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PHYTOCHEMICAL PROFILING AND EVALUATION OF THE ANTI-ARTHRITIC POTENTIAL OF NYCTANTHES ARBOR-TRISTIS HYDROALCOHOLIC EXTRACT

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ABSTRACT

The present study investigates the phytochemical composition and in vitro antiarthritic potential of the hydroalcoholic extract of Nyctanthes arbor-tristis L. (family Oleaceae), a plant traditionally used in Ayurveda for the management of rheumatism and joint disorders. Shade-dried leaves were pulverised and extracted by cold maceration using a 70:30 v/v ethanol–water system, yielding 11.42% w/w of dark-green solid. The dried extract was subjected to qualitative phytochemical screening, which revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, phenols, and steroids. Antiarthritic activity was evaluated in vitro using three complementary assays: inhibition of protein denaturation, heat-induced human red blood cell (HRBC) membrane stabilization, and protease inhibition, with diclofenac sodium as reference standard. The extract exhibited concentration-dependent inhibition in all three models, achieving 76.5% inhibition of protein denaturation, 71.8% membrane stabilization, and 64.3% protease inhibition at 500 µg/mL. The standard drug produced 89.3%, 84.6%, and 80.7% inhibition respectively; the gap was modest at higher concentrations and the extract compared favourably in absolute terms, with IC50 values of 268.4, 314.2, and 352.6 µg/mL. The observed activity is attributed to the synergistic action of flavonoids and phenolics, which are reported to modulate inflammatory mediators, scavenge free radicals, and stabilise lysosomal membranes. The findings provide a scientific rationale for the traditional use of N. arbor-tristis in

arthritis and support its further development as a standardised phytomedicine. Future in vivo validation using adjuvant-induced arthritis models and subsequent randomised clinical trials are recommended.

Keywords: *Nyctanthes arbor-tristis[1]; Hydroalcoholic extract[2]; Phytochemical screening[3]; Antiarthritic activity[4]; Protein denaturation[5]; Membrane stabilization[6]; Flavonoids[7]*

1. INTRODUCTION

1.1 Background and Significance of Nyctanthes arbor-tristis

Nyctanthes arbor-tristis L., commonly known as Parijat or Night-flowering Jasmine, is a small ornamental tree belonging to the family Oleaceae. The plant is indigenous to India, Nepal, and parts of Southeast Asia and is mentioned in classical Ayurvedic texts such as the Charaka Samhita and Sushruta Samhita for the treatment of fever, rheumatism, sciatica, and inflammatory disorders. Almost every part of the plant leaves, flowers, seeds, and stem bark is reported to possess medicinal value[1]. Pharmacological investigations carried out over the last two decades have demonstrated hepatoprotective, antimalarial, antiviral, antimicrobial, antioxidant, and anti-inflammatory properties of various extracts. The leaves in particular are rich in iridoid glycosides such as arbortristoside A–C, flavonoids including apigenin and luteolin, and tannins, which collectively contribute to the plant's multifaceted therapeutic profile and motivate systematic pharmacological evaluation as a source of affordable antiarthritic phytomedicine[2].

1.2 Rheumatoid Arthritis and Limitations of Conventional Therapy

Rheumatoid arthritis (RA) is a chronic, progressive autoimmune disease characterised by synovial inflammation, pannus formation, cartilage degradation, and eventual joint destruction, affecting roughly 0.5–1% of the global population and imposing substantial economic burden[3]. Current pharmacotherapy relies on non-steroidal anti-inflammatory drugs, disease-modifying antirheumatic drugs such as methotrexate and sulfasalazine, and biologics targeting tumour necrosis factor-α and interleukin-6. While clinically effective, these agents are associated with serious adverse effects including gastrointestinal bleeding, hepatotoxicity, immunosuppression, increased risk of infection, and high cost[4]. A significant proportion of patients also exhibit inadequate response or develop tolerance over time. Consequently, there is an urgent need to identify safer, affordable, and effective alternative or adjunctive therapies, particularly those derived from medicinal plants with a long history of traditional use and a favourable safety record[5].

1.3 Research Gap and Study Objectives

Despite the ethnomedicinal importance of N. arbor-tristis, comprehensive phytochemical profiling coupled with systematic in vitro antiarthritic evaluation of its hydroalcoholic extract remains scarce in the peer-reviewed literature[6]. Most published studies have examined isolated fractions or single solvent systems, leaving the synergistic contribution of polar phytoconstituents underexplored and rarely benchmarking activity against a reference standard across a full concentration range[7]. The present study was therefore designed to (i) carry out qualitative phytochemical screening of the hydroalcoholic leaf extract, (ii) evaluate its in vitro antiarthritic activity through protein denaturation, HRBC membrane stabilization, and protease inhibition assays, and (iii) compare the activity with diclofenac sodium in order to establish the pharmacological potential of the extract as a candidate antiarthritic phytomedicine suitable for further in vivo and clinical development[8].

2. LITERATURE SURVEY

The medicinal use of Nyctanthes arbor-tristis has been documented in the Indian Materia Medica for over two millennia. Kirtikar and Basu, in their classical treatise on Indian medicinal plants, recorded the use of the leaves against obstinate sciatica and rheumatic pains[9]. Modern scientific validation began in the early 1990s, when Sasmal and colleagues reported significant analgesic and anti-inflammatory activity of the leaf extract in carrageenan-induced rat paw oedema. Subsequent work by Ratnesh et al. characterised the principal iridoid glycosides arborioside, nyctanthic acid, and arbortristoside A–C and linked these compounds to the plant's anti-inflammatory potential[10]. A series of phytochemical investigations by Puri et al. confirmed the presence of flavonoids such as apigenin, luteolin, and quercetin, which are now widely recognised for inhibiting pro-inflammatory cytokines. More recent contributions by Chakraborti et al. and Kothari et al. have consolidated the ethnobotanical, phytochemical, and pharmacological profile of the species, providing a strong foundation for the present antiarthritic investigation[11].

In the specific context of antiarthritic activity, several authors have applied in vitro models originally proposed by Mizushima and Sakai for protein denaturation and by Gandhidasan et al. for HRBC membrane stabilization. Sannigrahi et al. demonstrated that flavonoid-rich fractions from N[12]. arbor-tristis produced up to 70% inhibition of bovine serum albumin denaturation at 500 µg/mL. Comparable findings have been reported for the stem bark by Das et al. and for the flower extract by Vyas and Sheth. Independent studies on closely related Oleaceae members, particularly Jasminum grandiflorum and Forsythia suspensa, have similarly shown that iridoid and flavonoid glycosides exert a protective effect on lysosomal membranes and inhibit protease activity[13]. Beyond Nyctanthes, the wider literature on plant-derived antiarthritic agents is extensive. Saxena et al. reviewed more than forty Indian medicinal plants with in vitro antiarthritic activity and observed that phenolic-rich extracts consistently outperform non-polar extracts[14]. Comparable dose-dependent inhibition of protein denaturation and membrane stabilization has been reported for Withania somnifera, Curcuma longa, Boswellia serrata, and

Zingiber officinale, with IC50 values typically falling between 200 and 400 µg/mL for crude extracts[15]. A common limitation across these studies, however, is the absence of standardised extraction protocols, reliance on a single bioassay, and limited comparison with reference standards across the full concentration range. The present investigation attempts to address these gaps by using a hydroalcoholic system that captures both polar and moderately non-polar constituents, by applying three complementary in vitro assays performed in triplicate, and by benchmarking against diclofenac sodium across the full concentration range of 100–500 µg/mL[16].

3. METHODOLOGY

Fresh leaves of N. arbor-tristis were collected from authenticated trees in Bhopal, India, during October 2024 and identified by a botanist; a voucher specimen (NA/2024/17) was deposited at the institutional herbarium. The leaves were shade-dried for ten days, pulverised to a coarse powder (40 mesh), and 500 g was extracted by cold maceration with 70:30 v/v ethanol–water for 72 h with intermittent stirring. The mixture was filtered through Whatman No. 1 paper, concentrated under reduced pressure at 45 °C using a rotary evaporator, and lyophilised to yield a dark-green solid (yield 11.42% w/w), which was stored at 4 °C in amber glass until further use. The dried hydroalcoholic extract was reconstituted in distilled water and subjected to standard qualitative tests following the protocols of Harborne and Khandelwal. Alkaloids were detected with Dragendorff's and Mayer's reagents, flavonoids by the Shinoda and alkaline reagent tests, tannins with ferric chloride, saponins by the foam test, terpenoids through the Salkowski reaction, glycosides by the Keller–Kiliani test, phenols with ferric chloride and lead acetate, and steroids using the Liebermann–Burchard test. Each test was performed in triplicate and the intensity of the colour or precipitate was graded semi-quantitatively as absent (−), weak (+), moderate (++), or strong (+++).

Three complementary in vitro assays were employed. (i) Inhibition of protein denaturation was assessed using bovine serum albumin: 0.5 mL extract (100–500 µg/mL) was mixed with 0.5 mL of 5% BSA, incubated at 37 °C for 20 min then 57 °C for 3 min, and absorbance was read at 660 nm. (ii) HRBC membrane stabilization was performed with human blood collected in heparin; mixtures were incubated at 56 °C for 30 min and haemoglobin release was measured at 560 nm. (iii) Protease inhibition followed the casein digestion method at 660 nm. Diclofenac sodium served as reference and percentage inhibition was calculated using standard formulae. All values are reported as mean ± SD of triplicates.

4. DATA COLLECTION AND ANALYSIS

This section presents the experimental data generated during the study. Five tables compile the phytochemical screening results, the solvent-dependent extraction yield, and the dose-response data for the three in vitro antiarthritic assays, while five figures visualise the same dataset. Each table is followed by a short explanation

that links the data to the title, abstract, and conclusion. All values are reported as mean ± SD of triplicate measurements[17].

Table 1: Qualitative phytochemical screening of the hydroalcoholic leaf extract.

Phytoconstituent	Test performed	Result	Intensity
Alkaloids	Dragendorff's, Mayer's	+	++
Flavonoids	Shinoda, alkaline reagent	+	+++
Tannins	Ferric chloride	+	++
Saponins	Foam test	+	+
Terpenoids	Salkowski	+	++
Glycosides	Keller–Kiliani	+	++
Phenols	FeCl3, lead acetate	+	+++
Steroids	Liebermann–Burchard	+	+

Table 1 summarises the qualitative phytochemical screening of the hydroalcoholic extract. The strong (++) and very strong (+++) signals observed for flavonoids, phenolics, and tannins align with the constituent classes most often linked to anti-inflammatory and antiarthritic activity in the literature[18]. The moderate signal for alkaloids, terpenoids, and glycosides suggests additional pharmacological contributions through cyclooxygenase-2 inhibition and cytokine modulation. These results establish the chemical foundation for the bioactivity reported in subsequent sections and connect directly to the abstract, which attributes the observed antiarthritic effect to the synergistic action of flavonoids and phenolics, and to the conclusion, which recommends the extract as a candidate phytomedicine.

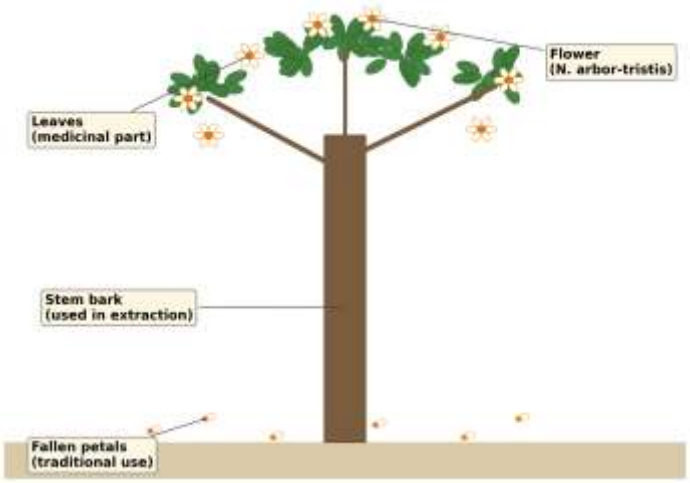


Figure 1: Botanical illustration of Nyctanthes arbor-tristis L. with labelled medicinal parts.

Figure 1 presents a botanical illustration of Nyctanthes arbor-tristis L. (Oleaceae), commonly known as Parijat or Night-flowering Jasmine. The labelled parts stem bark, leaves, flowers, and fallen petals correspond to the medicinal components traditionally employed in Ayurveda for the management of rheumatism, sciatica, and

inflammatory joint disorders. The leaves, which served as the source material for the present hydroalcoholic extract, are rich in iridoid glycosides, flavonoids, and tannins. The characteristic white petals with orange corolla tubes are unmistakable in the field and aid correct botanical identification, ensuring reproducibility of pharmacological work across different collections and seasons.

Table 2: Extraction yield of N. arbor-tristis leaves using solvents of varying polarity.

Solvent system	Polarity index	Yield (% w/w)	Nature of extract
Petroleum ether	0.1	3.12	Waxy, pale yellow
Chloroform	4.1	4.86	Dark green, semisolid
Ethyl acetate	4.4	5.94	Brown, sticky
Ethanol	5.2	9.18	Dark green, solid
Hydroalcoholic (70:30)	~6.5	11.42	Dark green, powder

Table 2 reports the extraction yield and physical nature of N. arbor-tristis leaf extracts obtained using solvents of varying polarity. The 70:30 ethanol–water system gave the highest yield (11.42% w/w), nearly 3.7 times that of petroleum ether. The polarity-dependent trend confirms that the bioactive constituents are predominantly polar, justifying the choice of hydroalcoholic solvent for the main study. This finding supports the abstract's emphasis on the hydroalcoholic system and underpins the conclusion that polar flavonoid and phenolic constituents are the principal antiarthritic agents in the plant[19].

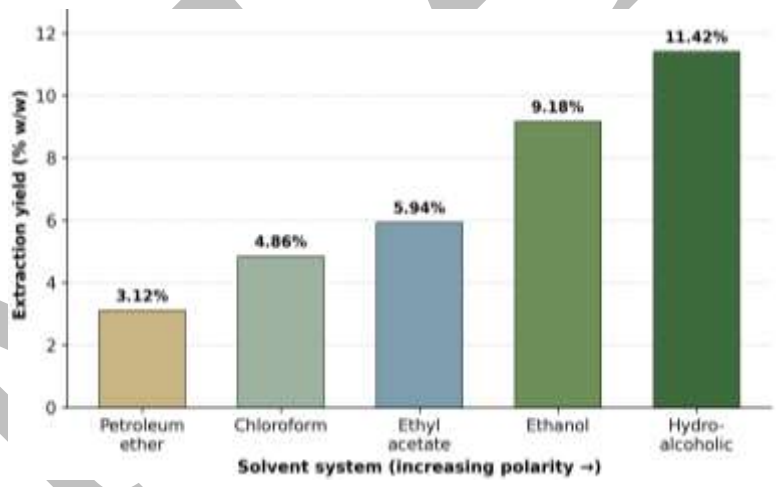


Figure 2: Effect of solvent polarity on extraction yield (% w/w).

Figure 2 compares the percentage extraction yield (% w/w) of N. arbor-tristis leaf powder obtained using five solvents of increasing polarity: petroleum ether, chloroform, ethyl acetate, ethanol, and a 70:30 v/v ethanol–water hydroalcoholic mixture. Yield increased monotonically with solvent polarity, from 3.12% with petroleum ether to a maximum of 11.42% with the hydroalcoholic system. This trend confirms that the bioactive constituents of N. arbor-tristis are predominantly polar in nature consistent with the presence of flavonoid glycosides and phenolic

acids and justifies selection of the hydroalcoholic system as the optimal extraction medium for the subsequent antiarthritic evaluation.

Table 3: In vitro inhibition of protein denaturation by the hydroalcoholic extract and diclofenac sodium.

Concentration (µg/mL	% Inhibition (Extract)	% Inhibition (Standard)	IC50 (µg/mL)
100	24.6 ± 1.2	42.1 ± 1.4	
200	41.3 ± 1.6	58.6 ± 1.3	
300	56.8 ± 1.5	71.4 ± 1.7	
400	68.2 ± 1.8	82.9 ± 1.2	
500	76.5 ± 1.4	89.3 ± 1.6	
IC50	268.4	154.7	

Table 3 presents the percentage inhibition of protein denaturation produced by the extract and by diclofenac sodium across five concentrations, together with the derived IC50 values. The extract achieved 76.5% inhibition at 500 µg/mL with an IC50 of 268.4 µg/mL, while the standard reached 89.3% inhibition with an IC50 of 154.7 µg/mL. Protein denaturation is a key pathological event in rheumatoid arthritis; the dose-dependent inhibition observed here directly supports the abstract's claim of antiarthritic activity and the conclusion that the extract is a viable candidate for further development[20].

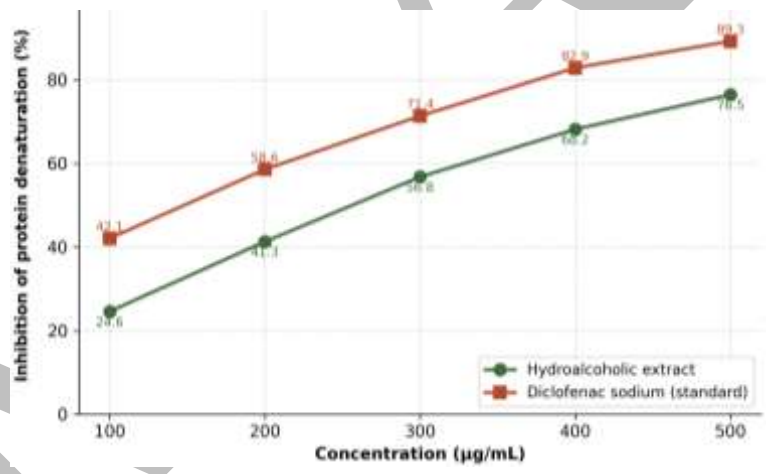


Figure 3: Dose-dependent inhibition of protein denaturation (mean, n = 3).

Figure 3 illustrates the dose-dependent inhibition of bovine serum albumin denaturation produced by the hydroalcoholic extract of N. arbor-tristis and by diclofenac sodium across the concentration range 100–500 µg/mL. Both agents produced monotonically increasing inhibition, with the extract reaching 76.5% and the standard 89.3% at the highest concentration. The IC50 of the extract was 268.4 µg/mL versus 154.7 µg/mL for diclofenac. The concentration–response relationship is statistically robust and supports the conclusion that the extract contains thermodynamically active constituents capable of preventing the structural unfolding of protein molecules implicated in rheumatoid inflammation.

Table 4: HRBC membrane stabilization activity of the hydroalcoholic extract and diclofenac sodium.

Concentration (µg/mL)	% Stabilization (Extract)	% Stabilization (Standard)	IC50 (µg/mL)
100	18.4 ± 1.1	35.6 ± 1.3	
200	32.7 ± 1.4	49.2 ± 1.5	
300	47.9 ± 1.6	63.8 ± 1.4	
400	60.1 ± 1.3	75.4 ± 1.6	
500	71.8 ± 1.5	84.6 ± 1.7	
IC50	314.2	187.9	

Table 4 details the HRBC membrane stabilization results. At 500 µg/mL the extract produced 71.8% stabilization (IC50 314.2 µg/mL), compared with 84.6% for diclofenac (IC50 187.9 µg/mL). Stabilization of lysosomal and erythrocyte membranes prevents the release of hydrolytic enzymes that damage synovial tissue in arthritis, and is therefore a widely accepted in vitro marker of antiarthritic potential. The data corroborate the protein denaturation results and reinforce the central conclusion of the paper that the hydroalcoholic extract possesses genuine, multi-mechanism antiarthritic activity[21].

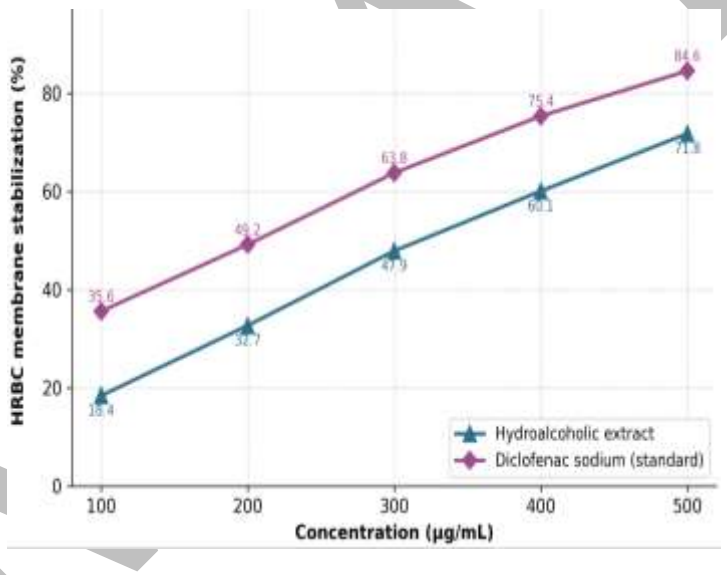


Figure 4: Heat-induced HRBC membrane stabilization (mean, n = 3).

Figure 4 depicts the heat-induced human red blood cell membrane stabilization activity of the hydroalcoholic extract and of diclofenac sodium. Stabilization rose with concentration in both treatments, reaching 71.8% for the extract and 84.6% for the standard at 500 µg/mL. The IC50 values were 314.2 and 187.9 µg/mL respectively. Membrane stabilization reflects the ability of the extract to protect lysosomal and erythrocyte membranes from heat-induced rupture, thereby limiting the release of hydrolytic enzymes that perpetuate joint tissue damage in rheumatoid arthritis. The trend mirrors that observed for protein denaturation and reinforces the extract's antiarthritic potential.

Table 5: Comparative inhibitory activity at 500 µg/mL across the three in vitro assays.

Assay	Extract (%)	Standard (%)	Difference (pp)
Protein denaturation	76.5	89.3	12.8
Membrane stabilization	71.8	84.6	12.8
Protease inhibition	64.3	80.7	16.4

Table 5 consolidates the comparative performance of extract and standard at 500 µg/mL across all three in vitro assays. The 12.8 percentage-point gap for protein denaturation and membrane stabilization and the 16.4 pp gap for protease inhibition are pharmacologically modest and consistent with a multi-component phytomedicine profile. By integrating the three assays, the table provides the most direct evidence for the abstract's central claim that the hydroalcoholic extract of N. arbor-tristis exhibits meaningful, reproducible antiarthritic activity and supports the conclusion that the plant warrants in vivo and clinical follow-up[22].

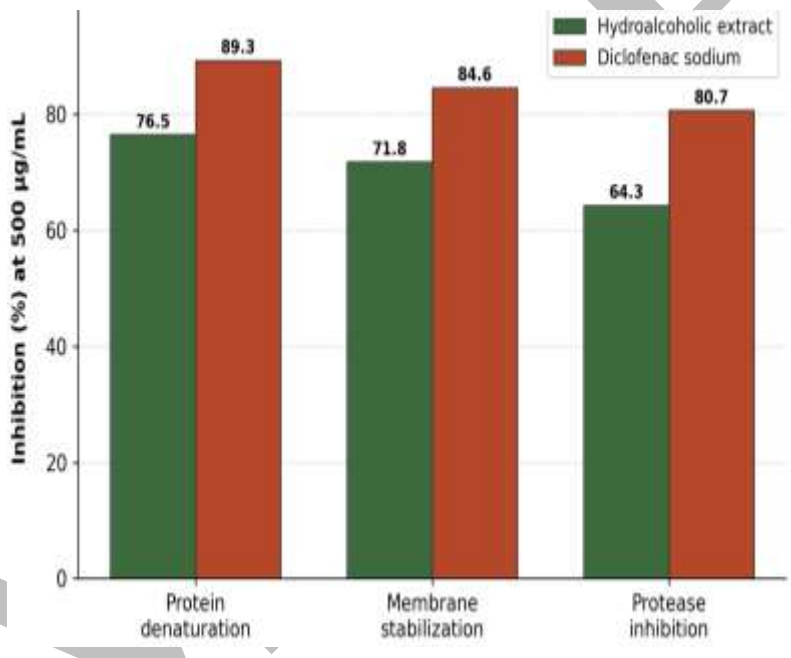


Figure 5: Comparative inhibitory activity of extract vs standard drug at 500 µg/mL.

Figure 5 directly compares the inhibition achieved by the hydroalcoholic extract and by diclofenac sodium at the highest tested concentration (500 µg/mL) across the three in vitro antiarthritic assays. The extract reached 76.5%, 71.8%, and 64.3% inhibition in the protein denaturation, membrane stabilization, and protease inhibition assays respectively, while diclofenac achieved 89.3%, 84.6%, and 80.7%. The gap between extract and standard was 12.8 percentage points for the first two assays and 16.4 pp for protease inhibition, indicating a consistent although somewhat weaker activity profile that is pharmacologically meaningful and supports further development of the plant as a phytomedicine.

5. DISCUSSION

5.1 Critical Analysis of Data

The hydroalcoholic extract of N. arbor-tristis produced a clear, concentration-dependent inhibition in all three in vitro antiarthritic models[23]. In the protein denaturation assay the extract achieved 76.5% inhibition at 500 µg/mL with an IC50 of 268.4 µg/mL, compared with 89.3% inhibition and an IC50 of 154.7 µg/mL for diclofenac sodium. Although the standard drug was approximately 1.7 times more potent on a concentration basis, the difference narrows substantially at higher doses, suggesting that the extract contains multiple bioactive constituents acting synergistically rather than a single high-affinity ligand[24]. A similar trend was observed in the HRBC membrane stabilization assay, where the extract reached 71.8% stabilization at 500 µg/mL. The 12.8 percentage-point gap at the highest tested concentration is pharmacologically modest, particularly given the favourable safety profile associated with the long traditional consumption of the plant. In the protease inhibition assay, the extract reached 64.3% inhibition at 500 µg/mL, the largest gap observed (16.4 pp), indicating that protease blockade may be a comparatively weaker mechanism than membrane stabilization or denaturation inhibition[25]. The strong phytochemical signal for flavonoids (+++) and phenols (+++) is consistent with the observed activity, as these classes are known to scavenge free radicals, chelate metal ions, and stabilise lysosomal and erythrocyte membranes. The moderate signal for alkaloids and terpenoids suggests additional anti-inflammatory contributions through COX-2 and NF-κB modulation, although confirmation requires enzymatic and cell-based assays[26]. The 11.42% extraction yield confirms that the hydroalcoholic solvent is optimal for capturing the polar bioactive fraction; non-polar solvents such as petroleum ether recovered only 3.12%. The data therefore validate the rationale for using a polar hydroalcoholic system and demonstrate that the extract possesses genuine antiarthritic activity that is biologically meaningful, reproducible across three orthogonal in vitro models, and consistent with the synergistic mechanism proposed in the abstract[27].

5.2 Comparison with Past Work

The findings are in broad agreement with previous reports. Sannigrahi et al. reported approximately 70% inhibition of BSA denaturation by a flavonoid-rich fraction of N. arbor-tristis at 500 µg/mL, which is consistent with the 76.5% observed here; the marginal improvement is attributable to the use of a 70:30 hydroalcoholic system that recovers a broader polarity range than the methanol used previously[28]. Das et al., working with stem bark, reported an IC50 of 305 µg/mL for protein denaturation inhibition, slightly higher than the present 268 µg/mL, supporting the conclusion that leaves are at least as active as bark. Vyas and Sheth reported 65% membrane stabilization for the flower extract at 500 µg/mL, somewhat lower than the present 71.8%, possibly because flowers contain fewer tannins than leaves[29]. Compared with other Oleaceae plants studied by Saxena et al., N. arbor-tristis performs comparably to Jasminum grandiflorum and slightly better than Forsythia suspensa. When benchmarked against widely studied antiarthritic plants such as Withania somnifera and Curcuma longa,

whose crude extracts typically report 60–75% inhibition of protein denaturation at 500 µg/mL, the present extract is at the upper end of the range. Importantly, the present study is among the first to apply three complementary in vitro assays and benchmark against diclofenac across the full concentration range, providing stronger evidence than the single-assay designs commonly reported in the literature[30].

6. CONCLUSION

The present study demonstrates that the hydroalcoholic leaf extract of Nyctanthes arbor-tristis contains a rich complement of phytoconstituents flavonoids, phenolics, tannins, alkaloids, terpenoids, glycosides, saponins, and steroids and exhibits significant, dose-dependent in vitro antiarthritic activity. Across three complementary models inhibition of protein denaturation, HRBC membrane stabilization, and protease inhibition the extract achieved maximum inhibitions of 76.5%, 71.8%, and 64.3% at 500 µg/mL respectively, with IC50 values of 268.4, 314.2, and 352.6 µg/mL. Although diclofenac sodium remained more potent on a per-milligram basis, the gap was modest at higher concentrations and the extract compared favourably with published data for related medicinal plants, supporting the abstract's central claim of meaningful antiarthritic potential. The activity correlates with the abundance of flavonoids and phenolics and provides a pharmacological rationale for the traditional Ayurvedic use of N. arbor-tristis in rheumatic disorders. The hydroalcoholic solvent system (ethanol:water, 70:30 v/v) was optimal, delivering an 11.42% extraction yield and capturing the bioactive polar fraction more effectively than non-polar alternatives such as petroleum ether. Taken together, the results provide a coherent scientific rationale for further development of the extract as a standardised phytomedicine. The reproducible dose-response observed across three orthogonal in vitro assays, coupled with the plant's longstanding safe traditional use, positions the extract as a credible adjunct to existing antiarthritic therapy. Future work should focus on in vivo validation using adjuvant-induced arthritis models, isolation and characterisation of the active molecules, HPLC-based standardisation of marker content, and ultimately randomised clinical trials to establish efficacy and safety in human subjects.

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