAE) per gram or millilitre of sample (Wolfe et al. 2023).

Determination of total flavonoid content
The determination of total flavonoid content is another common assay used to quantify the concentration of flavonoids in a sample, which serves as an indicator of its antioxidant potential and health benefits. Methanol, aqueous and ethyl acetate extract were prepared at varying concentration (20-100 µg/ml). Dissolve a known quantity of a flavonoid standard quercetin in the same solvent used for sample extraction to prepare a standard solution. Combine a measured volume of the sample extract with an appropriate colorimetric reagent, such as aluminum chloride (AlCl3) that reacts specifically with flavonoids to form colored complexes. Incubate the mixture for a specific period at room temperature or under controlled conditions to allow the formation of colored complexes.

Measure the absorbance of the resulting colored solution at a specific wavelength (typically around 415 nm for aluminum chloride method) using a spectrophotometer. Determine the total flavonoid content of the sample by comparing its absorbance to a standard curve generated using known concentrations of the flavonoid standard under the same conditions. Express the results as milligrams of the flavonoid standard equivalents per gram or milliliter of sample (Bahorun et al. 1996).

Determination of the effect of plant extracts on cell proliferation by MTT assay:

Cell culture:

The human synovial cell line SW982 was Procured from NCCS Pune. The SW982 cells were cultured in T-50 flasks with 10% DMEM (Dulbecco's Modified Eagle Medium), 10% FBS (Fatal Bovine Serum), and 5% CO2. Further, the cells were used for the cytotoxicity study of different extracts of plants, followed by an MTT assay (Morgan 1998).

MTT assay protocol:

Using the SW982 cell line (purchased from NCCS Pune), the cytotoxicity of the supplied samples was assessed using the MTT Assay. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is a common method used to measure cell viability and proliferation. Culture the cells of interest in an appropriate growth medium under standard conditions (e.g., 37°C, 5% CO2) until they reach the desired confluence or passage number. Plate the cells in 96-well plates at a density suitable for the assay. Typically, 5,000-10,000 cells per well seeded. After cells have adhered to the plate (usually overnight), treat them with the methanol, aqueous and ethyl acetate extract at varying concentrations (0, 1, 10, 50, 100, 250, 500, and 1000 µg/ml). After the desired treatment period, prepare a stock solution of MTT in phosphate-buffered saline (PBS) or growth medium. Typically, MTT is added at a final concentration of 0.5 mg/ml. Add the MTT solution (50 µl) to each well-containing cell and incubate the plate for 2-4 hours at 37°C to allow the formation of formazan crystals by metabolically active cells. After the incubation period, carefully remove the medium from the wells without disturbing the formazan crystals. Add a 100 µl dimethyl sulfoxide (DMSO) solubilizing agent to dissolve the formazan crystals formed by viable cells. This releases the purple-colored formazan solution. Using a microplate reader, measure the absorbance of the formazan solution in each well at a wavelength typically between 550-600 nm. Calculate the percentage of viable cells in each treatment group by comparing the absorbance values of treated wells to control wells (e.g., untreated cells). Graph and analyze the data using appropriate statistical methods to determine the effect of the treatments on cell viability (Morgan, 1998).

GC-MS analysis of Bark extracts:

Primary phenolic acids and flavonoids were identified using GC-MS in the methanolic aqueous and ethyl acetate extracts. GC/MS analysis of Bark extract was studied using SCIO-SQ436-GC with capillary column and helium as carrier gas. A solution of Bark extract was prepared with a concentration of 1 mg/ml. This solution was then injected into the column with a flow rate of 1 ml/min at a temperature of 400°C. After two minutes at 80°C, the oven temperature was raised by 8°C per minute until it reached 250°C. The temperature was then held steady for 25 minutes and then run at 280°C at a rate of 8°C/min rise with a total run time of 49 min (Kumari et al., 2020).

Statistical analysis:

Every experiment was carried out in triplicate, and the mean ± standard deviation was used to represent the findings. Graph Pad Prism-6 software was used to determine the IC50.

Results:

Antioxidant potential of *Nyctanthes arbor-tristis* Bark extracts:

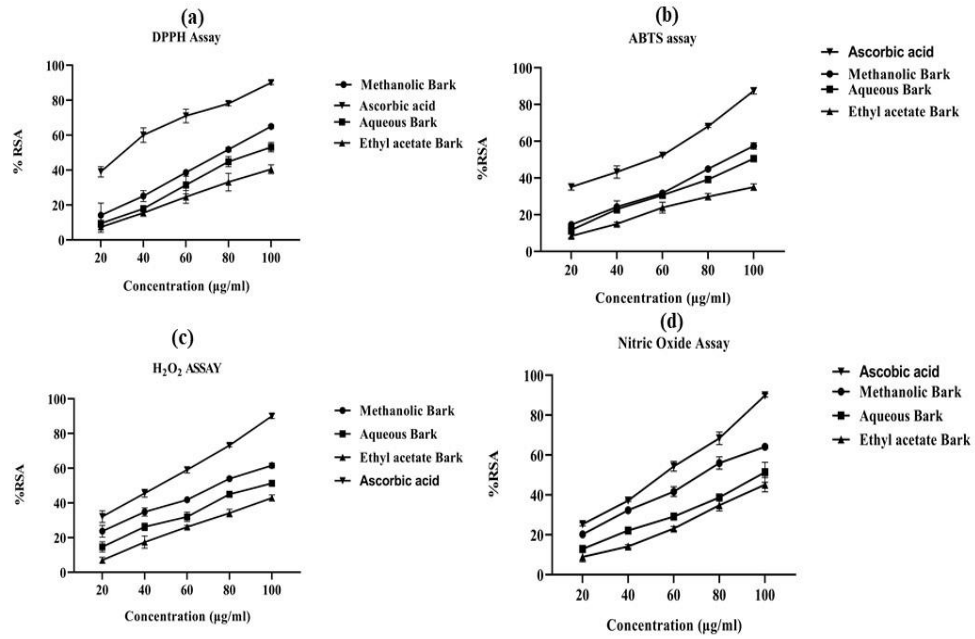

Fig. 1. Radical scavenging assays of *Nyctanthes arbortristis* Bark methanol, aqueous and ethyl acetate extracts compared with standard compound ascorbic acid (a) DPPH (b) ABTS (c) Nitric oxide (d) H_2O_2 assay

Antioxidant Activity:
The antioxidant potential of *Nyctanthes arbor-tristis* bark extracts was assessed using various in vitro biochemical assays, including DPPH, ABTS, Nitric Oxide (NO) scavenging, and Hydrogen Peroxide (H2O2) scavenging assays as depicted in fig 1. The IC50 values for the different extracts are summarized in Table 1.

Table 1: IC50 value of methanol, aqueous and ethyl acetate extracts of *Nyctanthes arbortristis* Bark various biochemical assays

Sr. no.	Plant extracts	DPPH IC50 (µg/ml)	ABTS IC50 (µg/ml)	Nitric oxide (NO) (µg/ml)	Hydrogen Peroxidase (H_2O_2) (µg/ml)
1	Ascorbic acid	30.31 ± 0.002	48.83 ± 0.004	53.77 ± 0.47	46.09 ± 0.05
2	Methanolic	77.28 ± 0.15	89.01 ± 0.15	72.83 ± 0.002	64.58 ± 0.01
3	Aqueous	82.30 ± 0.35	100.45 ± 0.015	101.02 ± 0.085	90.11 ± 0.04
4	Ethyl acetate	116.95 ± 0.03	141.02 ± 0.14	101.63 ± 0.23	80.56 ± 0.14

DPPH Radical Scavenging Activity:
The DPPH assay revealed that the methanolic extract had a significant radical scavenging activity with an IC50 value of 77.28 ± 0.15 µg/ml, followed by the aqueous extract with an IC50 of 82.30 ± 0.35 µg/ml.

The ethyl acetate extract exhibited the lowest antioxidant activity in this assay with an IC50 of 116.95 ± 0.03 µg/ml. Ascorbic acid, used as a standard, showed a much lower IC50 value (30.31 ± 0.002 µg/ml), indicating its superior radical scavenging ability.

ABTS Radical Scavenging Activity:
In the ABTS assay, the methanolic extract again demonstrated better antioxidant potential (IC50 = 89.01 ± 0.15 µg/ml) compared to the aqueous extract (IC50 = 100.45 ± 0.015 µg/ml) and the ethyl acetate extract (IC50 = 141.02 ± 0.14 µg/ml). However, ascorbic acid outperformed all plant extracts with an IC50 value of 48.83 ± 0.004 µg/ml.

Nitric Oxide Scavenging Activity:
The methanolic extract showed the best nitric oxide scavenging activity among the plant extracts, with an IC50 of 72.83 ± 0.002 µg/ml, while the aqueous and ethyl acetate extracts had IC50 values of 101.02 ± 0.085 µg/ml and 101.63 ± 0.23 µg/ml, respectively. Ascorbic acid again demonstrated a stronger scavenging ability with an IC50 of 53.77 ± 0.47 µg/ml.

Hydrogen Peroxide Scavenging Activity:
The hydrogen peroxide scavenging activity followed a similar trend, where the methanolic extract exhibited an IC50 value of 64.58 ± 0.01 µg/ml. The aqueous and ethyl acetate extracts had IC50 values of 90.11 ± 0.04 µg/ml and 80.56 ± 0.14 µg/ml, respectively. Ascorbic acid, with an IC50 value of 46.09 ± 0.05 µg/ml, showed the highest scavenging activity.

Total Phenolics Content (TPC):
Using the total phenolics content assay, the concentration of phenolic compounds in the *Nyctanthes arbortristis* bark extracts was determined. The polyphenol content in the bark extracts were observed in methanolic extract (92.45 ± 1.23 mg GAE/g), followed by aqueous extract (65.96 ± 0.69 mg GAE/g) and lowest in ethyl acetate extract (51.2 ± 2.36 mg GAE/g) as given in table 2.

Table 2: Total Phenolic contents of *Nyctanthes arbortristis* Bark extract

Plant Bark Extracts	Total Phenolic Content (mg GAE /g sample)	Total Flavonoid Content (mg QE/g sample)
Methanol	92.45 ± 1.23	110.22 ± 0.12
Aqueous	65.96 ± 0.69	40.30 ± 0.57
Ethyl acetate	51.2 ± 2.36	15.4 ± 0.45

Total Flavonoid Content (TFC):
The total flavonoid content is a quantitative phytochemical screening analysis to measure the amount or quantity of flavonoid phytochemical compounds in the *Nyctanthes arbortristis* bark extract. The results revealed that the methanolic bark extract contains flavonoid compounds with a 110.22 ± 0.12 mg quercetin E/g followed by the aqueous extract with 40.30 ± 0.57 mg quercetin E/g sample and ethyl acetate observed with low flavonoid content with 15.4 ± 0.45 mg quercetin E/g sample as given in table 2.

Cytotoxicity study of bark extract by MTT assay:
The evaluation of the toxicity of plant materials is an essential step that must be taken prior to the production or use of pharmaceuticals. In this work, the MTT test was utilized to investigate the cytotoxic effects of crude water, methanol, and bark ethyl acetate plant bark extracts on SW982 cells. According to the findings, the aqueous extracts, with a concentration of 688.8 ± 0.097µg/ml, exhibited a lower level of toxicity when compared to the methanolic extracts, which had a concentration of 192 ± 0.13µg/ml, and the bark ethyl acetate extract, which had a concentration of 66.01 ± 0.452µg/ml against the untreated control cells, as shown in table 3. When SW982 cells were subjected to extracts of methanol, water, and ethyl acetate, the viability of the cells decreased. According to the results of the MTT assay, the range of viability for methanolic, aqueous, and ethyl acetate was as follows: 86.12% to 36.73%, 90.25 to 47.42%, and 75.63 to 33.78%, respectively (Fig. 2). According to these findings, the cytotoxic profile of these extracts is one of the most important factors to consider when considering their prospective uses in the creation of drugs or in therapeutic usage.

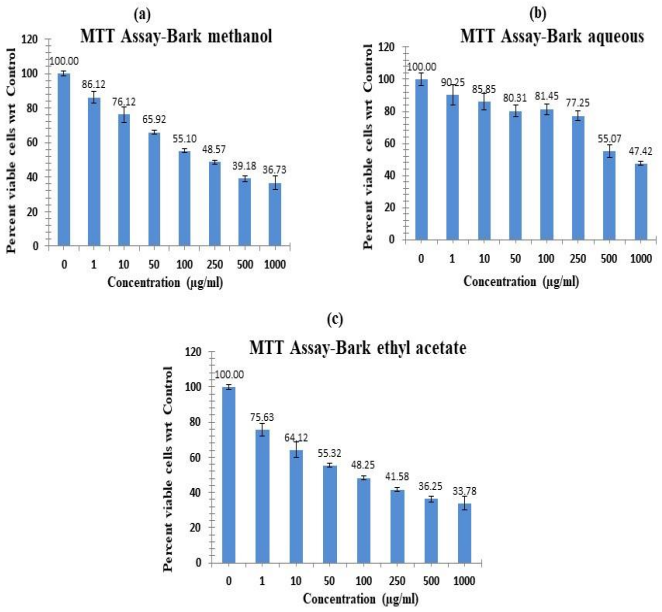

Fig. 2: MTT assay of bark extracts showing different cell viability at different concentrations (a) methanol (b) aqueous (c) ethyl acetate

Table 3: IC50 value of different Bark extracts of cytotoxicity by MTT assay

Sr. no.	Plant extract	MTT IC50 (µg/ml)
1	Methanolic	192 ± 0.13
2	Aqueous	688.8 ± 0.097
3	Ethyl acetate	66.01 ± 0.452

Phytochemical profiling of bark methanolic extract through GCMS:
Through the use of the GCMS analysis, eleven chemicals have been found in the bark methanol extract. These compounds were determined based on the percent area and retention time (RT) (Fig. 3 and table 4). Major phytochemical constituents include p-trimethylsiloxybenzaldehyde oxime, trimethylsulphyl with peak area (9.19%) (RT 11.458), phenol, 2-methoxy (2.08%) (RT 5.61), pyrazolidine-3,5-dione,4-phenyl (7.49%) (RT 12.22), dibutyl phthalate (4.92%) (RT 18.578), 4-Ethylcatechol (1.89%) (RT 10.691), 1,2-Benzenedicarboxylic acid,bis (2-methylpropyl)ester (1.84%) (RT 17.400), and other major phytochemical constituents.

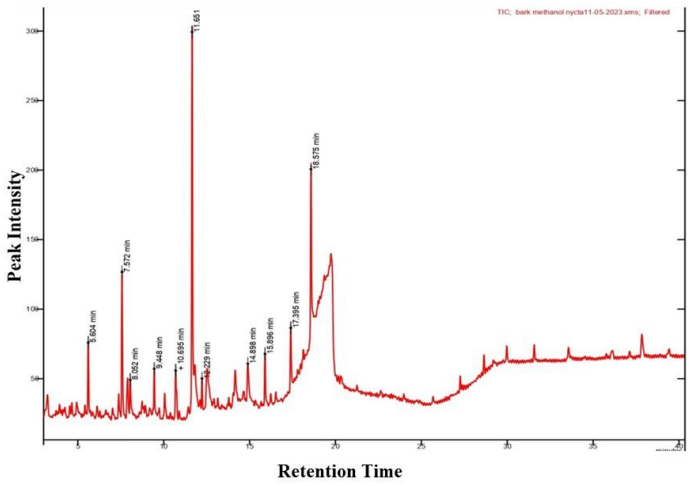

Fig. 3: GC-MS chromatogram of the methanolic extracts

Table 4: Phytochemical compounds present in bark methanol extract

Sr. No.	Retention time	Compound Name	Area	Molecular Formula	Molecular weight
1	5.611	Phenol, 2 -methoxy	2.08	C7H802	124
2	7.579	catechol	4.244e+8	C6H6O2	110
3	8.050	5-Hydroxymethylfurfural	1.103e+8	C6H6O3	126
4	9.446	2-Methoxy-4-vinylphenol	1.009e+8	C9H10O2	150
5	10.691	4-Ethylcatechol	1.89	C8H10O2	138
6	11.598	p-Trimethylsiloxybenzaldehyde oxime, trimethylsulphyl	9.19	C13H23NO2Si2	281
7	12.222	Pyrazolidine-3,5-dione,4-phenyl	7.49	C9H8N2O2	176
8	14.896	Benzeneacetic acid ,3,4-dihydroxy	1.556e+8	C8H8O4	168
9	15.888	4-[(1E)-3-Hydroxy-1-propenyl]-2-methoxyphenol	1.624e+8	C10H12O3	180
10	17.400	1,2-Benzenedicarboxylic acid,bis(2-methylpropyl)ester	1.84	C16H22O4	278
11	18.578	Dibutyl phthalate	4.92	C16H22O4	278

Bark aqueous:

There were nine bioactive chemicals found in the bark aqueous extract, and their percentage area and retention duration are represented in fig. 4 and table 5 respectively. The main bioactive constituents were 4-[4-(1-Methyl-2-piperidyl) methyl] (7.11%) (RT 3.31), 1,2-Cyclopentanedione,3-methyl (6.30%) (RT 4.17), Phenol,2-methoxy (1.92%) (RT 5.615), Catechol (2.27%) (RT 7.716), 2-[5-Diethylamino-2-pentylamino] (1.67%) (RT 7.92), Phenol,2,6-dimethoxy (1.03%) (RT 10.07), (3-Nitrophenyl) methanol, isopropyl (4.73%) (RT 11.717), Phenol,3,4,5-trimethoxy (4.55%) (RT 14.106) and 4-[(1E)-3-Hydroxy-1-propenyl] (1.66%) (RT 15.956).

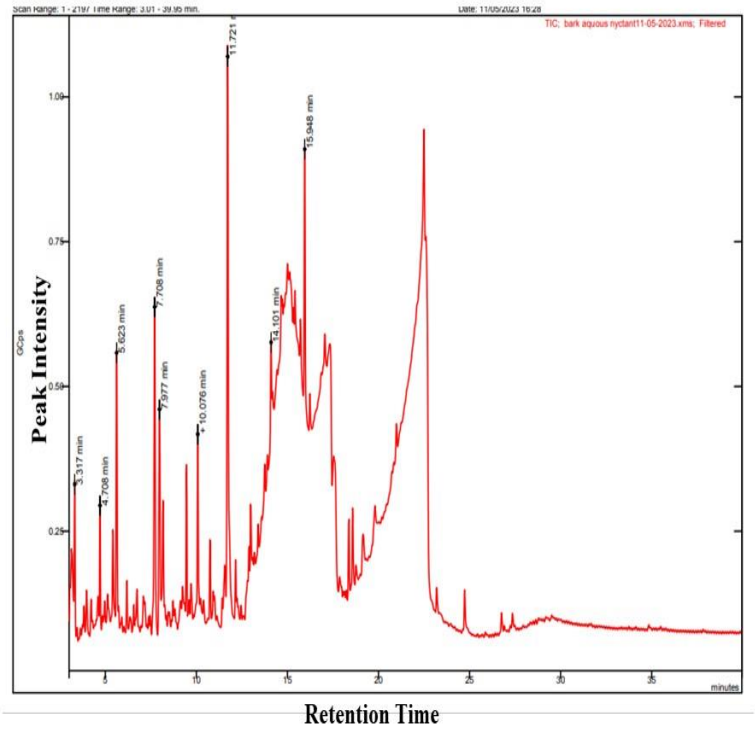

Fig. 4: GC-MS chromatogram of the methanolic extracts

Table 5: Phytochemical compounds present in bark aqueous extract

Sr. No.	Compound	Retention time	Area	Molecular Formula	Molecular weight
1	4-[4-(1-Methyl-2-piperidyl) methyl]	3.313	7.11	C20H24Cl3N5O	471
2	1,2-Cyclopentanedione,3-methyl	4.708	6.302	C6H8O2	112
3	Phenol,2-methoxy	5.615	1.92	C7H8O2	124
4	Catechol	7.716	2.57	C6H6O2	110
5	2-[5-Diethylamino-2-pentylamino]	7.984	1.67	C18H21F14N5	573
6	Phenol,2,6-dimethoxy	10.070	1.03	C8H10O3	154
7	(3-Nitrophenyl) methanol, isopropyl	11.717	4.73	C10H13NO3	195
8	Phenol,3,4,5-trimethoxy	14.106	4.55	C9H12O4	184
9	4-[(1E)-3-Hydroxy-1-propenyl]	15.956	1.66	C10H12O3	180

Discussion:

The evidence gathered from the antioxidant assays reveals that the methanolic extract of *Nyctanthes arbor-tristis* bark exhibited superior antioxidant activity compared to the aqueous and ethyl acetate extracts. This can be attributed to the higher solubility of phenolic and flavonoid compounds in methanol, which are well-known for their potent antioxidant properties (Pietta, 2000). These compounds act as effective free radical scavengers, hydrogen donors, and metal ion chelators, which are mechanisms that contribute to their antioxidant action (Rice-Evans et al., 1997). The lower antioxidant activity observed in the ethyl acetate extract may be due to the reduced solubility of certain bioactive compounds in this solvent, limiting their availability to exert antioxidant effects. The comparison with ascorbic acid, a standard antioxidant, validated the sensitivity and accuracy of the assays employed in this study, showing the efficacy of the methanolic extract in similar oxidative stress conditions (Singh et al., 2002).

The variation in IC50 values across the different extracts and assays emphasizes the importance of solvent selection in extracting bioactive compounds with antioxidant properties. Methanol, as an organic solvent, is known to extract a wide range of polyphenolic compounds, which correlates with the higher antioxidant activity observed in this study (Tung et al., 2009). The findings suggest that the methanolic extract of *Nyctanthes arbor-tristis* bark is a promising source of natural antioxidants, which could be further explored for pharmaceutical and nutraceutical applications (Wong et al., 2006). However, to fully understand the potential of this extract, further studies are needed to isolate and characterize the specific compounds responsible for the observed antioxidant activity and to assess their efficacy in vivo (Cai et al., 2004).

The cytotoxicity assays using the MTT method on the SW982 rheumatoid arthritis cell line indicated that the methanolic and ethyl acetate extracts of *Nyctanthes arbor-tristis* bark have a more significant cytotoxic effect compared to the aqueous extract. The higher cytotoxicity of the methanolic extract could be attributed to the presence of more potent bioactive compounds, which may induce cell death through mechanisms such as apoptosis or necrosis (Mosmann, 1983). The relatively lower cytotoxicity of the aqueous extract suggests that it may contain fewer bioactive compounds or that these compounds are less effective at inducing cell death in the SW982 cell line (Chanda & Rakholiya, 2011). These findings are crucial for evaluating the therapeutic potential of the bark extracts, particularly in developing treatments for conditions like rheumatoid arthritis, where controlling cell proliferation and inflammation is vital (Hwang et al., 2016).

The phytochemical analysis using GC-MS of the methanolic extract revealed a rich diversity of bioactive compounds, including phenols, catechols, and esters, known for their antioxidant, anti-inflammatory, and antimicrobial properties (Wong et al., 2006). The identification of compounds such as 5-Hydroxymethylfurfural (HMF) and 2-Methoxy-4-vinylphenol, which are recognized for their antioxidant and antimicrobial activities, further supports the therapeutic potential of the extract (Martins et al., 2011). The presence of dibutyl phthalate and 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, which have been associated with antimicrobial effects, suggests that the methanolic extract may also possess significant antimicrobial properties, which could be harnessed for treating infections (Singh et al., 2012).

Overall, the results from the GC-MS analysis corroborate the bioactive potential of the methanolic bark extract, particularly in terms of antioxidant, anti-inflammatory, and antimicrobial activities. The identification of these

compounds provides a basis for further investigation into their specific roles and mechanisms of action, which could lead to the development of new pharmaceutical agents derived from *Nyctanthes arbor-tristis* bark. This study highlights the importance of comprehensive phytochemical profiling in understanding the full therapeutic potential of medicinal plants.

Conclusion

The findings from this study underscore the significant antioxidant, cytotoxic, and phytochemical properties of *Nyctanthes arbor-tristis* bark extracts, particularly the methanolic extract. The superior antioxidant activity observed in the methanolic extract, compared to aqueous and ethyl acetate extracts, is likely due to its higher phenolic and flavonoid content, which are known for their potent free radical scavenging abilities. The cytotoxicity analysis also suggests that the methanolic extract could be a valuable candidate for further exploration in therapeutic applications, especially in the context of diseases characterized by oxidative stress and abnormal cell proliferation, such as rheumatoid arthritis.

Moreover, the GC-MS analysis provided a detailed phytochemical profile, revealing a diverse range of bioactive compounds, including phenols, catechols, and other potentially therapeutic compounds. These findings support the traditional use of *Nyctanthes arbor-tristis* in herbal medicine and highlight its potential as a source of natural antioxidants and other bioactive agents. Future research should focus on the isolation and characterization of these compounds to better understand their mechanisms of action and their potential applications in developing new pharmaceutical agents. The study lays a solid foundation for advancing the therapeutic use of *Nyctanthes arbor-tristis* bark in modern medicine

Abbreviations:

TFC- Total Flavonoid Content
TPC – Total Phenolic Content
GCMS – Gas Chromatography-Mass Spectrometry
DPPH – 1,1-diphenyl-2-picrylhydrazyl
ABTS – 2, 2'- azino-bis 3-ethylbenzthiazoline-6-sulphonic acid
NO – Nitric Oxide
H_2O_2- Hydrogen Peroxide
MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
ROS – Reactive Oxygen Species
OH – Hydroxyl Oxide
RT – Retention Time

Acknowledgement:

The author is grateful to the CSIR-UGC SRF for funding during the research work.

Declaration:

Ethical statement:
The animal-related research is not included in this article by any authors. None of the writers of this paper have conducted any studies on human subjects.

Conflict of interest:
Shammi Sharma has no conflict of interest, and Jaya Prakash Yadav has no conflict of interest

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