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Research Article

Phytopharmacology of Nyctanthes Arbor-Tristis

Evaluation of Phytochemicals, and Pharmacological Efficiency Studies of Nyctanthes Arbor-Tristis Linn.

Ponnusamy Kiruba*[1], Kuppusamy Palanisamy[1], Ponnuswamy Selvamaleeswaran[2], Michael Helan Soundra Rani[3] and Edward Gnanaraj Wesely[1]

[1]*PG and Research Department of Botany, Arignar Anna Government Arts College, Namakkal - 637002, Tamil Nadu, India*

[2]*Department of Biotechnology, Muthayammal College of Arts and Science, Rasipuram*

[3]*Department of Biotechnology, Sri Ramakrishna College of Arts & Science (Autonomous), Coimbatore - 641 006, Tamil Nadu, India*

Abstract: *Nyctanthes arbor-tristis* is a member of the Oleaceae family with various therapeutic characteristics. Different plant components are used in traditional medicine to treat illnesses such as sciatica, chronic fever, and skin diseases. It is today regarded as a rich source of numerous unique compounds for creating medicines against various diseases and certain industrial products. In general, the leaves, roots, flowers, and seeds of Parijat are used to cure several ailments in various dose forms, including juice and powder. The current paper discusses the Plant Profile of *N. arbor-tristis* Linn., as well as the Phytochemical Screening of Extract of *N. arbor-tristis* Leaves, physicochemical parameters, and the Extraction Procedure and Pharmacological Activities conducted on *N. arbor-tristis* Leaves. The study aims to analyze the antioxidant, antibacterial, and anti-inflammatory potential of petroleum ether extract of *N. arbor-tristis* leaves. DPPH scavenging assays and *in vitro* anti-inflammatory activity was evaluated using Albumin denaturation, Proteinase inhibitory assay and was subjected to GC-MS analysis to identify its active components. The antibacterial potential was analyzed using the agar well diffusion method against Gram-negative and Gram-positive bacteria. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, phenols, tannins, triterpenoids, Amino acids, Carbohydrate, and the absence of alkaloids, Anthraquinone, Phenols, Saponin, and tannin. Remarkably, free radicals scavenging ability was observed in DPPH with the IC_{50} of 610.71±13.6µg/mL, and the anti-inflammatory assay also observed Albumin denaturation with the IC_{50} of 781.93±13.6µg/mL and the proteinase inhibitory activity with the IC_{50} of 652.98±32.5µg/mL. In the antibacterial assay, the zone of inhibition was recorded as 20.6±1.6mm, 21.3±1.8mm, 21.3±1.8mm, and 17.9±1.5mm for the strains of *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas* spp., *Staphylococcus aureus*, and *Bacillus* spp. respectively. The highest inhibition zone was observed against *E. coli* (24.8±1.7 and 26.6±1.4mg/mL, respectively), and the least inhibitory was observed against *L. acidophilus* (19.5±1.1 and 21.7±1.7mg/mL) respectively. GC-MS profile exhibited the presence of 29 components highly accountable for its pharmaceutical activities. The results suggested the presence of phytochemicals in *Nyctanthes arbor-tristis* Leaves possess antioxidant and anti-inflammatory properties in the petroleum ether extract. This information can be used as a starting point in the search for natural-based drugs that are effective at alleviating inflammatory symptoms and antioxidant aspects.

Keywords: *Nyctanthes arbor-tristis* L, Antioxidant, Antibacterial, Anti-inflammatory, DPPH, Phytochemical profile, and GC-MS Analysis.

***Corresponding Author**

Ponnusamy Kiruba , PG and Research Department of Botany, Arignar Anna Government Arts College, Namakkal - 637002, Tamil Nadu, India

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1. INTRODUCTION

Plants are natural synthesizers that are essential for meeting humanity's daily needs. Out of the 4,22,000 flowering plants reported worldwide, more than 50,000 are reported to have medicinal and pharmacological use. India contains a diverse range of medicinal plants[1-2]. Plants have long been used as medicines, according to ancient literature. Many major medications of the modern era have been made possible by such documentation of the most important traditional information on medicinal plants. The Charaka Samhita (1000 B.C.), our earliest text on Indian medicine, has a collection of information regarding the benefits of herbal medicines and references the utilization of over 2000 distinct plants[3]. According to a WHO report, 80% of the population in developing countries rely almost totally on traditional medicine for their primary healthcare needs[4]. Based on this research into active plant chemicals and pharmacological screening, researchers may be able to discover leads for the production of novel medications. So yet, just 6% of the estimated 400,000 plant species have had their biological activity investigated[5]. *N. arbor-tristis*, commonly known as Harshingar, coral Jasmine, Night Jasmine, and the queen of the night. The genus name '*Nyctanthes*' is derived from two Greek words Nykhta (night) and Anthos (flower), and is consequently known as the night flower[6] It is also familiar as "*The sad tree*" or "*The tree of sorrow*" since the blossoms bloom only during the night and are shed throughout the day. *N. arbor-tristis* leaf has shown promising results as psourcel source for its bioactive compounds with multipotent active prevents and reduces risk factors for various dis enhances enhancing certain physiological functions[7-8]. The disease-preventing nature is due to the presence of antioxidants, such as flavonoids, hydrolyzable tannins, phenolic acids, etc. These react against free radicals caused by various biological and environmental sources caused by an imbalance of naturally occurring antioxidants. It further contributes to developing diseases [9] responsible for cancer, coronary heart diseases, and mutagenic and associated inflammation reactions[10]. Inflammation is frequently associated with pain and involves several occurrences, such as increased vascular permeability, protein denaturation, and membrane alteration. These occurrences can result in the loss of protein secondary and tertiary structure due to external stress or exposure to compounds like strong acids, bases, concentrated inorganic salts, organic solvents, or heat [11]. The immune modulation effect of anti-inflammatory agents includes modulating cytokine production and pro-inflammatory gene expression[12]. They effectively treat infectious diseases and alleviate many side effects often associated with synthetic drugs[13]. Numerous computational methods are used to discover novel and highly effective drug candidates and promising targets in various diseases[14]. Knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents but also because such information may be of great value in disclosing new sources of economic phytocompounds for synthesizing complex chemical substances and discovering the actual significance of folkloric remedies[15]. Only very few reports have been published on *N. arbor-tristis* leaf extracts, and no systematic work has been done on the phytochemical and antioxidant potential; hence the present study has been taken with the aim of extraction, phytochemical characterization, and evaluation of antimicrobial and antioxidant potential.

2. MATERIALS AND METHODS

2.1. Collection of plant material

The leaves of *Nyctanthes arbor-tristis* were collected from Kolli Hills, Namakkal district, South India (Lat:11.2485°N; Long:78.3387°E). Dr. P. Subramaniyam, Plant Taxonomist, Research Center in Botany, Aringar Anna Government Arts College, Namakkal, authenticated the plant. The voucher specimens (AGAR/BOT/001/2022 - 2023) were deposited in the Department of Botany, Arignar Anna Government Arts College, Namakkal, Tamil Nadu, India. Local Tamil names of the plants - Pavizhamalli or Parijata. The leaves were washed thoroughly with running tap water and distilled water, then shade-dried for 2-3 days, then oven dried and ground into a coarse powder using pestle-mortar. To prepare the petroleum ether extract, 30 g of a dried leaf was extracted in 300 mL of absolute petroleum ether using the Soxhlet apparatus for 8 hrs. The extracts were concentrated, stored at 4°C in a rotary evaporator, and freeze-dried for further use.

2.2. Preliminary phytochemical studies

The petroleum ether extract of *N. arbor-tristis* leaves was tested against microorganisms such as *Lactobacillus acidophilus*, *Escherichia coli*, *Salmonella typhi,* and *Klebsiella pneumonia*. The bacterial strains were maintained on nutrient agar slants at 4°C. A loopful of each bacterial strain was inoculated into 50 mL sterile nutrient broth. The flasks were incubated at 37°C for 24 hrs.

2.3. Phytochemical screening

Preliminary phytochemical analysis was carried out for the extracts as per standard methods[16]

2.3.1. Detection of Total Alkaloids Content

Mayer's test: Filtrates were treated with Mayer's reagent. The formation of a yellow cream precipitate indicates the presence of alkaloids.

2.3.2. Detection of Flavonoids

H_2SO_4 test: Extracts were treated with a few drops of H_2SO_4. The formation of orange color indicates the presence of flavonoids.

2.3.3. Detection of Steroids

Liebermann- Burchard test: 2mL of acetic anhydride was added to 0.5g of the extracts, each with 2mL of H_2SO_4. The color changed from violet to blue or green in some samples, indicating the presence of steroids.

2.3.4. Detection of Terpenoids

Salkowski's test: 0.2g of the extract of the whole plant sample was mixed with 2mL of chloroform, and concentrated H_2SO_4 (3mL) was carefully added to form a layer. A reddish-brown coloration of the inner face indicates the presence of terpenoids.

2.3.5. Detection of Anthroquinones

Borntrager's test: One gram (1 g) of the powdered seed was placed in a dry test tube, and 20 mL of chloroform was added. It was heated in a steam bath for 5 min. The extract was filtered while hot and allowed to cool. The filtrate was added with an equal volume of 10% ammonia solution. It was shaken, and the upper aqueous layer was observed for bright pink coloration to indicate the presence of Anthraquinones. In a test tube, control tests were done by adding 10 mL of 10 % ammonia solution in 5 ml chloroform.

2.3.6. Detection of Phenols

Ferric chloride test: Extracts were treated with a few drops of 5% ferric chloride solution. The formation of bluish-black color indicates the presence of phenol.

2.3.7. Detection of Saponins

Froth test: About 0.2g of the extract was shaken with 5 mL of distilled water. Formation of frothing (appearance of creamy stable persistent of small bubbles) shows the presence of saponins.

2.3.8. Detection of Tannins

Ferric chloride test: A small extract was mixed with water and heated in a water bath. The mixture was filtered, and 0.1% ferric chloride was added to the filtrate. A dark green color formation indicates the presence of tannins.

2.3.9. Detection of Carbohydrates

Fehling's test: 0.2gm filtrate is boiled in a water bath with 0.2mL each of Fehling solutions A and B. A red precipitate indicates the presence of sugar.

2.3.10. Detection of Oils and Resins

Spot test: Test solution of as little as 1 ng in a drop (ca. 50 µL) of the solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

2.4. Gas Chromatography-Mass Spectrometry (GC–MS) Analysis

Gas Chromatograph-Mass Spectrometer quantitatively performed the GC-MS analysis of petroleum ether leaf extract. Hp5-Ms column was used. High pure helium was the carrier gas, and the flow rate was 1ml/min. The temperature at the front inlet was 220°C. The oven temperature was maintained at 50°C to 250°C, gradually raised at 10°C/min. The ion chamber and GC interface temperature were maintained at 250°C. The identification of the peaks was based on computer matching of the mass spectra with the National Institute of Standards and Technology (NIST database) library and by direct comparison with published data[17].

2.5. *In vitro* Antioxidant Activities

2.5.3. DPPH Free Radical Scavenging Activity

The plant extract at different concentrations was diluted with DMSO to get a sample solution. 5 µL of the sample was seeded in a 96-well plate, followed by 195 µL DPPH working solution to each well. After 20 min reaction, the absorbance was measured at 515 nm. The free radical activity of the extracts was determined by comparing their absorbance with blank[18]. The scavenging ability of DPPH radical was expressed as percentage inhibition and calculated (µg/mL).

$$\text{DPPH scavenging activity (\%)} = (A_0 - A_1)/ A_0 \times 100$$

where A_0 - absorbance of the control, and A_1 - absorbance of the sample.

2.6. *In vitro anti-inflammatory activity of N. arbor-tristis*

2.6.1. Inhibition of albumin denaturation

The anti-inflammatory activity of the sample extract was studied by using the inhibition of albumin denaturation technique which was followed with minor modifications[19-20]. The reaction mixture consisted of test extracts and a 1% aqueous solution of bovine albumin fraction. pH of the reaction mixture was adjusted using a small amount of 1N HCl. The sample extracts were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes. After cooling the samples, the turbidity was measured at 660 nm. (UV Visible Spectrophotometer Model 371, Elico India Ltd) The experiment was performed in triplicate. The Percentage Inhibition of protein denaturation was calculated as follows:

Percent inhibition (%) = (OD of Sample - OD of Control/ OD of Control) X 100.

2.6.2. Inhibition of Antiproteinase action

The reaction mixtures (2 mL) contained 0.06 mg trypsin, 1 mL 20 mM Tris HCl buffer (pH 7.4), and 1 mL test samples, each of different concentrations (100 - 500 µg/mL). The mixtures were incubated at 37°C for 5 min, and then 1 mL of 0.8% (w/v) casein was added. The mixtures were incubated for an additional 20 min. 2 mL of 70% perchloric acid was added to arrest the reaction. The cloudy suspension was centrifuged, and the absorbance of the supernatant was read at 210 nm against the buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated as follows.

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control

3. RESULTS AND DISCUSSION

Phytochemical Screening

Because of their many pharmacological qualities, plants are considered natural resources. Indeed, many phytochemicals, such as alkaloids, terpenoids, phenols, tannins, and so on, can be related to pharmacological activities. The primary metabolites include carbohydrates, fats, proteins, and minerals; phytoconstituents such as phenols and flavonoids are secondary metabolites generated from the primary metabolites. Studies in the literature have linked the existence of secondary metabolites to the biological capacity of plants[21].

Table: 1 Phytochemical Analysis of *N. arbor-tristis*

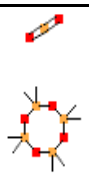
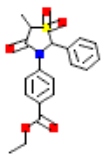
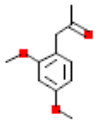
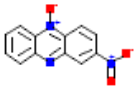
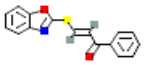
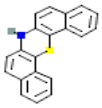
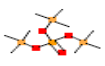
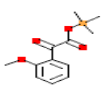
Phytochemicals	Observations	Inference
Alkaloids		-
Mayer's test	Cream color	
Wagner's test	Reddish brown solution/ precipitate	
Flavonoids		-
Lead acetate test	Yellow-orange	
H_2SO_4 test	Reddish brown / Orange color precipitate	
Steroids	Violet to blue or green color formation	+
Liebermann-Burchard test		
Terpenoids		+
Salkowski test	Reddish brown precipitate	
Arthroquinone		-
Borntrager's test	Pink color	
Phenols		-
Ferric chloride test	Deep blue to Black color formation	
Lead acetate test	White precipitate	
Saponin	Stable persistent	-
Tannin	Brownish green / Blue black	-
Carbohydrates	Yellow/brownish/blue/green color	+
Oils & Resins	Filter paper method	+

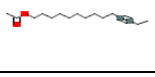
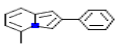
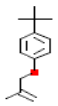
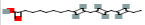
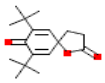
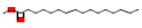
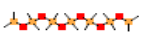
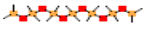
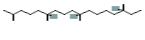
Fig: 1 Phytochemical analysis of *N. arbor-tristis*

Phytochemical screening of *N. arbor-tristis* leaves petroleum ether extract confirmed the presence of flavonoids, steroids, terpenoids, and carbohydrates oils & resins in different qualitative ranges. The negative sign depicted the absence of alkaloids, Arthroquinone, Phenols, Saponin, and tannin. The nine phytoconstituents, steroids, and terpenoids showed a strong presence depicting the pharmaceutical property of the *N. arbor-tristis* leaves aqueous extract. Hence all these secondary metabolites in the extracts were quantified further to assay the best extraction possessing the significant levels of bioactive components (Table 1 & Figure 1). In *N. arbor-tristis*, screening of phytochemicals in methanol and aqueous extracts revealed phytocompounds such as phenols, terpenoids, and tannins only in the methanolic extract. In contrast, alkaloids and steroids were only present in the aqueous extract obtained through the cold percolation technique[22]. Preliminary phytochemicals such as carbohydrates, alkaloids, cardiac glycosides, phenol, flavonoids, and physicochemical parameters were also encounted[23].

3.2. GC–MS Analysis

Table: 2 Phytocomponents of *Nyctanthes arbor-tristis* Leaves extract by GC–MS

R/T	Name of the Compound	Molecular Formula	M/W	Molecular Structure	Peak Area%	Biological Activity
4.342	o-Xylene	C_8H_{10}	106.16		1.12 %	Anti-proliferative
5.398	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_6Si_5$	356.7		1.35 %	Antimicrobial & antioxidant
6.198	Ethyl 4-(5-methyl-1,1-dioxide-4-	$C_{19}H_{19}NO_5S$	373.4		5.68 %	Analgesic
6.842	1-(2,4-Dimethoxyphenyl)-propan-2	$C_{11}H_{14}O_3$	194.23		21.51 %	Antimicrobial
7.087	Undecane	$C_{11}H_{24}$	156.31		1.62 %	Cytotoxic
8.375	Diethylene glycol mono-dodecyl ether	$C_{16}H_{34}O$	274.44		3.51 %	Anti-bacterial
9.009	Phenazine, 2-nitro-	$C_{12}H_7N_3O_3$	241.2		5.41 %	Anti-bacterial
10.286	Propenone, 3-(2-benzoxazolylthio	$C_{16}H_{11}NO_2S$	281.3		2.19 %	Antimicrobial & Anticancer
10.731	7H-Dibenzoc,h]phenothiazine	$C_{20}H_{13}NS$	299.4		2.42 %	Anticancer
10.875	Silanol, trimethyl-, phosphate	$C_9H_{27}O_4PSi_3$	314.54		2.91 %	Antioxidant & Antimicrobial
12.119	Octadecane, 1-bromo-	$C_{39}H_{79}BrO_2$	659.9		1.11 %	Antitumor
12.586	2-Methoxybenzoylformic acid, TMS	$C_{12}H_{16}O_4Si$	252.34		4.37 %	Anticancer

13.930	11-Tetradecyn-1-ol acetate	$C_{16}H_{28}O_2$	252.39		2.09 %	Antibacterial
14.152	Heneicosane, 3-methyl-	$C_{22}H_{46}$	310.6		2.74 %	Antioxidant & Antifungal
14.541	5-Methyl-2-phenylindolizine	$C_{15}H_{13}N$	207.27		1.10 %	Antifungal
14.986	Cyclopentane, 1,2-dimethyl-3-(1-	$C_{11}H_{20}$	152.28		2.63 %	Cytotoxic
15.352	Benzene, 1-(1,1-dimethylethyl)-4	$C_{14}H_{20}O$	204.31		1.72 %	Anti-proliferative
15.519	9,12,15-Octadecatrienoic acid, e	$C_{18}H_{30}O_2$	278.4		10.07 %	Antimicrobial & antioxidant
15.641	7,9-Di-tert-butyl-1-oxaspiro (4,5)	$C_{17}H_{24}O_3$	276.4		3.06 %	Antimicrobial & antioxidant
15.730	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5		1.23 %	Anti-inflammatory
16.774	Ethyladamantane-1-carboxylate	$C_{13}H_{20}O_2$	208.3		2.02 %	Antimycotic & Anti-cancer
17.152	2,3-Hexadiene, 4-diethylboryl-2	$C_{11}H_{21}B$	164.1		1. 66 %	Antimicrobial & antioxidant
19.818	Heptasiloxane, 1,1,3,3,5,5,7,7,9	$C_{14}H_{42}O_6Si_7$	503.07		1.17 %	Cytotoxic, Antimicrobial & antioxidant
19.974	Octasiloxane, 1,1,3,3,5,5,7,7,9	$C_{16}H_{48}O_7Si_8$	577.2		3.37 %	Antimicrobial
20.529	Heptasiloxane, 1,1,3,3,5,5,7,7,	$C_{14}H_{42}O_6Si_7$	503.07		1.33	Cytotoxic, Antimicrobial & antioxidant
20.896	N-Methyl-1-adamantaneacetamide	$C_{13}H_{21}NO$	207.31		1.66 %	Antibacterial & antioxidant
21.696	N-Methyl-1-adamantaneacetamide	$C_{13}H_{21}NO$	207.31		1.63 %	Antibacterial & Anti-fungal
21.940	2,6,10,14,18,22-Tetracosahexaene	$C_{24}H_{38}$	326.6		4.88 %	Antioxidant & Antifungal
22.451	Heptadecane, 2,6,10,15-tetramethyl	$C_{21}H_{44}$	296.6		3.01 %	Cytotoxic, Antimicrobial & antioxidant

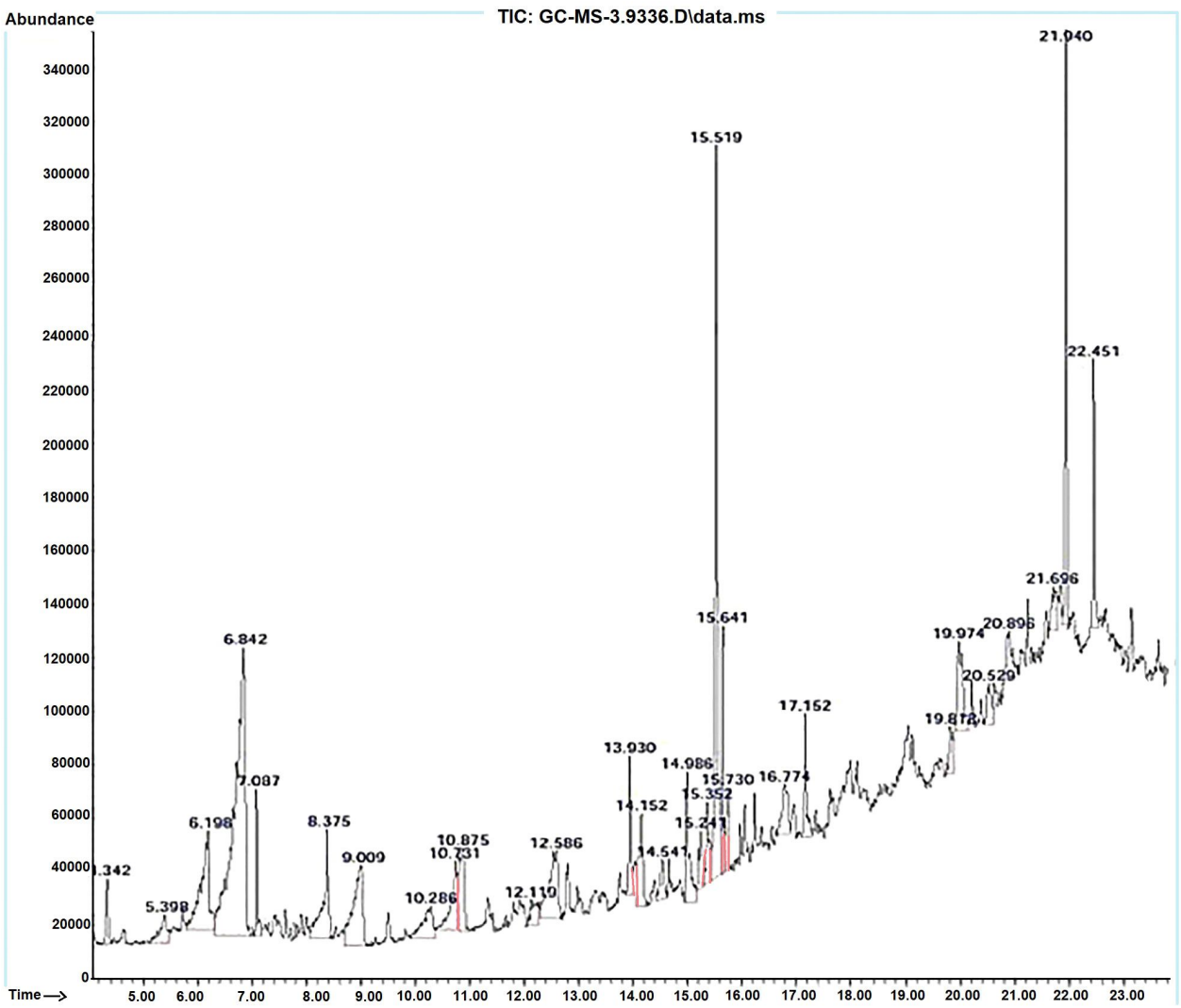


Fig: 2 GC–MS chromatogram of *N. arbor-tristis*

GC – MS results of petroleum ether extract leaves of *N. arbor-tristis* confirmed the presence of 29 components highly responsible for its antioxidant, antibacterial, antioxidant, and anti-cancer properties. The active biomolecules and their retention time (RT), peak area, molecular formula, molecular weight (MW), and structures obtained from PubChem sources are presented in Table 2. The first compound identified with less RT (4.342 min) was assigned as o-Xylene and the compound which took long RT (22.451min) was identified as Heptadecane, 2, 6, 10, 15-tetramethyl. Specifically, Vitamins, Sterols, and their derivative compounds possess a high percentage of peak area among the entire phytocompounds (Figure 2 & Table 2). The compounds elucidated in GC–MS spectra are known for their therapeutic properties[34]. GC-MS analysis of the methanolic extract from *N. arbor-tristis* L. leaves revealed the active compounds: Astragalin, Nicotiflorin, Nyctanthic acid, Friedeline, and Lupeol[35]. The study also supported the data, including the phytoconstituents such as D-mannitol, essential oil, and methyl salicylate. At the same time, the seeds contain glycerides of stearic, palmitic, oleic, linoleic, lignoceric, and myristic acids, lauric acid, D-glucose, and D-mannose. According to their reports, flower oil also includes 1-deconol anisaldehyde, Frie-del-1-ene-3-one, 1ahydroxyfriedel-4-(23)-en-3-one, 1-triacontanol, lignoceric acid, and pelargonic acid were extracted from *N. arbor-tristis* stems. Sigmasterol and n-Tetradecyl-b-D-glucopyranoside, a newly found rangy-olone. There are also additional compounds, such as 2-phenyl ethyl b-D-glucopyranoside. Some natural compounds collected from this plant species have been reported to improve various biological conditions. Some of these discovered and extracted compounds have been demonstrated to heal many biological diseases[36].

3.3. *In vitro* antioxidant Activity

3.3.1. DPPH assay

Plants contain powerful antioxidants, and the application of *N. arbor-tristis* leaves as a decoction in Ayurveda medicine for a variety of diseases has been connected to the anti-oxidant activity of groups of chemicals that help in scavenging the free radicals that are primarily responsible for pathogenesis. Various experimental setups such as DPPH ((1,1-diphenyl-2-picrylhydrazyl), hydroxyl and superoxide radicals, and H_2O_2 scavenging studies demonstrate the exceptional antioxidant activity of *N. arbor-tristis*. The free radical scavenging activity of different concentrations of *N. arbor-tristis* leaves extracts and of standard ascorbic acid was evaluated by using DPPH radical scavenging method[27] *in vitro* DPPH assay for the plant extract *N. arbor-tristis* were analyzed by different concentrations (50, 250, 500, 750 and 1000µL). Inhibitory Concentration (IC_{50}) values were calculated.

Table: 3 DPPH Assay for *N. arbor-tristis*			
S. No	Concentration (µ L)	IC_{50}%	IC_{50} (µ g /mL)
1	50	31.72±2.7	610.71±13.6
2	250	40.00±3.8	
3	500	48.97±3.4	
4	750	55.17±4.6	
5	1000	59.31±4.2	

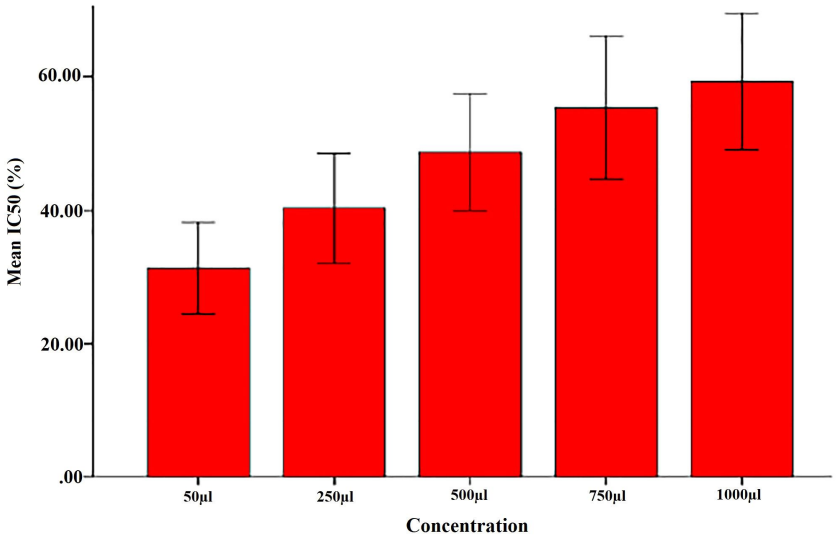


Fig: 3 *In vitro* DPPH assay of *N. arbor-tristis*

The inhibition concentration for the different plant concentrations was found as 31.72±2.7, 40.00±3.8, 48.97±3.4, 55.17±4.6 and 59.31±4.2% for the 50, 250, 500, 750, and 1000µL tested groups respectively. Finally, the overall inhibitory concentration of the plant *N. arbor-tristis* for DPPH assay is analyzed as 610.71±13.6µg/mL, increased concentration of plant extract showed increased scavenging activities against the reactive active species (Table 3 & Figure 2). The DPPH test of the methanolic extract revealed an inhibition percent of 69.42 at a 100g/mL concentration. However, aqueous extract only showed a 31.14 percent inhibition at the same concentration. Methanolic extracts were mostly effective at scavenging free radicals. The antioxidant potential data demonstrated inhibition in a dose-dependent way. The DPPH experiment revealed that the methanolic extract inhibited free radicals the most, whereas the aqueous extract inhibited them the least[21]. Various studies of free radical scavenging activity of various solvent extracts of dry and fresh flowers using multiple techniques, including such lipid peroxidation test, reducing activity, and H_2O_2 scavenging assay, as well as changing amounts of enzymatic and non-enzymatic antioxidants, discovered that methanol extracts of dry flowers possess total phenolics content and antioxidant activity. In contrast, the aqueous extract of dry flowers has high enzymatic activity. Ethanol extracts of *N. arbor-tristis* stem exhibit concentration-dependent antioxidant activity in numerous animals. These data suggest that different antioxidants occur in the leaves, stems, and flowers of *N. arbor-tristis*[28].

3.4. *Antibacterial Activity of N. arbor-tristis leaves petroleum ether extract.*

Antimicrobial activities of the plant extract are the essential parameters for the active compound analysis in phyto sources[29]. By disc diffusion method, the antimicrobial activity of the plant extract *N. arbor-tristis* is analyzed against four bacterial (*L. acidophilus, E. coli, S. typhi,* and *K. pneumoniae*) species tested. Three concentrations (40, 60, and 80mg/mL) and control groups are maintained.

Table 4: Antibacterial Activity of *N. arbor-tristis*				
Organisms	Control	Inhibition zone (in mm)		
		40mg/mL	60mg/mL	80mg/mL
Lactobacillus acidophilus	17.8±1.1	18.8±1.9	19.5±1.1	21.7±1.7
Escherichia coli	20.6±1.6	21.3±1.8	24.8±1.7	26.6±1.4
Salmonella typhi	16.4±1.7	17.9±1.5	19.8±1.5	21.9±1.6
Klebsiella pneumoniae	19.2±1.5	20.0±1.7	22.7±1.8	24.8±1.3

Compared to the control, all the treated groups showed high antimicrobial activity. In the control group, the highest microbicidal activity is exhibited against *E. coli* at 20.6±1.6mm, whereas the least micro-bacterial activity was observed against *S. typhi* at 16.4±1.7mm (Figures 4 & 5). In the 40mg/mL group, high inhibition was found against *E. coli* at 21.3±1.8mm, and the least inhibition was found against *S. typhi* at 17.9±1.5mm. In 60 and 80mg/mL groups, the highest inhibition zone are observed against *E. coli* (24.8±1.7 and 26.6±1.4mg/mL, respectively), and the least inhibition activity was observed against *L. acidophilus* (19.5±1.1 and 21.7±1.7mg/mL) respectively. Significantly increased concentration of plant extract showed significant inhibition against the testing microbes (Table 4).

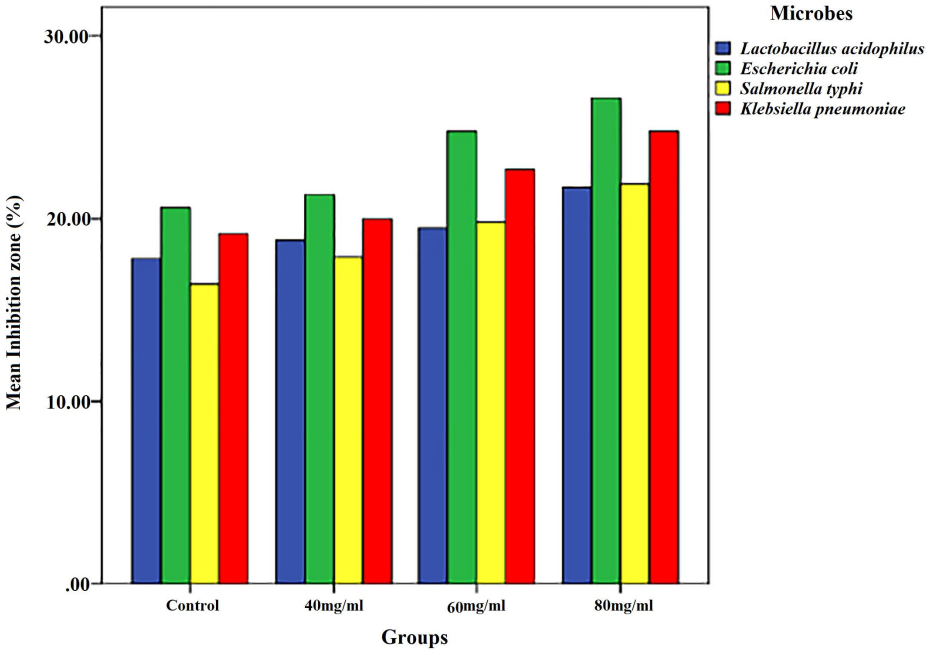


Fig: 4 *In vitro* Antibacterial Activity of *N. arbor-tristis*

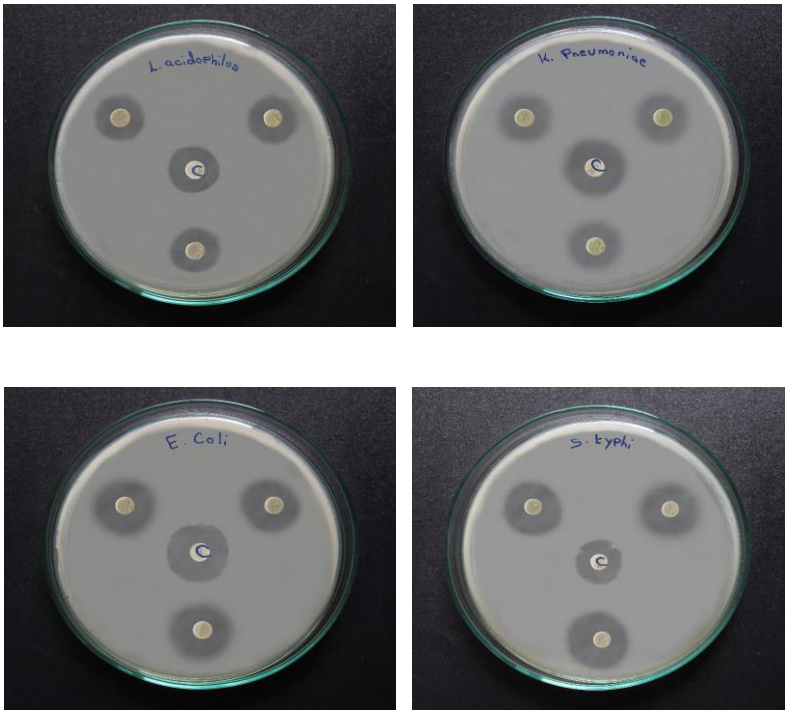


Fig: 5 Minimum Inhibitory Concentration of *N. arbor-tristis* extracts

3.5. Anti-inflammatory Activity

3.5.1. Inhibition of albumin denaturation

Denaturation of proteins is a well-certified cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, the capability of extract to inhibit protein denaturation was studied[30 - 31]. Albumin denaturation inhibition activity for the plant extract *N. arbor-tristis* was analyzed by different concentrations (50, 250, 500, 750, and 1000µL).

Table 5: *in vitro* Inhibition of albumin denaturation of *N. arbor-tristis*

S. No	Concentration (µ L)	IC_{50}%	IC_{50} (µ g /mL)
1	50	26.97±2.85	781.93±13.6
2	250	32.89±3.13	
3	500	41.45±3.51	
4	750	49.34±4.21	
5	1000	56.58±3.86	

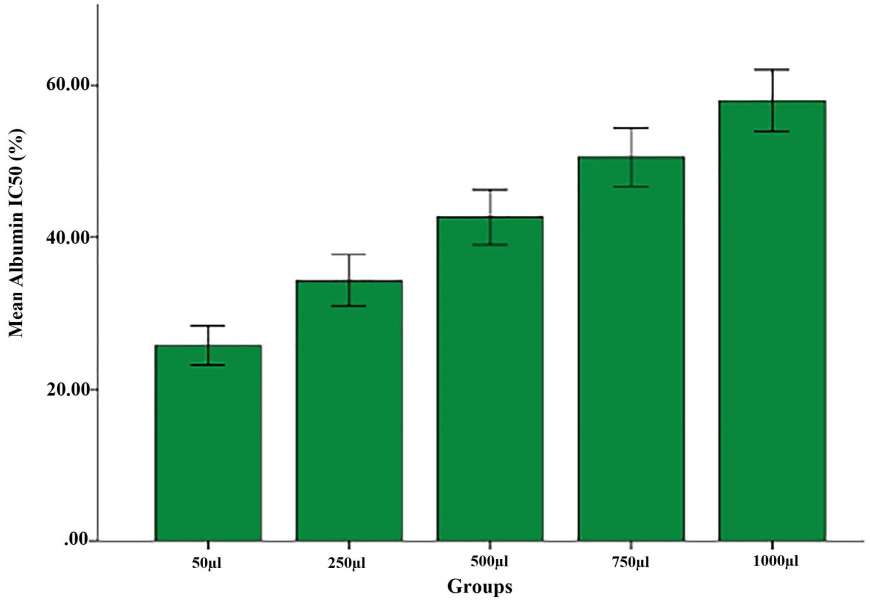


Fig: 6 *In vitro* Inhibition of albumin denaturation of *N. arbor-tristis*

The inhibition concentration for the different plant concentrations was found as 26.97±2.85, 32.89±3.13, 41.45±3.51, 49.34±4.21 and 56.58±3.86% for the 50, 250, 500, 750, and 1000µL tested groups respectively. Finally, the overall albumin denaturation inhibitory concentration of the plant *N. arbor-tristis* is 781.93±13.6µg/mL. Increased concentration of plant extract showed increased inhibition of albumin denaturation activities (Table 5 & Figure 6).

3.6. Proteinase inhibitory activity

Neutrophils are a rich resource of serine proteinase and are localized at lysosomes. It was previously reported that leukocyte proteinase plays an important role in the development of tissue damage during inflammatory reactions. A significant level of proteinase was provided by proteinase inhibitors[32], and the Petroleum ether extract of *N. arbor-tristis* whole plant exhibited significant anti-proteinase inhibition activity for the plant extract of *N. arbor-tristis* are analyzed by different concentrations (50, 250, 500, 750, and 1000µL).

Table 6: *in vitro* Inhibition of Anti-proteinase action of *Nyctanthes arbor-tristis*

S. No	Concentration (µ L)	IC_{50}%	IC_{50} (µ g/mL)
1	50	32.26±1.99	652.98±32.5
2	250	41.29±2.31	
3	500	45.81±2.75	
4	750	53.55±2.41	
5	1000	58.06±1.90	

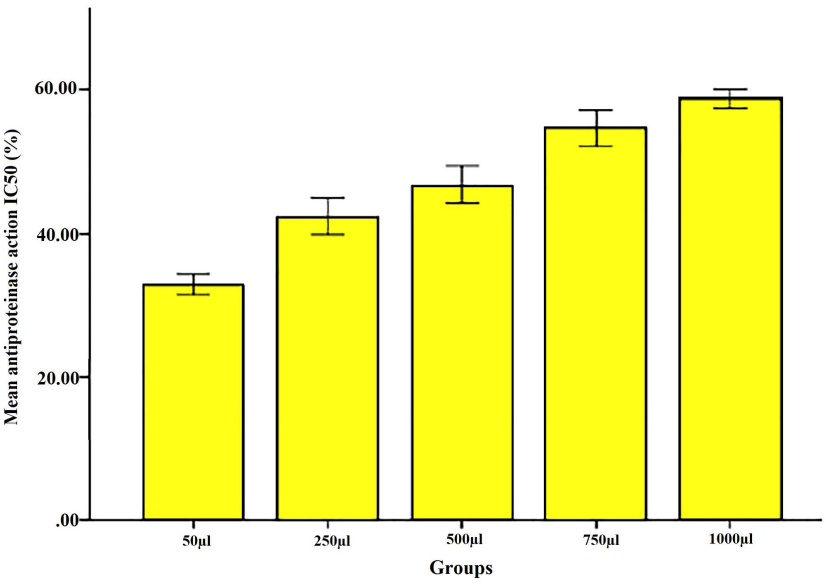


Fig: 7 In vitro Inhibition of Anti-proteinase action of Nyctanthes arbor-tristis

The inhibition concentration for the is found as 32.26±1.99, 41.29±2.31, 45.81±2.75, 53.55±2.41 and 58.06±1.90% for the 50, 250, 500, 750, and 1000µL tested groups respectively. Finally, the overall albumin denaturation inhibitory concentration of the plant *N. arbor-tristis* is 652.98±32.5µg/mL. Increased concentration of plant extract showed increased inhibition of anti-proteinase activities. (Table 6 & Figure 7).

4. CONCLUSION

According to the results of *in-vitro* and *in-vivo* investigations, *N. arbor-tristis* is a potentially enormous therapeutic in the field of herbal medicine, notably in the management of diseases including malaria, fevers, arthritis, and protozoan infections such as leishmaniasis. It has also been established that every part of this plant has therapeutic potential. Despite using crude extract, the only research that has undergone clinical trials is for treating malaria. Though the potentiality may widen, the studies faced limitations in exploring their research aspects. Furthermore, more detailed safety data on acute, subacute, cardiac, and immunotoxicity for crude and purified extracts is required. The present study highlights the petroleum ether extracts of the leaf of *N. arbor-tristis* possess multipotent activities. It is a potential antibacterial agent against pathogenic bacteria and may help interact with various plant diseases and human allergies. *N. arbor-tristis* has a better inhibitory effect on trypsin and a good inhibitory effect on protein denaturation and good antioxidants. Wherein GC–MS analysis revealed, 29 components in *N. arbor-tristis* leaves petroleum ether extract. The plant material could be exploited as an alternative antibiotic drug discovery toward combating the global disease burden. The potential economic and social impact of the use of *N. arbor-tristis* in the management of various diseases. Thus, the importance of continued research in this area and the potential benefits that could be derived from herbal medicine.

5. ACKNOWLEDGEMENTS

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6. AUTHORS CONTRIBUTION STATEMENT

Ms. Ponnusamy Kiruba & Dr. Michael Helan Soundra Rani collected the samples from the Kolli Hills, Namakkal district, and sketched the concepts in processing & extraction procedures. Dr. Ponnuswamy Selvamaleeswaran designed and performed the anti-inflammatory activity and GCMS characterization study. Dr. Edward Gnanaraj Wesely and Dr. Kuppusamy Palanisamy analyzed the data regarding Methodology & Validate the original draft writing with suggestions and inputs from all authors.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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