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Anti-inflammatory potential of *Catharanthus roseus*: A systematic review of *In vitro* investigations

Ayu Sari
Department of Pediatric Nursing, Faculty of Medicine, Universitas Lambung Mangkurat, Indonesia

Abstract
Inflammation is a critical biological response to injury or infection, but chronic inflammation is implicated in the pathogenesis of numerous diseases, including arthritis, cardiovascular disorders, and cancer. The search for safe and effective anti-inflammatory agents has increasingly turned toward medicinal plants. *Catharanthus roseus* (commonly known as Madagascar periwinkle) is a well-known medicinal plant traditionally used for its diverse pharmacological properties. This systematic review aims to critically evaluate the anti-inflammatory potential of *Catharanthus roseus* based on evidence from *In vitro* studies.

A comprehensive literature search was conducted across electronic databases including PubMed, Scopus, Web of Science, and Google Scholar, focusing on studies published up to August 2025. Eligible studies were selected based on predefined inclusion criteria, including *In vitro* experimental designs assessing anti-inflammatory activity of *C. roseus* extracts or compounds. Data extraction focused on study design, extract type, experimental models used (e.g., macrophage cell lines, LPS-induced inflammation), biomarkers assessed (e.g., TNF-α, IL-6, COX-2, NO), and outcomes reported.

The review included 18 *In vitro* studies demonstrating that various parts of *C. roseus*—notably leaf, stem, and root extracts—exhibit significant anti-inflammatory activity. Mechanisms of action observed included inhibition of pro-inflammatory cytokines, suppression of nitric oxide production, and downregulation of inflammatory mediators such as COX-2 and iNOS. Several bioactive compounds, including alkaloids and flavonoids, were identified as likely contributors to the observed effects. Ethanol and methanol extracts were among the most effective solvent systems used for extraction.

In conclusion, *In vitro* evidence strongly supports the anti-inflammatory potential of *Catharanthus roseus*, attributed to its rich phytochemical composition and multi-targeted mechanisms of action. These findings provide a scientific basis for the traditional use of *C. roseus* in inflammatory conditions and support further *In vivo* and clinical investigations to validate its therapeutic efficacy and safety.

Keywords: Catharanthus roseus, Anti-inflammatory Activity, *In vitro* Studies, Medicinal Plants, Phytochemicals, Cytokine Inhibition, COX-2 Suppression, Natural Products

Introduction

Background and Context

Inflammation is a fundamental physiological response triggered by harmful stimuli such as pathogens, damaged cells, or chemical irritants. In the acute phase, it serves as a protective mechanism, promoting healing and restoring homeostasis. However, when inflammation becomes chronic, it underlies a wide array of pathological conditions—including rheumatoid arthritis, atherosclerosis, neurodegenerative disorders, inflammatory bowel disease, and even certain cancers. Chronic inflammation is characterized by a sustained release of pro-inflammatory mediators (e.g., cytokines like TNF-α, IL-1β, IL-6), reactive oxygen species (ROS), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2)—all of which can perpetuate tissue damage. [1]

Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are standard treatments that dampen inflammatory responses. Yet, their long-term use is limited by adverse effects such as gastrointestinal bleeding, renal impairment, hypertension, and immune suppression. [2] This has prompted interest in discovering safer and more effective anti-inflammatory agents. Natural products—particularly plant-derived phytochemicals—have drawn significant attention due to their favorable safety profiles, accessibility, and multi-targeted mechanisms. [3]

Catharanthus roseus (Madagascar periwinkle), a member of the Apocynaceae family, has been widely cultivated and used in traditional medicine systems such as Ayurveda and folk remedies. It is best known for its anticancer alkaloids (vincristine, vinblastine), but historical texts also cite its use in treating fever, diabetes, wound healing, and inflammatory conditions [4]. Modern phytochemical investigations reveal that *C. roseus* is rich in alkaloids, flavonoids, terpenoids, and phenolic acids—all of which have documented bioactivity [5]. This converges on the hypothesis that *C. roseus* may harbor potent anti-inflammatory constituents.

Importance of the Research

There are several compelling reasons why a systematic evaluation of *C. roseus*'s anti-inflammatory potential is of scientific and practical importance:

1. **Bridging Traditional Knowledge and Modern Science**
   Documenting and experimentally validating traditional claims helps integrate ethnobotanical wisdom into evidence-based medicine. Demonstrating anti-inflammatory effects strengthens the value of traditional uses and may lead to novel therapeutic leads.

2. **Need for Safer Therapeutics**
Given the adverse effects associated with conventional anti-inflammatory drugs, identifying plant-derived compounds with multi-mechanistic action and lower toxicity profiles holds promise for safer long-term patient care.

3. Unmet Therapeutic Need

Chronic inflammatory diseases remain prevalent and often poorly controlled. Novel agents that modulate inflammation through different molecular pathways may be especially valuable for patient's refractory to current therapies.

4. Phytochemical Diversity and Multi-target Mechanisms

The chemical richness of *C. roseus* allows the possibility of targeting multiple inflammatory pathways simultaneously. Its complex mixture of bioactives—even in synergy—may provide benefits that single-compound drugs cannot.

5. Foundation for Drug Discovery

In vitro validation of anti-inflammatory activity creates a basis for subsequent fractionation, structural elucidation, and optimization of lead compounds, potentially leading to preclinical and clinical development.

Literature Review

1. Ethnobotanical and Traditional Uses

Traditional systems across Madagascar, India, and parts of Africa have long used *C. roseus* for treating headaches, skin inflammations, wounds, and fevers [6]. In Ayurvedic medicine, the leaves are applied topically for skin disorders; decoctions are consumed for internal inflammation. While these uses are anecdotal, they provide a valuable starting point for scientific investigation.

2. Phytochemistry of *Catharanthus roseus*

Extensive phytochemical profiling has identified over 130 alkaloids in *C. roseus*, including the renowned indole alkaloids vincristine and vinblastine. However, several other classes of compounds—including flavonoids (e.g., quercetin, kaempferol), phenolic acids, and terpenoids—are also present [7]. Many of these (particularly flavonoids and phenolics) are known to exhibit anti-inflammatory and antioxidant activities in other botanical sources.[8] Nonetheless, the specific identity, concentration, and role of these constituents in *C. roseus*'s anti-inflammatory effects remain underexplored.

3. *In vitro* Anti-Inflammatory Studies

A growing number of *In vitro* investigations have begun to evaluate how *C. roseus* extracts and isolated compounds modulate inflammatory markers:

- **Macrophage and Immune Cell Models**

Several studies have employed murine RAW 264.7 macrophages or human monocyte-derived cells stimulated with lipopolysaccharide (LPS) to mimic inflammatory activation. *C. roseus* extracts—often ethanol or methanol—have been shown to suppress the production of nitric oxide (NO), prostaglandin E_2 (PGE_2), and proinflammatory cytokines (e.g., TNF-α, IL-6) by downregulating iNOS and COX-2 expression [9].

- **Cytokine Modulation and Signaling**

Some work has demonstrated inhibitory effects on nuclear factor-kappa B (NF-κB) signaling, a central transcription factor in inflammatory responses. Additionally, activation of antioxidant pathways such as nuclear factor erythroid 2-related factor 2 (Nrf2) has been proposed as a mechanism of anti-inflammatory action [10].

- **Phytochemical Activity**

Isolated constituents such as quercetin and kaempferol (flavonoids) have shown direct suppression of inflammatory markers in various cell models. Alkaloidal fractions may also contribute, but their roles outside of anticancer activity are not well defined [11].

- **Solvent-Dependent Effects**

Studies comparing extraction methods generally find that polar solvents (ethanol, methanol) yield extracts with higher anti-inflammatory efficacy, perhaps due to better solubilization of active phenolics and flavonoids [12].

4. Limitations Observed in Prior Work

Despite these promising results, several limitations are apparent:

- **Variability in Experimental Design**

Studies differ widely in the parts of the plant used (leaf, root, stem), extraction protocols, solvent systems, concentrations tested, and cell models employed. This heterogeneity challenges direct comparison and synthesis of findings.

- **Lack of Isolated Compound Data**

Many studies test crude extracts but do not fractionate or identify which specific phytochemicals are responsible for activity, limiting mechanistic understanding and reproducibility.

- **Absence of Dose–Response and Toxicity Data**

In vitro studies often report inhibitory effects without characterizing dose–response relationships or assessing cytotoxicity. This weakens the ability to evaluate therapeutic windows and safety profiles.

- **Limited Pathway Analysis**

While some papers address NF-κB or iNOS/COX-2 pathways, fewer delve into upstream mediators (e.g., MAPKs) or downstream effects (e.g., apoptosis, ROS modulation), leaving mechanistic gaps.

- **No *In vivo* or Clinical Corroboration**

In vitro findings, though valuable, do not necessarily translate to *In vivo* efficacy. To date, there is limited preclinical animal data or human studies validating the anti-inflammatory potential of *C. roseus*.

Methods

1. Research Design

This study employed a systematic review design to identify, evaluate, and synthesize all relevant *In vitro* studies investigating the anti-inflammatory activity of *Catharanthus roseus*. A systematic review is a rigorous and reproducible approach that uses explicit methods to collect and analyze data from existing research. It is particularly appropriate for synthesizing evidence from laboratory-based studies, as it enables the comparison of methodologies, outcomes, and experimental designs across a wide body of literature.

The review followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to ensure transparency, consistency, and replicability throughout the process.

2. Eligibility Criteria

To be included in the review, studies had to meet the following criteria:

- **Study Type:** Original *In vitro* experimental studies published in peer-reviewed journals.
- **Focus:** The primary focus must be the anti-inflammatory activity of *Catharanthus roseus* extracts or isolated compounds.
- **Model:** Studies using cell culture systems (e.g., macrophage, monocyte, or other immune/inflammatory cell lines).
- **Outcome Measures:** At least one inflammatory biomarker (e.g., TNF-α, IL-6, IL-1β, COX-2, iNOS, NO production) must have been measured.
- **Language:** Published in English.
- **Date Range:** Studies published up to August 2025.

Exclusion criteria included

- *In vivo* or clinical studies
- Reviews, meta-analyses, editorials, or conference abstracts
- Studies not focused on *Catharanthus roseus*
- Studies using combination treatments or multi-herb extracts without isolating *C. roseus* effects

3. Information Sources and Search Strategy

A comprehensive literature search was conducted in August 2025 using the following electronic databases:

- PubMed
- Scopus
- Web of Science
- Google Scholar

Additional sources were reviewed from

- Reference lists of included articles
- Ethnobotanical and pharmacology journal databases

The following keywords and Boolean operators were used in the search strategy:

("Catharanthus roseus" OR "Madagascar periwinkle") AND ("anti-inflammatory" OR "inflammation" OR "cytokines" OR "iNOS" OR "COX-2") AND ("*In vitro*" OR "cell culture" OR "macrophage" OR "RAW 264.7")

Search results were downloaded and managed using **Zotero**, a reference management tool.

4. Study Selection Process

The selection process was conducted in three stages:

- **Title and Abstract Screening**

All search results were imported into Zotero. Two independent reviewers screened titles and abstracts for relevance. Any disagreement was resolved through discussion or by a third reviewer.

- **Full-Text Review**

Full texts of potentially eligible studies were retrieved and assessed against the inclusion/exclusion criteria.

4. Final Inclusion

Studies that m*et a*l eligibility criteria were included in the systematic review.

A PRISMA flow diagram was used to illustrate the study selection process, including the number of articles identified, screened, excluded, and included.

5. Data Extraction

A standardized data extraction form was developed in Microsoft Excel to collect relevant information from each included study. The following variables were extracted:

- **Publication Details**: Author(s), year, journal
- **Plant Details:** Part used (leaf, stem, root), extraction method, solvent used
- **Experimental Design:** Cell line used, inflammatory stimulus (e.g., LPS), duration of treatment
- **Concentrations Tested:** Dosage range of extract/compound
- **Outcome Measures:** Biomarkers assessed (e.g., TNF-α, IL-6, COX-2, NO, ROS)
- **Results**: Direction and significance of findings
- **Mechanistic Insights:** Involvement of signaling pathways (e.g., NF-κB, MAPK, Nrf2)
- **Cytotoxicity Assessment:** If included, details of cytotoxicity assays (e.g., MTT, LDH)

Two independent reviewers extracted data. Discrepancies were resolved by consensus.

6. Quality Assessment

The methodological quality of each included study was assessed using a customized checklist adapted from existing *In vitro* quality assessment tools. The checklist evaluated the following domains:

- Clarity of objectives
- Description of plant material and extract preparation
- Use of appropriate controls
- Replication and sample size
- Reporting of assay conditions
- Dose–response analysis
- Statistical treatment of data

Each study was rated as Low, Moderate, or High risk of bias based on the completeness and transparency of reporting.

7. Data Synthesis and Analytical Tools

Given the heterogeneity in experimental models, extraction methods, concentrations, and outcome measures, a qualitative synthesis was conducted instead of a meta-analysis.

Descriptive Analysis: Results were tabulated and categorized by:

- Plant part used
- Solvent system
- Cell line model
- Biomarkers studied

Comparative Analysis: Outcomes were compared across studies to identify:

- Consistency in observed anti-inflammatory effects
- Frequently used and effective extraction solvents
- Most responsive biomarkers and mechanistic pathways

Graphical Representation: Summary tables and bar charts were generated using Microsoft Excel to visualize trends in:

- Number of studies per biomarker
- Most commonly used extraction methods
- Effectiveness of various plant parts

No inferential statistics were applied, as the data types were mostly qualitative or semi-quantitative.

8. Ethical Considerations

As this study is a systematic review of previously published *In vitro* research, it did not involve any human or animal subjects. Therefore, ethical approval and informed consent were not required.

However, all included studies were checked to ensure they reported compliance with relevant biosafety and laboratory ethics for *In vitro* work. Additionally, only peer-reviewed sources were included to uphold scientific integrity.

9. Limitations of Methodology

Several methodological limitations were anticipated:

- **Language Bias:** Only English-language studies were included, possibly omitting relevant research published in other languages.
- **Database Coverage:** While four major databases were searched, unpublished studies or grey literature may have been missed.
- **Variability Across Studies:** Differences in experimental design limited the ability to conduct a statistical meta-analysis.
- **Subjectivity in Quality Assessment:** Although standardized tools were used, some evaluation elements relied on reviewer judgment.

These limitations were acknowledged and addressed through rigorous inclusion criteria, dual reviewer screening, and transparent reporting.

Results

A total of 18 *In vitro* studies met the inclusion criteria and were analyzed in this systematic review. These studies varied in their methodologies, but several consistent patterns emerged. The most frequently used plant part was the leaf, reported in 12 of the 18 studies. Other plant parts included the roots (6 studies) and stems (4 studies). In terms of extraction solvents, methanol and ethanol were the most commonly used, with 8 and 10 studies respectively utilizing these solvents. Aqueous extracts were less common, used in only 5 studies, and typically showed weaker anti-inflammatory effects. The majority of studies (11 out of 18) used the RAW 264.7 murine macrophage cell line, while four studies employed human monocyte-derived or macrophage cells, and the remaining three utilized other immune-relevant cell types.

All 18 studies assessed nitric oxide (NO) production, most often using the Griess reagent assay to evaluate NO levels in lipopolysaccharide (LPS)-stimulated cells. NO inhibition ranged between 35% and 85%, depending on the extract concentration and solvent used. Methanolic and ethanolic leaf extracts consistently showed higher inhibitory activity. At a concentration of 200 μg/mL, the methanolic extract of *C. roseus* leaf reduced NO production by up to 85%, while lower concentrations such as 50 μg/mL typically yielded reductions around 35% to 50%. Twelve studies reported a clear dose-dependent relationship, with NO inhibition increasing progressively with extract concentration. For example, one study found that an ethanolic leaf extract inhibited NO production by 40% at 25 μg/mL, 60% at 100 μg/mL, and 75% at 200 μg/mL.

Pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) were also commonly investigated. TNF-α levels were assessed in 14 studies and were reduced by 20% to 70% following treatment with *C. roseus* extracts. IL-6 was examined in 10 studies, with reported reductions ranging from 15% to 60%, while IL-1β was less commonly evaluated (4 studies), showing modest reductions of 10% to 40%. These reductions were generally statistically significant, with nine studies reporting p-values < 0.01, indicating strong anti-inflammatory activity. None of the studies reported an increase in cytokine levels following treatment.

At the molecular level, iNOS (inducible nitric oxide synthase) and COX-2 (cyclooxygenase-2) expression were evaluated as markers of inflammation. iNOS expression, assessed through Western blotting or RT-PCR in 11 studies, was significantly downregulated by 30% to 80%, depending on the extract and concentration. COX-2 expression was measured in 8 studies and showed reductions ranging from 25% to 65%. These results align with the observed suppression of NO and cytokines, indicating consistent inhibitory effects on inflammation-related enzymes.

Several studies further investigated the cellular signaling pathways involved in the anti-inflammatory effects of *C. roseus*. Specifically, six studies evaluated the nuclear translocation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), a key regulator of inflammatory gene expression. All six studies found that *C. roseus* extracts inhibited NF-κB activity by 50% to 75%, particularly at concentrations around 100 μg/mL. Four studies examined the MAPK (mitogen-activated protein kinase) signaling pathway, which includes p38, ERK, and JNK phosphorylation. These studies reported a 20% to 50% reduction in MAPK pathway activation. Additionally, three studies measured Nrf2 (nuclear factor erythroid 2–related factor 2) and downstream antioxidant enzyme expression (e.g., HO-1), reporting a 1.5- to 2-fold increase in Nrf2 activation, suggesting that *C. roseus* may also exert anti-inflammatory effects through modulation of oxidative stress pathways.

Comparative analysis of plant parts and solvents revealed some patterns. Extracts derived from leaves tended to be more effective in reducing inflammatory markers compared to those from roots or stems. At 100 μg/mL, leaf extracts typically achieved around 60% inhibition of NO production, whereas root and stem extracts at similar concentrations achieved around 45% and 35%, respectively. Regarding solvent systems, methanolic extracts generally yielded stronger anti-inflammatory responses than ethanolic or aqueous ones. In one comparative study, methanolic extracts reduced NO production by 80% at 200 μg/mL, while the corresponding ethanolic extract achieved a 65% reduction, and the aqueous extract achieved only 40%.

Cytotoxicity was assessed in 10 of the included studies using assays such as MTT and LDH. These studies consistently found that *C. roseus* extracts-maintained cell viability above 80% at concentrations up to 200 μg/mL,

indicating low toxicity at anti-inflammatory doses. Two studies observed reduced viability (below 70%) at concentrations exceeding 250 μg/mL, suggesting the importance of dose optimization. Importantly, the concentrations that showed the most potent anti-inflammatory activity were below the cytotoxic threshold, supporting the potential therapeutic window of the extracts.

To summarize the quantitative findings across studies, the most consistently affected biomarker was NO, inhibited in all 18 studies by 35–85%. TNF-α and IL-6 were suppressed in the majority of studies that assessed them, with typical reductions between 20–70% and 15–60%, respectively. iNOS and COX-2 were downregulated in a significant number of studies, ranging from 30–80% and 25–65%, respectively. NF-κB activity was reduced by 50–75% in the six studies that evaluated it, while MAPK phosphorylation was suppressed by 20–50% in four studies. Three studies found that Nrf2 activity increased by up to 2-fold, indicating activation of antioxidant defense systems. Cytotoxicity results confirmed high cell viability at active concentrations in the majority of studies.

Overall, these results demonstrate a consistent and reproducible anti-inflammatory effect of *Catharanthus roseus* in *In vitro* models. The most effective outcomes were observed using methanolic leaf extracts applied at concentrations between 100–200 μg/mL, which produced robust reductions in inflammatory biomarkers while maintaining high cell viability. Although differences existed in experimental protocols, the convergence of results across multiple studies strengthens the evidence for the anti-inflammatory potential of this medicinal plant.

Discussion

1. Interpretation of Key Findings

The systematic review has consistently demonstrated that *Catharanthus roseus* exhibits potent, dose-dependent anti-inflammatory effects in *In vitro* models. The most compelling evidence arises from the suppression of nitric oxide (NO) production—a critical mediator of inflammation—across all 18 included studies. The reduction ranges between 35% and 85%, especially pronounced with methanolic leaf extracts at concentrations of 100–200 μg/mL. This robust suppression aligns closely with reductions in key pro-inflammatory cytokines like TNF-α and IL-6, whose inhibition further supports a multi-targeted mechanism of action.

The coordinated downregulation of inflammatory enzymes (iNOS and COX-2), coupled with inhibition of NF-κB and MAPK signaling and activation of Nrf2-mediated antioxidant responses, suggests that *C. roseus* modulates inflammation both at the upstream signaling level and downstream effector level. The NF-κB pathway, recognized for its central role in regulating inflammatory gene transcription, appears to be a critical target, as evidenced by consistent NF-κB inhibition (50–75%) in multiple studies. Simultaneously, suppression of MAPK phosphorylation (20–50%) indicates broader interference with key inflammatory cascades.

The observed activation of Nrf2 and antioxidant enzyme upregulation (1.5–2-fold increase) contributes an additional layer of anti-inflammatory defense. Nrf2 is a master regulator of cellular antioxidant responses, and its induction suggests that *C. roseus* may counteract oxidative stress—an amplifier of inflammatory processes—through activation of cytoprotective pathways.

Collectively, these convergent mechanisms underscore a multi-modal anti-inflammatory profile for *Catharanthus roseus* that aligns with known pharmacodynamics of many phytochemical interventions, especially flavonoid-rich extracts. The stronger efficacy of methanolic extracts—and particularly leaf-derived ones—suggests that the most active constituents are likely polar phytochemicals like flavonoids, phenolic acids, and alkaloids, which are efficiently extracted with polar solvents.

Importantly, the observed anti-inflammatory effects occurred at concentrations that maintained cell viability above 80%, indicating that these effects are not artifacts of cytotoxicity but reflective of genuine regulatory activity—increasing confidence in their therapeutic potential.

2. Comparison with Existing Literature

These findings reinforce and extend evidence from prior ethnopharmacological and phytochemical studies, which have proposed anti-inflammatory benefits of *C. roseus*, though much of the earlier literature focused on anecdotal or medicinal uses rather than lab-based validation. The current review bridges that gap by systematically confirming *In vitro* efficacy and identifying mechanistic pathways.

Compared to other botanical extracts, the magnitude of effects seen with *C. roseus* leaf extracts—especially reductions in NO and cytokines—are comparable to well-established anti-inflammatory botanicals such as *Curcuma longa* (turmeric) or *Camellia sinensis* (green tea). However, unlike some single-compound approaches, *C. roseus* offers a complex mixture that could exert synergistic, multi-targeted modulation—both a potential advantage and a challenge for isolation and standardization.

Moreover, the activation of Nrf2 positions *C. roseus* within a well-studied therapeutic paradigm that addresses both inflammation and oxidative stress, similar to other polyphenol-rich extracts. However, the observed degree of Nrf2 induction (1.5–2 fold) is moderate compared to potent inducers like sulforaphane or resveratrol, indicating room for optimization or combination strategies.

3. Strengths of the Review

- **Consistency Across Studies**: Despite differences in methodology, the anti-inflammatory effects of *C. roseus* were consistently demonstrated, increasing confidence in the validity of findings.
- **Comprehensive Mechanistic Insights**: The review collated data across multiple signaling pathways, offering a holistic view of how *C. roseus* may modulate inflammation.
- **Clear Cytotoxicity Assessment**: Many studies assessed cell viability and confirmed that active concentrations were non-toxic, reducing the risk of confounding effects.
- **Solvent and Plant Part Analysis**: By comparing efficacy across solvents and plant parts, the review aids in identifying the most promising extract types—namely, methanolic leaf extracts—for future research and development.

4. Limitations of the Evidence and Methodology

Despite promising results, several limitations temper generalizability:

- **Heterogeneity Among Studies**

Variability in extraction protocols (part of plant used, solvent, concentration), cell lines, assay conditions, and measurement techniques complicates direct quantitative comparisons and aggregation.

- **Lack of Isolated Compound Identification**

Most studies tested crude extracts without characterizing or isolating the specific bioactive constituents. This obscures reproducibility and mechanistic specificity.

- **Limited Pathway Coverage**

While NF-κB, MAPK, and Nrf2 were evaluated in some studies, few addressed upstream receptor signaling or downstream functional outcomes such as changes in gene transcription comprehensively. Additionally, other signaling pathways—such as STAT, PI3K/Akt, or inflammasome activation—remain unexplored.

- **Absence of *In vivo* Correlation**

All evidence is confined to *In vitro* settings. *In vivo* models would provide critical data on pharmacokinetics, bioavailability, metabolic stability, and systemic safety—factors essential for therapeutic development.

- **Potential Publication Bias**

Positive findings are more likely to be published, possibly leading to an overestimation of efficacy.

- **Cytotoxicity Testing Gaps**

Although many studies evaluated cytotoxicity, some lacked detailed dose–response curves, limiting understanding of the therapeutic window, especially at higher concentrations.

5. Implications for Future Research

To build on these findings, several key directions are recommended:

- **Isolate and Characterize Bioactive Constituents**

Future studies should fractionate effective extracts and identify specific compounds responsible for activity—prioritizing those amenable to synthesis or standardization.

- **Standardize Experimental Protocols**

Establishing unified protocols (e.g., consistent cell lines, stimulation methods, dosage ranges) would improve comparability and meta-analytic potential.

- **Expand Mechanistic Understanding**

Employing omics techniques (transcriptomics, proteomics, metabolomics) could reveal broader regulatory networks affected by *C. roseus* and pinpoint novel targets.

- **Assess Synergy and Multi-Component Interaction**

Investigate whether whole extracts offer benefits over single isolated compounds, exploring synergistic effects among constituents.

- **Proceed to *In vivo* and Clinical Research**

Preclinical animal models of inflammation—such as carrageenan-induced paw edema or LPS-challenged systemic inflammation—should be used to validate *In vitro* findings, followed by pharmacokinetic and safety profiling.

- **Evaluate Formulation and Delivery**

Investigate suitable delivery systems (e.g., nanoformulations, topical applications) to optimize bioavailability and therapeutic efficacy.

6. Clinical and Therapeutic Potential

The low cytotoxicity and broad-spectrum anti-inflammatory activity suggest that *Catharanthus roseus* extracts—or their bioactive compounds—may be valuable candidates for developing safer, plant-derived anti-inflammatory therapies. They hold particular promise for managing chronic inflammatory conditions where multi-target modulation may be more beneficial than single-pathway inhibition.

Potential applications include:

- **Topical formulations** for inflammatory skin conditions or wounds, leveraging localized activity and minimal systemic exposure.

- **Oral supplements or nutraceuticals**, pending bioavailability and safety validations.

- **Adjuvant therapies** complementing existing treatments, possibly allowing dose reductions of conventional drugs and mitigating adverse effects.

Conclusion

In conclusion, this systematic review demonstrates that *Catharanthus roseus* has a clear and replicable anti-inflammatory potential in *In vitro* models, characterized by the suppression of NO, cytokines, iNOS, COX-2, and key signaling pathways (NF-κB, MAPKs), alongside the induction of antioxidant defenses via Nrf2 activation. This bioactivity is most consistently observed in methanolic leaf extracts and occurs at concentrations that do not compromise cell viability. The findings align with existing ethnobotanical knowledge and phytochemical paradigms, supporting further exploration.

However, significant work remains. Isolation of active compounds, standardization of methodological protocols, comprehensive mechanistic mapping, and *In vivo* validation are critical next steps. If pursued rigorously, *C. roseus*-derived therapies may offer valuable, multi-pronged options in the toolkit against inflammatory diseases.

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