Review Article

Human Metapneumovirus: Antiviral Potential of Traditional Plant Species

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ABSTRACT

Human metapneumovirus (HMPV) has emerged as a significant respiratory pathogen affecting vulnerable populations including children under five years, elderly individuals, and immunocompromised patients [1][2]. Despite its clinical importance, no specific antiviral treatment or preventive vaccine currently exists, creating urgent demand for alternative therapeutic strategies [3]. This review comprehensively examines the antiviral properties of four traditional medicinal plants: Stypopodium zonale (brown seaweed), Ginkgo biloba (maidenhair tree), Zanthoxylum bungeanum (Sichuan pepper), and Cryptonemia seminervis (red seaweed). Each plant species contains distinct bioactive compounds demonstrating inhibitory effects against HMPV replication through multiple mechanisms including virucidal activity, viral entry interference, and penetration prevention [4][5]. S. zonale meroditerpenoids (epitaondiol and atomaric acid) achieve >99.99% HMPV inhibition, while ginkgolic acid from G. biloba specifically targets early viral entry stages [6][7]. Zanthoxylum bungeanum's γ-Fagarine reduces viral loads by 67% in Vero-E6 cells, and Cryptonemia seminervis sulfated galactans inhibit viral attachment and cellular receptor binding [8][9]. This review synthesizes current knowledge on HMPV biology, epidemiology, and molecular mechanisms of plant-derived antiviral compounds, emphasizing the potential of phytochemistry-based approaches in viral disease management and the necessity for continued translational research toward clinical application.

INTRODUCTION

1.1 Respiratory Viral Infections and Global Health Burden

Respiratory tract infections represent one of the most significant threats to public health globally, contributing to substantial morbidity, mortality, and economic burden [10]. The respiratory system faces persistent challenges from numerous viral

pathogens, including influenza virus, respiratory syncytial virus (RSV), rhinovirus, adenovirus, and the recently recognized human metapneumovirus [11]. These viruses collectively cause an estimated 33 million cases of acute lower respiratory tract infections annually, resulting in significant hospitalization rates and mortality, particularly in vulnerable populations [2].

1.2 Classification and Emergence of Human Metapneumovirus

Human metapneumovirus, taxonomically classified as a member of the family Pneumoviridae, genus Metapneumovirus, species Metapneumovirus hominis, was first identified in 2001 by Dutch researchers during retrospective analysis of respiratory samples from children [12]. Although newly identified, serological evidence indicates HMPV has circulated in human populations for at least 50-60 years, suggesting it represents a recently recognized rather than newly emerged pathogen [1]. Phylogenetic analysis has identified HMPV into four primary genotypes (A1, A2, B1, B2) with recent emergence of variant strains featuring G gene duplications (A2b, A2c), which demonstrate enhanced transmissibility compared to historical strains [3].

1.3 Clinical Significance and Epidemiological Impact

HMPV infections manifest as a spectrum of respiratory illnesses ranging from mild upper respiratory tract infections to severe lower respiratory tract disease including bronchiolitis, pneumonia, and acute respiratory distress syndrome [13]. Recent epidemiological data from the Chinese Center for Disease Control and Prevention reported HMPV accounted for 6.2% of positive respiratory illness test results in December 2024, surpassing COVID-19, rhinovirus, and adenovirus in prevalence [2]. In 2018, HMPV was associated with approximately 11.1 million acute lower respiratory tract infections in children under five years, resulting in 502,000 hospitalizations and 11,300 deaths, underscoring the substantial pediatric disease burden [14].

1.4 Therapeutic Challenges and Current Treatment Limitations

Currently, no specific antiviral agents or effective vaccines exist for HMPV prevention or treatment, with clinical management remaining largely supportive and focused on symptom management [3][15]. Ribavirin, while showing in vitro activity against HMPV comparable to RSV, demonstrates limited clinical efficacy and remains controversial, with no rigorous human clinical trials establishing therapeutic benefit [6]. This therapeutic vacuum necessitates exploration of alternative treatment strategies, including investigation of natural products derived from traditional medicinal systems.

1.5 Objectives of the Present Review

This comprehensive review examines the antiviral potential of four medicinal plant species against HMPV through systematic analysis of: (1) HMPV molecular virology and replication mechanisms; (2) epidemiological patterns and clinical manifestations; (3) phytochemical composition and bioactive compounds from selected plant species; (4) in vitro and in vivo evidence of antiviral efficacy; and (5) proposed mechanisms of viral inhibition by plant-derived compounds. The integration of traditional botanical knowledge with contemporary molecular virology aims to identify promising candidates for therapeutic development and clinical translation.

2. HMPV: Molecular Virology and Replication

2.1 Viral Genome Organization and Structural Proteins

Human metapneumovirus possesses a negative-sense, single-stranded RNA genome of approximately 13.2 kilobases, organized in 3′-to-5′ polarity, encoding eight genes that specify nine distinct proteins [16]. The genomic order comprises 3′-N-P-M-F-M2-SH-G-L-5′, where each gene encodes specific viral proteins essential for replication and pathogenesis. Unlike most Pneumoviridae members, the M2 gene encodes two proteins (M2-1 and M2-2) through overlapping open reading frames, representing a unique genomic feature [1].

Structural proteins include the fusion glycoprotein (F), attachment glycoprotein (G), small hydrophobic protein (SH), and matrix protein (M), which mediate viral entry, assembly, and immune evasion [17]. The F protein, produced as a fusogenically inactive precursor (F0), undergoes proteolytic cleavage by cellular proteases including transmembrane protease serine subtype 2 (TMPRSS2) to generate the active disulfide-linked F1-F2 heterodimer [16]. This F protein remains highly conserved, immunogenic, and essential for viral membrane fusion, representing a critical target for therapeutic and vaccine development strategies.

2.2 Non-Structural and Replication Proteins

Non-structural proteins (N, P, M2-1, M2-2, L) function in nucleocapsid protection, replication complex regulation, and immune antagonism [17]. The nucleoprotein (N) encapsidates viral RNA forming the ribonucleoprotein complex, simultaneously protecting genomic RNA from pattern recognition receptors including RIG-I and MDA5[18]. The phosphoprotein (P) serves as a cofactor stabilizing the viral RNA-dependent RNA polymerase (L protein), facilitating ribonucleoprotein formation during replication [1].

M2-1 functions as a putative transcription factor enhancing mRNA synthesis while reducing TLR and RIG-I activation, whereas M2-2 regulates the transcription-replication switch controlling viral RNA production dynamics [19]. The L polymerase catalyzes both RNA-dependent RNA synthesis and genome replication while limiting double-stranded RNA intermediate accumulation, reducing immunological detection.

2.3 Viral Replication Cycle

HMPV replication occurs predominantly in the cytoplasm of respiratory epithelial cells, initiating with attachment through glycoprotein-mediated binding to cellular glycosaminoglycans [20]. Following attachment, the F protein facilitates membrane fusion via integrin αvβ1-RGD motif interaction at neutral or low pH, permitting ribonucleoprotein release into the cytoplasm [16]. The viral polymerase complex, assembled from N, P, and L proteins, then transcribes negative-sense viral RNA into mRNA for translation of viral proteins essential for replication.

Genome replication proceeds through positive-sense RNA intermediate synthesis, followed by negative-sense genomic RNA production to create progeny ribonucleoproteins [21]. Assembly involves nucleoprotein-encapsidated genomes complexing with matrix protein beneath the host plasma membrane where glycoproteins F, G, and SH are incorporated [22]. Virion release occurs through ESCRT-mediated budding, permitting efficient escape while maintaining host cell structural integrity to maximize viral spread.

3. Epidemiology and Clinical Manifestations

3.1 Global Epidemiological Patterns

HMPV demonstrates worldwide distribution with seasonal predominance in temperate climates during late winter and spring, synchronous with RSV and influenza circulation [11]. Prevalence increases with distance from the equator, reflecting seasonal respiratory virus dynamics in northern latitudes. Since its initial discovery in the Netherlands in 2001, HMPV has been documented across all inhabited continents including North America, Europe, Australia, and Asia, establishing its global presence [23].

Research conducted at the Vanderbilt Vaccine Clinic reported HMPV accounted for 12% of acute respiratory illness in healthy children at United States outpatient clinics, with 15% and 8% prevalence in hospitalized community-acquired pneumonia cases in children below and above five years respectively (2010-2012) [24]. Serological evidence indicates HMPV infection becomes nearly universal by age five, with nearly all children experiencing at least one infection during childhood, establishing HMPV as a ubiquitous childhood pathogen.

3.2 Population-Specific Disease Burden

Pediatric populations experience disproportionate HMPV disease burden, with highest incidence in children under five years [25]. Approximately 25% of HMPV infections occur in infants younger than six months, while 49% manifest in six-month to one-year-old infants, establishing infancy as the period of maximum vulnerability [14]. Elderly individuals aged 65 and older represent a secondary population at substantially elevated risk for severe disease progression, hospitalization, and mortality following HMPV infection [23].

Immunocompromised individuals, including transplant recipients, HIV-positive patients, and those receiving immunosuppressive therapy, experience particularly severe disease manifestations including prolonged viral shedding, secondary bacterial superinfection, and multi-organ dysfunction [26]. The male-to-female ratio in HMPV lower respiratory tract infection approximates 1.8:1, with average patient age of 11.6 months and median age of 6.5 months among pediatric cases [14].

3.3 Clinical Presentations and Respiratory Manifestations

Symptomatic HMPV infection typically manifests 3-6 days following viral exposure with common presentations including fever, cough, rhinorrhea or nasal congestion, and wheezing or other respiratory abnormalities [27]. Less frequent manifestations include sore throat, voice changes, and transient rash, with some infected individuals remaining asymptomatic despite documented viral infection [28].

Severe manifestations include bronchiolitis (particularly affecting children under two years), viral pneumonia with potential respiratory failure in infants under one year, and hypoxemia requiring supplemental oxygen or mechanical ventilation [13]. Post-invasion complications in elderly populations include ARDS, chronic respiratory impairment simulating COPD exacerbation, and ICU admission requirements [25]. Neurological complications including febrile seizures and encephalitis have been reported in children, reflecting potential CNS involvement during systemic infection.

4. Traditional Medicinal Approaches and Phytochemistry

4.1 Historical Context of Plant-Based Antiviral Therapy

Nature has provided medicinal plants for disease prevention and health maintenance throughout

recorded history, with botanical remedies comprising a cornerstone of traditional medicine systems across diverse cultures [29]. Recent literature identifies approximately 9,000 plant species recognized globally for medicinal properties, with extensive research documenting antiviral, antimicrobial, antifungal, and immunomodulatory activities [30]. The integration of traditional botanical knowledge with contemporary molecular pharmacology represents a promising approach for identifying novel antivirals, particularly given the therapeutic vacuum for emerging viral pathogens.

4.2 Rationale for Marine Pharmacognosy

Marine pharmacognosy, representing the investigation and identification of medically important marine organisms, has expanded recognition of the sea's extraordinary therapeutic potential. Approximately 79% of Earth's surface is covered by water, yet marine biodiversity remains substantially underexplored, offering vast undiscovered resources for pharmaceutical development. The 2004 FDA approval of ziconotide from marine cone snail venom pioneered marine-derived therapeutics, establishing feasibility of translating marine compounds into clinical agents [29].

Marine algae represent particularly promising sources of bioactive compounds, producing complex polysaccharides, terpenoids, and other metabolites that exhibit antiviral properties. The distinct evolutionary pressures and unique chemical environments of marine ecosystems have generated secondary metabolites with structures and activities rarely encountered in terrestrial plants, suggesting enhanced potential for novel antiviral mechanisms.

5. Stypopodium zonale: Marine Brown Algae with Anti-HMPV Activity

5.1 Taxonomy, Distribution, and Morphology

Stypopodium zonale (Dictyotaceae family, class Phaeophyceae) represents a brown marine alga distributed throughout tropical and subtropical waters including the Caribbean, Western Atlantic, Indian Ocean, and Bay of Bengal [4]. The thallus exhibits characteristic greenish-brown coloration, fan-shaped (flabellate) morphology, measuring 5-30 cm in length with distinctive surface zonation evident in 1-6 cm spaced bands. The organism attaches via a compact, rhizomatous holdfast (1-5 mm length) and reaches depths of 20-30 meters in Caribbean environments and up to 55 meters in depth ranges.

5.2 Chemical Composition and Meroditerpenoid Isolation

Stypopodium zonale produces diverse secondary metabolites encompassing meroditerpenoids, diterpenes, and phlorotannins with pronounced biochemical variability depending on collection locality. Major compounds identified include atomaric acid and epitaondiol (meroditerpenoids), stypoldione and stypotriol (related diterpenes), and variable levels of phlorotannins functioning in defense against herbivory [25]. These metabolites demonstrate antiviral, antitumoral, and insecticidal properties with significant chemovariability across geographic populations.

5.3 In Vitro Anti-HMPV Activity: Virucidal Properties

Reverse transcription-polymerase chain reaction (RT-PCR) analysis of S. zonale extracts and isolated meroditerpenoids revealed dose-dependent HMPV inhibition with remarkably potent efficacy [27]. The crude S. zonale extract

demonstrated 97.7% viral RNA inhibition (ED50 = 1.48 µg/mL), while atomaric acid achieved 99.4% inhibition (ED50 = 3.52 µg/mL), and epitaondiol demonstrated >99.99% inhibition (ED50 = 1.01 µg/mL) [6][27]. The methyl ester derivative of atomaric acid showed 99.4% inhibition with ED50 value of 2.66 µg/mL [27].

Virucidal activity assays established that epitaondiol achieved 99.27% inhibition when directly incubated with viral suspensions at 37°C for two hours, while parent atomaric acid demonstrated 99.9% inhibition, exceeding the precursor compound's 61.2% activity [27]. These results indicate meroditerpenoid compounds irreversibly inactivate extracellular HMPV particles independent of cellular infection mechanisms.

5.4 Penetration and Post-Entry Mechanisms

Penetration assays demonstrated that epitaondiol and parent atomaric acid effectively inhibited viral particle entry into LLC-MK2 cells, with atomaric acid achieving 51.4% inhibition of penetration [28]. S. zonale extract and atomaric acid exhibited post-penetration stage activity, suggesting inhibitory effects on early intracellular viral replication stages following successful cell entry [29]. These findings establish that meroditerpenoid action extends beyond virucidal effects to encompass multiple stages of viral replication cycle including attachment, penetration, and post-entry processes.

6. Ginkgo biloba: Flavonoid-Rich Antiviral Agent

6.1 Taxonomy, Distribution, and Botanical Characteristics

Ginkgo biloba (Ginkgoaceae family, order Ginkgoales) represents a living fossil species native to China and Japan, now cultivated globally for ornamental and medicinal purposes. The deciduous gymnosperm exhibits characteristic bilobed (hence "biloba") fan-shaped leaves with open dichotomous venation patterns, unique among seed plants. Trees attain heights of 20-35 meters with pyramidal or columnar morphology, deep root systems providing wind resistance, and documented lifespans exceeding 3,500 years in some specimens.

6.2 Phytochemical Composition

Ginkgo biloba leaves contain approximately 110 identified flavonoids representing seven distinct structural classes including flavanones, isoflavones, flavones, biflavones (sciadopitysin, ginkgetin, isoginkgetin, amentoflavone, bilobetin), flavan-3-ols, and flavonols (kaempferol, quercetin, isorhamnetin). Additionally, ginkgolides (diterpenoid lactones Q, P, N, M, L, K, J, C, B, A) and bilobalides represent unique nor-terpenoid compounds exclusive to Ginkgo species. Alkylphenols, carboxylic acids (ferulic acid, caffeic acid, gallic acid), lignans (pinoresinol), proanthocyanidins (prodelphinidin, procyanidin), and ginkgolic acids constitute additional bioactive constituents.

6.3 Ginkgolic Acid: Mechanism of Anti-HMPV Activity

Recent molecular investigations by Columbia University researchers identified ginkgolic acid (GA), a major alkylphenolic compound from G. biloba, as a potent HMPV inhibitor [7]. Fluorescence microscopy analysis using HMPV-GFP virus demonstrated dose-dependent suppression of viral infection in both A549 and Vero E6 cell lines when pre-incubated with ginkgolic acid prior to viral challenge. Flow cytometry analysis established ginkgolic acid's

IC50 values in both cell culture systems, enabling quantitative assessment of antiviral potency.

Mechanistic studies revealed ginkgolic acid exerts antiviral effects specifically during early viral life cycle stages, interfering with viral entry and attachment processes without affecting post-entry events including viral gene transcription, genome replication, virion assembly, or particle release [7]. This selective targeting of viral entry mechanisms distinguishes ginkgolic acid from other antiviral agents, potentially reducing cellular toxicity while maintaining antiviral efficacy.

7. Zanthoxylum bungeanum: Alkaloid-Mediated Antiviral Effects

7.1 Taxonomy, Distribution, and Botanical Properties

Zanthoxylum bungeanum Maxim (Rutaceae family, order Sapindales), commonly designated Sichuan pepper or Chinese prickly ash, represents a deciduous shrub distributed throughout East and Southeast Asia including China, Japan, Korea, Vietnam, Myanmar, India, and Nepal. The plant exhibits characteristic prickled stems (3-8 cm diameter) with branched woody architecture, reaching 3-7 meters height, with small globular fruits measuring 4-5 mm diameter displaying distinctive warty oil dots and purplish-red coloration.

7.2 Chemical Composition and Compound Diversity

Approximately 198 distinct compounds have been isolated and characterized from Z. bungeanum, encompassing volatile oils, amide compounds (sanshools), flavonoids (>40 types including quercetin, rutin), alkaloids (quinoline, isoquinoline, benzophenanthridine derivatives including γ-Fagarine, kappa fagarine, dictamnine, nitidine), coumarins (bergapten, herniarin), lignans (asarinin, eudesmin, sesamin), and phenylpropanoids. This remarkable chemical complexity reflects the plant's ecological and medicinal significance in traditional Asian medicine systems.

7.3 γ-Fagarine: Potent HMPV Inhibitor with Cellular Selectivity

γ-Fagarine, a benzophenanthridine alkaloid isolated from Z. bungeanum roots, has emerged as a particularly promising HMPV inhibitor demonstrating cellular selectivity and measurable efficacy in both in vitro and in vivo experimental systems [8]. Reverse transcription quantitative PCR (RT-qPCR) analysis of HMPV F gene copies demonstrated dose-dependent viral inhibition with increasing γ-Fagarine concentration in both Vero-E6 and 16HBE cell cultures [8]. At maximum tested concentration (200 µM), γ-Fagarine achieved 67% HMPV F gene copy reduction in Vero-E6 cells and 55% reduction in 16HBE cells without apparent cytotoxic effects.

Viral replication kinetics analysis revealed no apparent inhibitory effect during the initial 6 hours of infection, yet significant suppression ($p < 0.001$) emerged at 12, 24, and 48 hours post-infection, suggesting γ-Fagarine interferes with early to mid-stage viral replication processes. This temporal pattern indicates the compound requires intracellular accumulation and/or processing to exert antiviral effects, distinguishing it from purely virucidal agents.

7.4 In Vivo Efficacy in Murine HMPV Infection Model

Intranasal HMPV inoculation in mouse models followed by oral γ-Fagarine administration (25 mg/kg daily) demonstrated reduced viral titers in lung tissues compared to untreated HMPV-

infected controls (p<0.05). Weight loss observed in HMPV-infected animals reached 83% of baseline weight, whereas γ-Fagarine-treated animals exhibited significantly attenuated weight loss, indicating reduced systemic viral burden and improved physiological status. These in vivo results establish γ-Fagarine's therapeutic potential beyond cellular culture systems, supporting translational development toward clinical trials.

8. Cryptonemia seminervis: Sulfated Polysaccharide Antivirals

8.1 Taxonomy, Distribution, and Morphological Features

Cryptonemia seminervis (Halymeniaceae family, order Halymeniales, class Florideophyceae) represents a red marine alga endemic to Indo-Pacific regions including Southeast Asia, Australia, Indian Ocean territories, and associated waters. The membranous thallus exhibits characteristic flat, irregularly lobed morphology, measuring 10-15 cm in length and 1-2 cm width, with reddish-brown to purplish-red coloration and attachment via small holdfast structures. Anatomically, cells are compactly arranged with single stellate chloroplasts containing pyrenoids, organized in pseudo-parenchymatous filamentous tissue.

8.2 Galactan Polysaccharide Composition

Cryptonemia seminervis produces a family of d, l-hybrid galactans with characteristic 3-linked β-d-galactopyranosyl→4-linked α-d- and α-l-galactopyranosyl alternating sequences [9]. Major constituents include α-d- and α-l-galactose as primary polymer backbone components, 3,6-anhydro-d- and l-galactose as significant secondary components, 3,6-anhydro-2-O-methyl-l-galactose as lesser constituents, and 2-O-methyl-, 4-O-methyl-, and 6-O-methylgalactoses as minor modifications. Sulfate groups represent major substituent groups, while pyruvic acid ketals and glycosyl side-chains including 4-O-methyl galactopyranosyl and xylosyl moieties provide additional structural complexity [9].

8.3 Multiple Anti-HMPV Mechanisms: Virucidal to Receptor Interference

Comprehensive analysis of C. seminervis sulfated galactan fractions identified multiple distinct anti-HMPV mechanisms operating at different viral replication stages [9]. Virucidal activity assays demonstrated that S, DS, DS-1, and DS-3 fractions exhibited 67.9%, 56.9%, 99.1%, and 99.9% viral inactivation respectively, while DS-2e fraction showed no direct virucidal activity. This finding established that sulfated polysaccharides can physically inactivate extracellular viral particles independent of cellular processes.

Cell receptor interference assays demonstrated that DS-2e and S fractions produced 68.4% and 16.6% inhibition of HMPV replication respectively by preventing viral adsorption to LLC-MK2 cell surfaces. The retention of inhibitory activity following removal of polysaccharides prior to infection established that these fractions shield or interact with cellular receptors, preventing subsequent viral attachment. Penetration assays revealed that only the depolymerized DS-3 fraction inhibited viral penetration (72.7% inhibition), demonstrating that structural depolymerization generates compounds with novel penetration-blocking properties.

8.4 Therapeutic Applications for Respiratory Disease

The protective mechanisms of C. seminervis sulfated galactans against HMPV extend potential applications beyond viral infection prevention to encompass treatment of HMPV-associated

respiratory disease manifestations [9]. By interfering with viral attachment and replication, these polysaccharides may reduce airway inflammation driven by direct viral cytopathology and associated inflammatory cascade activation. Antioxidant properties inherent to the polysaccharide structure may further mitigate reactive oxygen species-associated airway damage characteristic of severe HMPV infection.

9. Comparative Analysis of Plant-Based Antivirals

9.1 Relative Potency and Efficacy Comparison

The four medicinal plant species examined demonstrate varying efficacy profiles against HMPV, with distinctions in potency, mechanism specificity, and toxicological profiles relevant to therapeutic development. Stypopodium zonale meroditerpenoids achieve the highest absolute viral inhibition rates (>99.99% for epitaondiol) with lowest ED50 values (1.01 µg/mL for epitaondiol) [27], establishing unparalleled virucidal potency [6]. However, high lipophilicity and demonstrated cytotoxicity limit in vivo application potential [28].

Ginkgo biloba ginkgolic acid demonstrates selective early-stage viral entry inhibition through a distinct mechanism pathway without late-stage viral process interference [7], potentially permitting development of treatment strategies with reduced host cell toxicity. This mechanism selectivity represents an important distinction from non-selective antivirals, as it preserves cellular antiviral defense mechanisms while specifically blocking HMPV entry.

Zanthoxylum bungeanum γ-Fagarine demonstrates moderate in vitro potency (55-67% F gene inhibition) with excellent cellular tolerance, establishing feasibility for in vivo application with established murine efficacy ($p < 0.05$ viral load reduction) [8]. The moderate potency combined with proven in vivo efficacy without apparent toxicity identifies Z. bungeanum as a particularly promising candidate for clinical development.

Cryptonemia seminervis sulfated galactans exhibit variable potency depending on structural fraction (56.9-99.9% virucidal activity) with multiple complementary mechanisms including virucidal, receptor interference, and penetration inhibition [9]. The multi-mechanism approach and reduced expected cytotoxicity from polysaccharide structure establish C. seminervis as a secondary promising candidate for therapeutic development.

9.2 Mechanism Diversity and Therapeutic Implications

The four plant species target distinct viral replication stages, suggesting potential for combination therapy approaches exploiting multiple mechanism pathways. Stypopodium zonale affects virucidal, penetration, and post-entry stages; Ginkgo biloba targets early entry mechanisms; Zanthoxylum bungeanum interferes with mid-stage replication; and Cryptonemia seminervis blocks attachment and penetration stages [4][7][8][9]. This mechanistic diversity suggests additive or synergistic effects from combined administration, potentially enabling therapeutic efficacy enhancement while permitting dose reduction and toxicity mitigation.

9.3 Translational Development Priorities

Evidence synthesis prioritizes Zanthoxylum bungeanum and Cryptonemia seminervis for immediate clinical translation given demonstrated in vivo efficacy, established safety profiles, reduced apparent cytotoxicity, and feasibility for pharmaceutical formulation [8][9]. Ginkgo biloba

represents a secondary priority given its commercial availability as nutritional supplement, established regulatory history, and selective mechanism profile permitting mechanistic investigation [7]. Stypopodium zonale warrants continued research for structure-activity relationship studies and toxicity mitigation strategies given its extraordinary viral inhibition potency [6][27].

10. Future Directions and Research Imperatives

10.1 Preclinical Development Pathway

Immediate research priorities encompass standardization of plant extract composition and bioactive compound isolation to establish consistent pharmaceutical formulations [3]. High-performance liquid chromatography (HPLC) and mass spectrometry should quantify meroditerpenoid, alkaloid, and polysaccharide content across cultivars, seasons, and geographic sources to identify optimal material for therapeutic development [29].

Structure-activity relationship studies should synthesize or isolate compound analogs with enhanced potency, selectivity, and reduced cytotoxicity. Cell culture systems examining dose-response relationships across therapeutic-relevant concentration ranges will establish efficacy profiles and identify optimal therapeutic windows. Primary respiratory epithelial cells and three-dimensional organoid cultures should replace immortalized cell lines to establish physiological relevance [2].

10.2 In Vivo Efficacy Models and Safety Evaluation

Animal models including mice, guinea pigs, and non-human primates should establish dose-response relationships, pharmacokinetic parameters, and biodistribution characteristics following oral, intranasal, or parenteral administration. Particularly, intranasal administration mimics natural infection route, permitting direct assessment of respiratory tract bioavailability and local therapeutic delivery. Histological examination should quantify pulmonary inflammation, mucus production, epithelial damage, and immune cell infiltration following HMPV challenge in treated versus control animals [25].

Comprehensive toxicology studies must establish maximum tolerated doses, identify dose-limiting toxicities, characterize organ system effects, and establish safety margins between therapeutic and toxic dose ranges. Genotoxicity, reproductive toxicity, and immunotoxicity should receive particular attention given intended vulnerable population targets.

10.3 Clinical Translation and Regulatory Pathways

Following successful preclinical studies, phase I clinical trials should establish maximum tolerated dose, pharmacokinetic parameters, and preliminary safety in healthy human volunteers. Phase II trials in HMPV-infected patients should evaluate efficacy endpoints including viral load reduction, symptom amelioration, hospitalization prevention, and complications reduction. Respiratory syncytial virus co-infection should receive particular attention given frequent HMPV-RSV co-infection patterns [14].

Registration pathways may include investigational new drug (IND) applications for novel synthetic derivatives or botanical drug development pathways for plant extracts, dependent on regulatory strategy and commercial development plans [30]. Pediatric formulation development

should address specific challenges in young children including palatability, appropriate dosing, and safety margins.

CONCLUSION

This comprehensive review establishes that four traditional medicinal plant species Stypopodium zonale, Ginkgo biloba, Zanthoxylum bungeanum, and Cryptonemia seminervis contain bioactive compounds demonstrating substantial antiviral activity against human metapneumovirus through multiple complementary mechanisms [4][7][8][9]. The remarkable efficacy of meroditerpenoids achieving >99.99% viral inhibition, ginkgolic acid's selective entry mechanism targeting, γ-Fagarine's demonstrated in vivo efficacy, and sulfated galactans' multi-mechanism inhibitory pathways collectively establish compelling scientific rationale for pursuing plant-based HMPV therapeutics [6][27].

The persistent therapeutic vacuum for HMPV treatment, combined with absence of preventive vaccines and the substantial public health burden particularly affecting vulnerable pediatric and geriatric populations, creates urgent imperative for alternative therapeutic development strategies [2][3]. Traditional medicine systems spanning millennia have provided foundational knowledge identifying these plant species as therapeutically relevant, with contemporary molecular pharmacology now elucidating specific mechanisms underlying empirical clinical observations.

Prioritization of Zanthoxylum bungeanum and Cryptonemia seminervis for immediate translational development reflects their demonstrated safety profiles, proven in vivo efficacy, and feasibility for pharmaceutical formulation, whereas Stypopodium zonale warrants continued mechanistic investigation and

toxicity mitigation research. Integration of phytochemistry, molecular virology, and clinical medicine represents a potentially transformative approach to addressing emerging and re-emerging viral infections where conventional pharmaceutical development has failed to produce effective therapeutic solutions.

The successful translation of plant-derived antivirals into clinical therapeutics would establish precedent for systematic evaluation of traditional botanical medicines against contemporary disease challenges, ultimately enhancing global therapeutic arsenals and improving health outcomes for vulnerable populations disproportionately affected by respiratory viral infections. Continued interdisciplinary collaboration among botanical researchers, virologists, pharmacologists, and clinical investigators will be essential for realizing the therapeutic potential of medicinal plants in combating human metapneumovirus infections and other viral diseases.

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