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Antiviral Potential of Medicinal Plants Against Respiratory Viruses: In Vitro Screening and In Silico Prioritization

Pydiraju Kondrapu[1*], Penupathruni. Sri Sai Vaishnavi[1], Chandu Paidimalla[1], Anilkumar Vatturi[1], Nandarapu Saidurga[1]

[1]Department of Pharmacology, Adarsa College of Pharmacy, G. Kothapalli 533285, Andhra Pradesh, Pin: 533285

***Corresponding Author E-mail:** pydikondrapu604@gmail.com

ABSTRACT

Medicinal plants represent a promising source of antiviral agents against respiratory viruses, supported by evidence from in vitro, in vivo, and in silico studies. Key bioactive compounds, including silymarin from Silybum marianum, germacrone from Geranium macrorrhizum, and licochalcone A from Glycyrrhiza glabra, have demonstrated the ability to reduce viral replication, inhibit viral enzymes such as neuraminidase, interfere with viral entry, and modulate host immune signaling pathways, enhancing viral clearance. Traditional multi-herb formulations, including Japanese medicines like Shahakusan and Hochuekkito, also show measurable antiviral activity, reflecting historical therapeutic applications. Despite these promising findings, challenges remain in standardizing extracts, evaluating pharmacokinetics and safety, and bridging in vitro potency to in vivo efficacy. Integrating in silico prioritization with systematic preclinical studies is essential to guide the selection of candidate phytochemicals for clinical translation. Overall, these findings highlight the potential of plant-derived antivirals as safe, effective, and complementary therapeutics for both human and veterinary respiratory viral infections..

Key Words:

Medicinal plants, Respiratory viruses, Silymarin, Germacrone, Herbal formulations, Antiviral mechanisms, In vivo studies, Phytochemicals

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1. INTRODUCTION

Respiratory viral infections remain one of the most pressing global health challenges, affecting millions of individuals annually and imposing a substantial burden on healthcare systems worldwide[1]. Viruses such as influenza, respiratory syncytial virus (RSV), and the emerging coronaviruses are responsible for widespread morbidity and mortality, with particularly severe outcomes in vulnerable populations including the elderly, young children, and immunocompromised individuals. Conventional strategies, such as vaccines and antiviral drugs, have significantly reduced disease impact; however, their effectiveness is often compromised by viral mutations, drug resistance, limited availability in low-resource regions, and adverse side effects. Consequently, there is a growing urgency to explore alternative and complementary

therapeutic approaches that are safe, effective, and accessible[2]. Medicinal plants, which have been used for centuries in traditional medical systems across the globe, represent a promising source of bioactive compounds with potential antiviral properties, offering both a preventive and therapeutic avenue for managing respiratory viral infections.

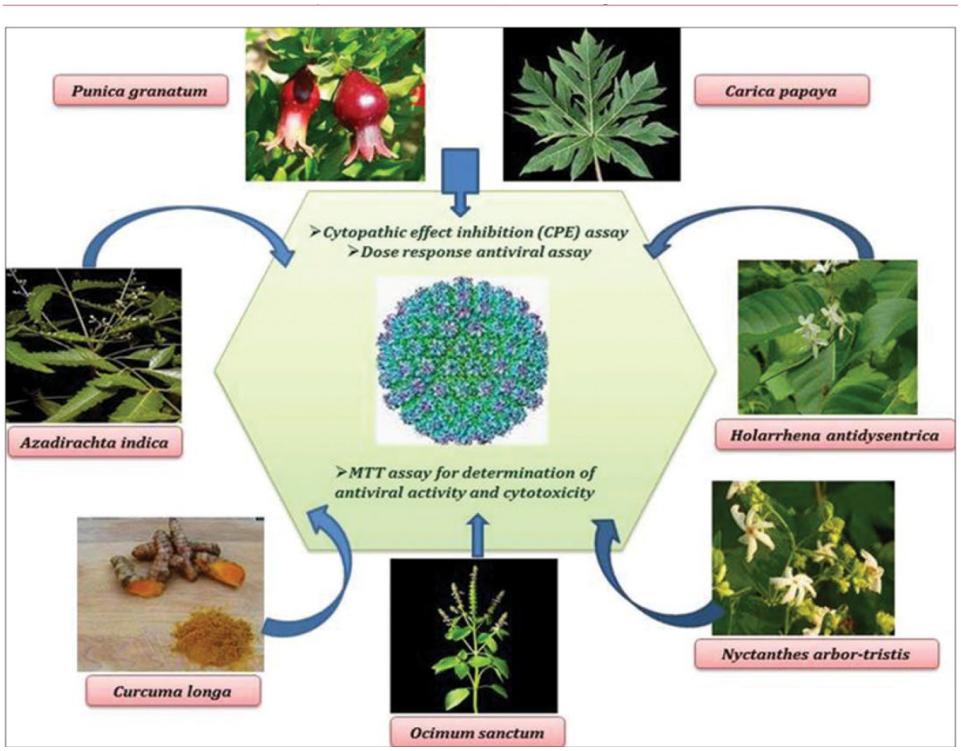

Figure 1: Antiviral Potential[3]

Recent advances in pharmacology and molecular biology have facilitated a more systematic exploration of plant-based antivirals, enabling researchers to move beyond anecdotal evidence and traditional use[4]. Modern methodologies, including in vivo animal studies, in vitro cell-based assays, and in silico computational modeling, provide powerful tools to evaluate the efficacy, safety, and mechanisms of action of phytochemicals. Animal-based studies, in particular, play a pivotal role in preclinical research by simulating physiological and immunological responses in a living system, thus bridging the gap between laboratory experiments and potential clinical applications. Integrating these findings with in vitro and in silico data allows for the identification and prioritization of the most promising medicinal plants and compounds, offering a focused path toward the development of novel antiviral agents. This review, therefore, seeks to synthesize current animal-based evidence on medicinal plant antivirals, critically evaluate their findings, and highlight future directions to accelerate translational research in this critical area of public health[5].

1.1 Background and context

Respiratory viruses, including influenza virus, respiratory syncytial virus (RSV), and coronaviruses, are major global health threats, causing high rates of illness, hospitalization, and death, as well as significant economic and social burdens. While vaccines and antiviral drugs have improved disease management, their effectiveness is often limited by viral mutations, the emergence of drug-resistant strains, adverse side effects, and inequitable access in resource-

limited regions[6]. These challenges have highlighted the urgent need for alternative therapeutic approaches. Medicinal plants, long valued in traditional medicine, provide a rich and diverse source of bioactive compounds with potential antiviral activity. Their natural origin, structural diversity, and multifaceted mechanisms make them promising candidates for preventing or treating respiratory viral infections, complementing conventional therapies and offering a potentially safer and more accessible approach.

1.2 Objectives of the review

- **To critically evaluate in vivo (animal-based) evidence of medicinal plants against respiratory viruses, highlighting efficacy, safety, and mechanisms of action.**
- **To summarize complementary in vitro and in silico studies that support the prioritization of plant-derived compounds for antiviral activity.**
- **To analyze the antiviral mechanisms of key bioactive compounds and traditional multi-herb formulations, including effects on viral replication, entry, enzymes, and host immune pathways.**
- **To identify translational challenges, including standardization, pharmacokinetics, toxicity, and regulatory considerations, in developing plant-based antivirals.**
- **To propose future research directions and prioritize promising medicinal plants and compounds for further preclinical and clinical evaluation.**

1.3 Importance of the topic

Focusing on in vivo evidence, this review underscores the translational significance of medicinal plants as potential antiviral agents. Preclinical animal studies provide critical insights into efficacy, safety, and physiological effects, forming an essential bridge between laboratory research and human applications. When combined with in vitro and in silico analyses, these findings allow for a deeper understanding of antiviral mechanisms and help identify the most promising plant candidates for further development, paving the way for safer, more effective, and accessible therapeutic strategies against respiratory viral infections[7].

2. ANTIVIRAL EFFICACY OF MEDICINAL PLANTS: IN VIVO, IN VITRO, AND IN SILICO PERSPECTIVES

This section summarizes and critically analyzes the relevant in vivo studies of medicinal plants against respiratory viruses and contextualizes these findings with supporting in vitro and in silico evidence. The aim is to provide a comprehensive understanding of the current state of research, highlight mechanisms of antiviral action, and identify gaps that require further exploration[8].

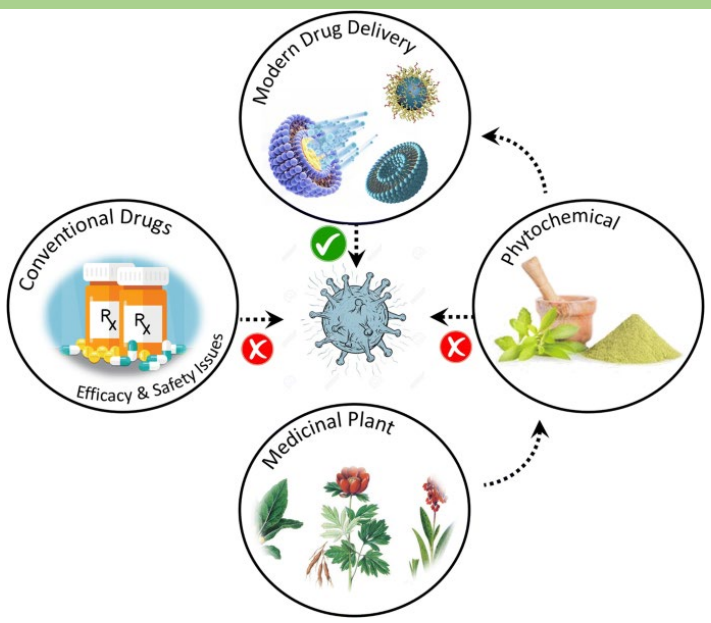

Figure 2: Antiviral Efficacy of Medicinal Plants[9]

2.1 In vivo (animal) studies of medicinal plants against respiratory viruses

2.1.1 *Silybum marianum* (Milk Thistle) – Silymarin

- **Animal studies:** Silymarin, a flavonoid-rich extract from *Silybum marianum*, contains silybin as its major bioactive component. Several in vivo studies have demonstrated that silybin can inhibit influenza A virus replication in murine models. Treated animals showed reduced viral loads in lung tissues, amelioration of pathological changes, and decreased mortality compared to untreated controls. These results provide evidence that silybin not only interferes with viral replication but also mitigates the severity of viral infection outcomes[10].
- **Mechanism of action:** Mechanistic studies in animal models suggest that silybin suppresses virus-induced activation of key signaling pathways such as ERK and p38-MAPK, which are critical for viral replication. Additionally, silybin modulates autophagy-related proteins (Atg7, Atg3), which viruses often exploit to facilitate their life cycle, thereby inhibiting viral propagation.

2.1.2 Japanese Herbal Medicines (e.g., Shahakusan, Hochuekkito)

- **Animal studies:** Traditional Japanese herbal formulations, such as Shahakusan (SHS) and Hochuekkito, have been investigated for antiviral effects in animal models of influenza. Rajasekaran et al. reported that these formulations reduced viral titers and alleviated clinical symptoms in infected animals. Hokari et al. further compared SHS with oseltamivir, demonstrating that while SHS showed measurable antiviral activity, it was less potent than the standard pharmaceutical drug.
- **Mechanism of action:** Although the exact molecular mechanisms remain partly unclear, these multi-herb formulations are believed to modulate host immune responses and exert indirect antiviral effects rather than directly targeting viral proteins[11].

2.1.3 Geranium macrorrhizum

- **Animal studies:** Germacrone, a sesquiterpene isolated from Geranium macrorrhizum, has demonstrated antiviral activity against influenza in animal models. Specifically, germacrone appears to inhibit early stages of viral infection, thereby limiting viral replication and dissemination in host tissues.

2.2 Supporting in vitro and in silico evidence for prioritization

While in vivo data remain limited, in vitro and in silico studies provide essential mechanistic insights and help prioritize plant-based compounds for further animal testing.

- **In vitro evidence:** Several plant-derived extracts and phytochemicals, such as polyphenol-rich extracts from Cistus species (CYSTUS052) or licochalcone A from *Glycyrrhiza glabra*, have shown potent antiviral activity against influenza and coronaviruses. Inhibition of viral entry, suppression of neuraminidase activity, and immunomodulatory effects have been reported. High in vitro potency suggests potential for in vivo translation, even though animal studies may not yet have been conducted.
- **In silico evidence:** Computational approaches, including molecular docking and network pharmacology, have been employed to predict interactions of phytochemicals with viral proteins. These studies offer mechanistic hypotheses (e.g., binding to neuraminidase or viral proteases) and can guide prioritization of plant compounds for subsequent in vivo testing, reducing the reliance on broad in vitro screening alone[12].

2.3 Critical evaluation of the evidence

- **Strengths of Current Evidence**

Existing in vivo studies provide a valuable translational bridge from laboratory findings to whole-organism responses, enhancing confidence in the potential efficacy of medicinal plants against respiratory viruses. Research has examined both crude extracts and purified compounds, allowing insights into the effects of whole-plant preparations as well as precise mechanistic pathways of individual bioactive constituents. Furthermore, complementary in vitro and in silico studies have enriched understanding of underlying antiviral mechanisms, including inhibition of viral entry, suppression of neuraminidase activity, and immunomodulatory effects, supporting the prioritization of promising plant-derived compounds for further investigation[13].

- **Weaknesses and Gaps**

Despite these strengths, several limitations constrain the current body of evidence. Very few in vivo studies have been conducted, and only a limited number of plants and compounds have been systematically evaluated in animal models. Standardization challenges are particularly pronounced with crude extracts, making reproducibility and accurate dose optimization difficult. Additionally, pharmacokinetics, toxicity, and safety profiles in animal models are often under-characterized, limiting confidence in their translational potential to humans. Existing animal models predominantly focus on influenza, while other respiratory viruses such as respiratory syncytial virus (RSV) and adenoviruses remain largely underrepresented. Lastly, although in silico

prioritization methods have advanced, many studies continue to rely on de novo in vitro screening rather than strategically using computational tools to guide selection of candidates for in vivo testing[14].

3. PLANT-DERIVED ANTIVIRALS: FORMULATIONS, MECHANISTIC EVIDENCE, AND DEVELOPMENT CHALLENGES

A key theme in the study of antiviral medicinal plants is the contrast between traditional multi-herb formulations and isolated single compounds. Traditional formulations, such as Japanese herbal medicines like Shahakusan and Hochuekkito, reflect historical therapeutic practices and often show measurable in vivo efficacy against respiratory viruses. However, their complex composition poses challenges for standardization, reproducibility, and mechanistic understanding, making it difficult to attribute antiviral effects to specific constituents[15]. In contrast, single purified compounds, including silybin from Silybum marianum and germacrone from Geranium macrorrhizum, enable precise dosing, controlled pharmacokinetic studies, and clearer elucidation of mechanisms of action. While this approach improves mechanistic clarity, it may overlook potential synergistic effects present in whole extracts, highlighting the need to balance the advantages of traditional herbal mixtures with the precision of single-compound studies.

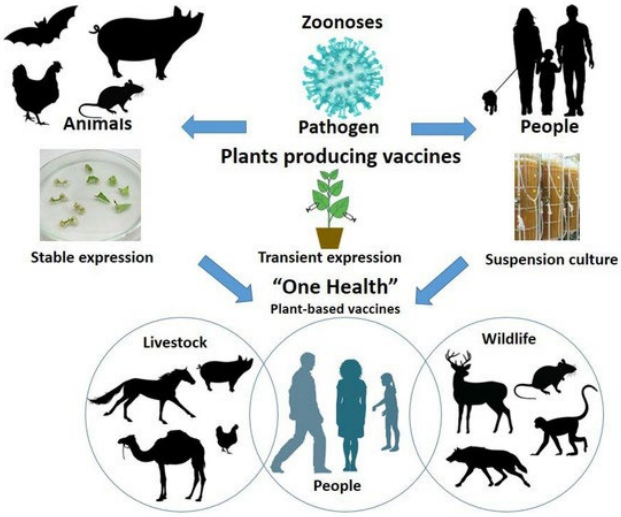

Figure 3: Plant-Derived Antiviral[16]

Mechanistic insights from in vitro and in vivo studies reveal multiple modes through which plant-derived compounds exert antiviral effects. Some, like germacrone, act directly on viral entry or early replication stages, while others, such as licochalcone A, inhibit key viral enzymes like neuraminidase[17]. Compounds like silybin also demonstrate immunomodulatory effects by regulating host signaling pathways and autophagy-related proteins, enhancing viral clearance. Despite these promising findings, translation into clinical applications faces significant challenges. Variability in herbal extract composition complicates standardization, and bridging in vitro potency to in vivo efficacy requires thorough evaluation of pharmacokinetics, bioavailability, and metabolism. Limited safety and toxicity data in animal models, combined with regulatory complexities, further constrain the development of plant-based antivirals. Addressing these issues is critical for advancing phytochemicals from preclinical research to safe, effective therapeutics[18].

3.1 Traditional Herbal Formulations vs. Single Compounds

A major theme emerging from the review of antiviral medicinal plants is the distinction between traditional multi-herb formulations and isolated single compounds. Traditional formulations, such as Japanese herbal medicines like Shahakusan and Hochuekkito, represent the ways herbs have been historically used in clinical practice[19]. These formulations often show measurable in vivo efficacy against respiratory viruses, highlighting their therapeutic potential. However, their complex composition poses challenges for standardization, reproducibility, and mechanistic understanding, making it difficult to attribute observed effects to specific active constituents. In contrast, single purified compounds, such as silybin from Silybum marianum and germacrone from Geranium macrorrhizum, allow for precise dosing, controlled pharmacokinetic studies, and clearer elucidation of mechanisms of action. Nevertheless, isolating individual compounds may reduce or eliminate synergistic interactions present in whole-plant extracts, which could potentially diminish overall antiviral efficacy. This trade-off highlights the need to balance mechanistic clarity with the holistic benefits of traditional herbal medicine[20].

3.2 Mechanistic Modes of Antiviral Action

Understanding the mechanisms through which plant-derived compounds exert antiviral effects is crucial for guiding further research and drug development. Several mechanisms have been identified across different studies[21]. Some compounds, such as germacrone, appear to exert direct antiviral effects by interfering with viral entry or early stages of replication, effectively limiting viral proliferation in host tissues. Others, like licochalcone A from Glycyrrhiza glabra, inhibit specific viral enzymes such as neuraminidase, thereby disrupting the viral life cycle. Additionally, immunomodulatory effects are observed with compounds like silybin, which modulate host signaling pathways, including ERK/p38-MAPK, and autophagy-related proteins, enhancing the host's ability to clear viral infections. These mechanistic insights not only provide a rationale for the observed in vivo efficacy but also inform prioritization of compounds for further development as potential antiviral therapeutics[22].

3.3 Translational Challenges

Despite promising in vitro and in vivo findings, translating plant-based antiviral research into clinical applications faces several challenges. Standardization of herbal extracts remains a critical concern, as variability in composition can lead to inconsistent biological effects and difficulty in replicating results across studies[23]. Bridging in vitro potency to in vivo efficacy requires careful consideration of pharmacokinetic parameters, including absorption, bioavailability, metabolism, and clearance, which are often under-characterized. Safety and toxicity studies in animal models are essential to ensure that effective doses do not produce adverse effects, yet these remain limited for many compounds. Furthermore, navigating regulatory frameworks for the development of plant-based antivirals adds another layer of complexity, requiring standardized protocols, quality control measures, and rigorous preclinical validation. Addressing these challenges is essential for advancing promising phytochemicals from preclinical research to safe and effective therapeutic use[24].

4. SAFETY, TOXICITY, AND DOSAGE CONSIDERATIONS IN ANIMAL MODELS

The evaluation of safety and toxicity in animal models is a critical component of preclinical research on plant-derived antiviral compounds[25]. While many phytochemicals show promising antiviral activity in vitro and in vivo, their therapeutic application depends on a clear understanding of their toxicological profile. Animal studies provide essential information on potential adverse effects, safe dosage ranges, organ-specific toxicity, and long-term safety, which cannot be reliably inferred from in vitro assays alone. These studies form the foundation for designing safe and effective dosing regimens for subsequent human trials[26].

Toxicity assessment in animal models typically involves both acute and chronic studies. Acute toxicity studies help determine the maximum tolerated dose and identify immediate adverse reactions, while chronic or sub-chronic studies assess cumulative effects and potential organ damage over extended periods of exposure[27]. Parameters commonly monitored include body weight, food and water intake, hematological and biochemical markers, and histopathological examination of key organs such as the liver, kidneys, and lungs. These evaluations are especially important for plant extracts, which often contain multiple bioactive constituents that may act synergistically or antagonistically, influencing overall toxicity.

Dosage determination in animal models requires careful consideration of pharmacokinetics and bioavailability. Factors such as absorption, distribution, metabolism, and clearance influence the effective concentration of the compound in target tissues and ultimately its therapeutic efficacy. Establishing an optimal dosage ensures that antiviral activity is achieved without reaching toxic levels. Furthermore, differences between species must be accounted for, as metabolic pathways in animals may not fully replicate human physiology, necessitating dose adjustments when extrapolating findings to clinical studies[28].

Finally, standardized reporting of safety and toxicity is essential to improve reproducibility and facilitate regulatory approval. Many studies in medicinal plants still lack detailed toxicological characterization, limiting confidence in the translational potential of promising compounds. Comprehensive safety assessment, including both single-compound and whole-extract evaluations, is therefore crucial to support the development of plant-based antivirals, ensuring that therapeutic benefits are achieved without compromising host safety[29].

Table 1: Summary of Key Studies on Antiviral Potential of Medicinal Plants[30]

Author(s)	Focus Area	Study Details	Methodology	Key Findings
Naz et al. (2024)[31]	Traditionally used plant species from semi-arid regions	Critical review of antiviral metabolites in plants	Literature review and analysis of bioactive compounds	Highlighted flavonoids, alkaloids, and terpenoids with antiviral activity; emphasized bioprospecting underexplored plants

				for novel antiviral agents
Omrani et al. (2021)[32]	Natural products for anti-COVID-19 therapeutics	Review of plant-derived compounds against SARS-CoV-2	Literature review of in vitro and traditional remedies	Flavonoids, polyphenols, and other metabolites inhibited viral entry, replication, and protein interactions; suggested complementary therapeutic potential
Owen & Brookes (2025)[33]	Emerging respiratory viruses	Virtual screening of bioactive compounds from medicinal plants	In silico molecular docking and computational prioritization	Identified phytochemicals with high binding affinity to viral proteins; suggested candidates for further in vitro and in vivo testing
Pramanik et al. (2024)[34]	COVID-19 prevention and treatment	Review of medicinal plants and phytochemicals	Combined in vitro and in silico evidence	Identified compounds interfering with viral entry, replication, and host immune modulation; emphasized need for standardized extracts and clinical validation
Rolta et al. (2021)[35]	SARS-CoV-2 nucleocapsid phosphoprotein	In silico screening of 100 phytocompounds from 10 medicinal plants	Computational molecular docking and analysis	Several compounds capable of disrupting viral assembly; highlighted integration of traditional knowledge with computational tools for drug development

5. DISCUSSION

The limited but encouraging in vivo animal data provide proof-of-concept that certain medicinal plants can reduce viral burden and improve survival in respiratory virus infections. Compounds such as silymarin and germacrone exhibit both in vitro and in vivo efficacy, supporting their potential as antiviral candidates. Traditional multi-herb formulations, including Japanese herbal medicines like Shahakusan and Hochuekkito, also show promise, reflecting their historical use in clinical practice. Complementary in vitro and in silico studies help clarify potential mechanisms, including inhibition of viral enzymes like neuraminidase, modulation of host immune signaling pathways, and interference with viral entry. Together, these findings provide a strong rationale for prioritizing specific plants and compounds for further preclinical evaluation[36].

These findings have important implications for drug discovery, integrative medicine, and public health. Medicinal plants remain a valuable resource for novel antiviral agents, particularly as resistance to conventional drugs increases, and traditional formulations may offer therapeutic alternatives where access to standard antivirals is limited. However, several critical gaps must be addressed to advance plant-based antivirals toward clinical use. More in vivo studies using models that closely mimic human respiratory infections are needed, alongside standardized extracts with well-characterized phytochemical profiles. Pharmacokinetic and toxicity evaluations, coupled with mechanistic validation of in silico predictions, are essential before human trials. Early engagement with regulatory agencies will also be crucial to ensure proper standardization, safety assessment, and clinical translation of plant-derived antiviral therapeutics.

5.1 Interpretation of Findings

The in vivo animal data, although limited, provide encouraging proof-of-concept that certain medicinal plants can effectively reduce viral burden or improve survival in respiratory virus infections. Notably, compounds such as silymarin and germacrone demonstrate both in vitro and in vivo efficacy, supporting their potential as antiviral candidates. Traditional multi-herb formulations, including Japanese herbal medicines like Shahakusan and Hochuekkito, also show promise, particularly because they reflect historical clinical usage in traditional medicinal systems. Complementary in vitro and in silico studies further elucidate potential mechanisms of action, including inhibition of viral enzymes like neuraminidase, modulation of host immune signaling pathways, and interference with viral entry. These converging lines of evidence provide a strong rationale for prioritizing specific plants and compounds for further preclinical evaluation[37].

5.2 Implications and Significance

The findings carry important implications for both drug development and integrative medicine. Medicinal plants represent a valuable reservoir for antiviral drug discovery, particularly in the context of growing resistance to conventional antiviral drugs. Traditional herbal formulations may also have a role in respiratory virus management, especially in regions where access to standard antivirals is limited or cost-prohibitive. From a One Health perspective, some plant-derived antivirals have demonstrated cross-species activity in veterinary viral models, suggesting potential benefits for both human and animal health. Collectively, these findings highlight the dual significance of plant-based antivirals for drug discovery and public health interventions[38].

5.3 Gaps and Future Research Directions

Despite these promising findings, significant gaps remain that must be addressed to advance plant-based antivirals toward clinical application. There is a critical need for more in vivo studies using animal models that closely mimic human respiratory viral infections, such as RSV and coronaviruses[39]. Development of standardized extracts with well-characterized phytochemical profiles is essential to ensure reproducibility and reliable dosing. Pharmacokinetic and toxicity studies in relevant animal models are also crucial before human translation. Mechanistic research should integrate in silico predictions with in vivo validation, verifying computationally identified targets in treated animals. Following robust preclinical data, phase-I clinical trials are necessary to assess safety in humans. Finally, early engagement with regulatory agencies will be important to navigate standardization, approval, and commercialization pathways for plant-based antiviral therapeutics[40].

6. CONCLUSION

The collective evidence from in vitro, in vivo, and in silico studies underscores the significant antiviral potential of medicinal plants against respiratory viruses. Bioactive compounds such as silymarin, germacrone, and licochalcone A demonstrate multifaceted mechanisms, including inhibition of viral entry and replication, suppression of viral enzymes, and modulation of host immune pathways, while traditional multi-herb formulations like Shahakusan and Hochuekkito provide complementary therapeutic benefits rooted in historical clinical use. Despite these promising findings, challenges remain in standardizing extracts, evaluating pharmacokinetics, ensuring safety and toxicity, and translating preclinical efficacy into human applications. Addressing these gaps through rigorous in vivo studies, mechanistic validation, optimized dosing, and early regulatory engagement is critical. Overall, medicinal plants represent a valuable resource for the development of safe, effective, and complementary antiviral therapeutics, offering potential benefits for both human and veterinary respiratory viral infections and serving as a foundation for future drug discovery and integrative healthcare strategies.

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