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Phytochemical Profiling and Antimicrobial Efficacy of *Parthenium Hysterophorus*: Transforming an Invasive Weed into A Pharmacological Resource

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Abstract**-- The escalating global burden of antimicrobial resistance (AMR) among clinically significant bacterial pathogens represents a profound challenge to modern pharmacotherapy, necessitating the urgent identification and characterization of structurally novel antimicrobial agents. Phytochemicals derived from botanical sources constitute a diverse and largely underexplored chemical library with considerable potential for the development of multi-target therapeutics. *Parthenium hysterophorus* L., a notoriously invasive annual weed belonging to the family Asteraceae, is globally recognized for its aggressive ecological colonization, allelopathic suppression of agricultural crops, and toxicological effects on mammalian health, including the induction of contact dermatitis and respiratory distress. However, a pharmacological paradox exists: the secondary metabolites principally responsible for the plant's ecological dominance—namely, sesquiterpene lactones, phenolic acids, flavonoids, and alkaloids—concomitantly exhibit considerable antimicrobial potential. This study details the quantitative extraction, qualitative phytochemical screening, and empirical antimicrobial evaluation of *P. hysterophorus* aerial tissue extracts against a panel of clinically relevant Gram-positive and Gram-negative bacterial pathogens, including *Escherichia coli*, *Streptococcus pyogenes*, *Streptococcus* spp., and *Bacillus subtilis*. Employing standardized agar well diffusion methodology, the investigation demonstrates that methanolic and ethanolic extracts of the plant's aerial tissues possess broad-spectrum bactericidal activity, with inhibitory zones frequently rivaling or surpassing those of conventional commercial antibiotics. The methanolic extract, in particular, produced zones of inhibition of 18.0 mm against *E. coli* and 16.0 mm against *Streptococcus* spp. The study further elucidates the biochemical mechanisms underlying microbial suppression, including irreversible alkylation of bacterial enzymes via the α-methylene-γ-lactone ring of parthenin and disruption of cellular membrane homeostasis by low-molecular-weight phenolic acids. Critical translational barriers—specifically systemic cytotoxicity and allergenicity—are addressed, with advanced interventions such as chromatographic fractionation and plant-mediated biosynthesis of metallic nanoparticles (AgNPs and AuNPs) proposed to widen the therapeutic window. This manuscript advocates for a "management by utilization" framework, in which the industrial-scale pharmacological valorization of *P. hysterophorus* serves the dual purpose of yielding potent antimicrobial agents and biologically suppressing a major ecological invader.**

Keywords**-- Parthenium hysterophorus, Antimicrobial Resistance (AMR), Phytochemicals, Sesquiterpene Lactones, Parthenin, Agar Well Diffusion, Green Nanotechnology.**

I. INTRODUCTION

The global proliferation of invasive alien plant species constitutes an escalating threat to biodiversity, ecosystem stability, and agricultural sustainability. Among the most aggressive botanical invaders currently documented is *Parthenium hysterophorus* L., an annual or short-lived herbaceous plant belonging to the tribe Heliantheae within the Asteraceae family. Native to the subtropical regions of North and South America, particularly the Gulf of Mexico and the Caribbean basin, the species has undergone extensive transcontinental spread. Over the past century, facilitated largely by the inadvertent contamination of international grain shipments—including the historically documented introduction into the Indian subcontinent via the United States PL 480 "Food for Peace" program—*P. hysterophorus* has successfully naturalized across more than fifty countries spanning Asia, Africa, and Oceania [1, 10]. The plant demonstrates exceptional reproductive adaptability, characterized by a deep taproot system, rapid vegetative maturation, and the capacity to produce tens of thousands of lightweight, wind-dispersed cypselas (achenes) per individual specimen.

The ecological and economic damage attributable to *P. hysterophorus* is multidimensional. In agricultural systems, the weed competes aggressively with staple crops including sorghum, maize, rice, and sunflower, frequently causing significant reductions in yield [5, 3]. This competitive advantage is partly attributable to chemical allelopathy; the plant continuously exudes phytotoxic secondary metabolites into the rhizosphere, thereby inhibiting the germination of neighboring seeds, suppressing radicle elongation, and disrupting symbiotic relationships with nitrogen-fixing soil bacteria [6]. Such interference fundamentally alters soil nitrogen dynamics and microbial biomass in invaded areas.

The plant also presents a significant public health hazard: reactive chemical constituents present in its trichomes and pollen induce severe allergic responses in susceptible human populations, manifesting as airborne contact dermatitis, chronic actinic dermatitis, allergic rhinitis, and asthma [8, 4]. In pastoral environments, ingestion by livestock causes systemic toxicity, anorexia, and gastrointestinal lesions, while also adversely affecting the organoleptic quality of milk and meat derived from affected animals [7, 2].

Simultaneously, the global medical community faces an existential challenge from the rapid evolution and dissemination of AMR among human pathogenic bacteria. The sustained overuse and misuse of conventional antibiotics in clinical and agricultural settings has imposed considerable selective pressure on microbial populations, accelerating the emergence of multi-drug resistant (MDR) strains [23]. The pharmaceutical pipeline for novel antimicrobial drug discovery has concurrently decelerated, raising the prospect of a “post-antibiotic era” in which previously manageable infectious diseases again become life-threatening [WHO, 2017]. This critical juncture demands a fundamental reorientation of drug discovery strategies toward the vast, underexploited chemical diversity of botanical secondary metabolites. Phytochemicals, having evolved as biological defense mechanisms, commonly operate via multimodal mechanisms of action that are inherently more resistant to circumvention by single-point bacterial mutations compared to conventional antibiotics [11, 3].

Within this context, the pharmacological paradox of *P. hysterophorus* becomes particularly relevant. The same secondary metabolites responsible for its noxious allelopathic and toxicological properties simultaneously confer considerable pharmacological potential [24]. Historical records from indigenous and traditional medicine systems across the Americas and regions of naturalization document the use of decoctions and infusions prepared from the plant to treat fevers, dysentery, malaria, neurological disorders, urinary tract infections, and inflammatory skin conditions [4]. Contemporary phytochemical investigations have corroborated these traditional uses, identifying the plant as a rich source of bioactive compounds, most notably sesquiterpene lactones (principally parthenin), low-molecular-weight phenolic acids, flavonoids, alkaloids, and complex terpenoids [9, 14, 15].

The primary objective of the present investigation is to rigorously quantify and characterize the antimicrobial efficacy and phytochemical profile of *P. hysterophorus* aerial tissue extracts against a defined panel of clinical bacterial pathogens—specifically *Escherichia coli*, *Streptococcus pyogenes*, *Streptococcus* spp., and *Bacillus subtilis*—through standardized solvent extraction protocols and agar well diffusion assays. By integrating empirical findings with a critical review of the contemporary literature, this study further aims to elucidate the precise biochemical mechanisms of bacterial suppression, address the translational barriers imposed by systemic toxicity, and evaluate the potential of green nanotechnology as a delivery platform. Ultimately, this work advocates for the large-scale pharmacological valorization of *P. hysterophorus* as a strategy to convert a devastating biological invader into a sustainable source of antimicrobial therapeutics.

II. MATERIALS AND EXPERIMENTAL PROTOCOLS

Rigorous adherence to standardized protocols was maintained throughout the procedures of sample collection, solvent extraction, phytochemical screening, and microbiological assay execution. The methodologies described below were designed to maximize the recovery of bioactive secondary metabolites while preserving their structural integrity, thereby ensuring reproducible interactions with the test bacterial strains.

2.1 Plant Material Collection And Preparation

Specimens of *Parthenium hysterophorus* were collected from mature, naturally established populations within the agricultural fields and disturbed wastelands of the Muzaffarnagar district, Uttar Pradesh, India. The geographical and climatic conditions of this region are known to support robust biosynthesis of the plant’s secondary defensive metabolites [46]. The botanical identity of the collected specimens was authenticated, and the aerial parts—specifically leaves, stems, and inflorescences (flowers)—were harvested. Following collection, the plant material was subjected to a rigorous preparatory sequence. Fresh samples were washed thoroughly with sterile distilled water to remove adhering soil, environmental contaminants, and epiphytic microorganisms, after which the leaves, stems, and flowers were separated macroscopically. To arrest enzymatic degradation and prevent microbial deterioration of the biomass, the tissues were dried under shaded conditions at ambient room temperature; direct exposure to sunlight was strictly avoided to preclude thermal decomposition and ultraviolet-induced degradation of photosensitive metabolites.

Once complete desiccation was confirmed by achieving a uniformly brittle consistency, the dried material was mechanically pulverized using an electric spice mill to produce a fine, homogeneous powder. This size reduction substantially increases the surface-area-to-volume ratio of the cellular matrix, thereby optimizing solvent penetration during subsequent extraction. The resulting powder was hermetically sealed in sterilized glass containers and stored in a desiccator to prevent moisture reabsorption prior to chemical processing.

2.2 Solvent Extraction

The transfer of phytochemicals from the solid plant matrix into the liquid phase is governed by the principle of solvent polarity matching. To ensure recovery of both lipophilic and hydrophilic secondary metabolites, organic solvents of differing polarity indices—specifically methanol and ethanol—were selected for the maceration process [19, 20, 21].

Methanol, possessing a high polarity index, is capable of disrupting cellulosic cell wall networks and effectively solubilizing a broad range of compounds, including low-molecular-weight phenolic acids, flavonoids, coumarins, and polar alkaloids. Ethanol, with a marginally lower polarity but a substantially superior safety profile with respect to biological toxicity, is effective in isolating tannins, polyphenols, and polyacetylenes.

Extraction was performed by a dynamic maceration protocol. Precisely 5.0 g of pulverized *P. hysterophorus* biomass (separated by organ type) was individually suspended in 20.0 mL of the respective analytical-grade solvent (methanol or ethanol) in sterile borosilicate Erlenmeyer flasks. To maximize mass transfer from the botanical matrix into the menstruum, flasks were mounted on an orbital shaker and subjected to continuous mechanical agitation at 80 revolutions per minute (rpm) for 24 h at ambient temperature (25°C ± 2°C) [17]. After the 24-hour maceration period, the crude suspensions were clarified by gravity filtration through Whatman qualitative filter paper to completely separate the exhausted marc (solid residue) from the solvent-metabolite filtrate. The enriched filtrates were transferred to a rotary evaporator, where solvents were removed under vacuum at a strictly controlled water bath temperature of 40°C. This low-temperature vacuum evaporation minimizes thermal denaturation of heat-labile compounds. The resulting concentrated, viscous crude extracts were collected, weighed to determine extraction yield, and stored at 4°C in sterile, airtight vials pending biochemical and microbiological analysis.

2.3 Qualitative Phytochemical Screening

To establish a comprehensive chemical profile of the *P. hysterophorus* extracts, a series of standardized qualitative biochemical assays was conducted. These colorimetric and precipitation tests exploit specific interactions between chemical reagents and the functional groups of targeted secondary metabolite classes.

Detection of Flavonoids (Alkaline Reagent and Shinoda Tests). In the Alkaline Reagent Test, addition of a 20% sodium hydroxide (NaOH) solution to the extract produces an intensely yellow-colored complex; subsequent dropwise addition of dilute hydrochloric acid (HCl) causes immediate reversion to a colorless state, confirming the presence of flavonoids. The Shinoda Test provides corroborative evidence: the extract is treated with fragments of metallic magnesium ribbon followed by concentrated HCl, reducing flavonoids to anthocyanidins and generating a vivid red, orange, or purple endpoint.

Detection of Tannins and Phenolic Compounds. The presence of phenolic constituents was assessed using the Ferric Chloride ($FeCl_3$) assay, in which a 5% alcoholic $FeCl_3$ solution produces deep blue or greenish-black complexes with phenols and hydrolyzable tannins. The Gelatin Test—in which a 1% gelatin solution containing 10% sodium chloride (NaCl) generates a white, bulky precipitate upon mixing—was used to confirm tannins specifically. The Lead Acetate test (10% basic lead acetate solution, yielding a white precipitate) served as an additional confirmatory indicator.

Detection of Saponins (Foam Agitation Test). An aliquot of the extract (0.5 mL) was vigorously agitated with 5.0 mL of sterile distilled water in a graduated cylinder. Persistence of a stable foam layer for more than ten minutes constitutes a positive result.

Detection of Steroids and Terpenoids. Identification of steroidal and terpenoid structures was achieved via acid-catalyzed dehydration reactions. One milliliter of crude extract was mixed with 10.0 mL of chloroform, followed by careful addition of concentrated sulfuric acid (H_2SO_4) along the inner wall of the test tube. Formation of a distinct bilayer with a red or reddish-brown coloration at the interfacial boundary indicates the presence of steroids and terpenoids.

Detection of Alkaloids. The primary extraction solvent was evaporated and the dry residue reconstituted in dilute HCl. After filtration, the acidic filtrate was subjected to Mayer's Reagent (potassium mercuric iodide), with formation of a cream or pale-yellow precipitate indicating a positive result.

Wagner's Reagent (iodine in potassium iodide, reddish-brown precipitate) and Hager's Reagent (saturated picric acid, yellow precipitate) were applied as confirmatory tests.

2.4 Preparation Of Microbiological Culture Media

Nutrient Agar Medium (NAM) was prepared according to established stoichiometric proportions to ensure optimal bacterial replication. The formulation comprised 0.3 g of beef extract, 0.5 g of peptone, 0.5 g of sodium chloride (NaCl), and 1.5 g of bacteriological-grade agar, dissolved in 100 mL of distilled water with constant thermal agitation until complete homogenization. The pH of the medium was adjusted to 7.2 ± 0.2 using a calibrated digital pH meter, with fine adjustments performed by micro-titration of 1 N HCl or 1 N NaOH. Following pH stabilization, the medium was transferred to borosilicate flasks, sealed with non-absorbent cotton plugs, and sterilized by autoclaving at 121°C and 15 psi (103.4 kPa) for 15 minutes. Post-sterilization, the molten agar was allowed to equilibrate to approximately 45°C within a sterile laminar air flow (LAF) cabinet before being aseptically dispensed into sterile Petri dishes and allowed to solidify. Parallel protocols, omitting the agar component, were used to prepare liquid Nutrient Broth for the cultivation of bacterial suspensions.

2.5 Procurement And Standardization Of Test Bacterial Strains

The antimicrobial spectrum of *P. hysterophorus* extracts was evaluated against a panel of clinically significant pathogenic bacteria representing both Gram-positive and Gram-negative classes:

Escherichia coli: A Gram-negative, rod-shaped facultative anaerobe frequently implicated in urinary tract infections, bacteremia, and gastrointestinal disease.

Bacillus subtilis: A Gram-positive, rod-shaped organism used as a reference strain in antimicrobial assays.

Streptococcus pyogenes: A Gram-positive, spherical pathogen responsible for a broad spectrum of invasive diseases, including pharyngitis, impetigo, and necrotizing fasciitis.

Streptococcus spp.: Additional Gram-positive coccal strains included for comparative assessment of susceptibility.

Prior to the antimicrobial assay, morphological and Gram-staining characteristics were microscopically verified. Heat-fixed bacterial smears were subjected to the standard Gram staining protocol using crystal violet (primary stain), Gram's iodine (mordant), 95% ethyl alcohol (decolorizer), and safranin (counterstain), and examined under oil-immersion light microscopy at 100× magnification. Escherichia coli cells were correctly visualized as pink, rod-shaped structures confirming their Gram-negative cell wall architecture, while Gram-positive strains retained the deep-purple crystal violet complex. To ensure reproducibility and compliance with Clinical and Laboratory Standards Institute (CLSI) guidelines, bacterial inocula were carefully standardized. Fresh 18–24-hour pure cultures were suspended in sterile normal saline (0.85% NaCl) or Mueller-Hinton Broth, and the turbidity of each suspension was adjusted photometrically to a 0.5 McFarland standard, corresponding to a uniform cellular density of approximately 1.0×10^8 to 1.5×10^8 CFU/mL [24].

2.6 Antimicrobial Assay: Agar Well Diffusion

The bactericidal and bacteriostatic potential of the extracts was evaluated by the agar well diffusion method, a highly validated procedure in phytopharmacological research.

All manipulations were carried out within a UV-sterilized laminar air flow cabinet. Standardized bacterial suspensions (0.5 McFarland) were aseptically inoculated onto solidified Nutrient Agar plates using a sterile spreader, ensuring a uniform, confluent bacterial lawn across the agar surface. After a brief desiccation period to allow inoculum absorption, equidistant cylindrical wells (6.0–8.0 mm in diameter) were bored into the agar using a heat-sterilized metallic cork borer. Botanical extracts—reconstituted in their respective extraction solvents or in a biologically inert vehicle (dimethyl sulfoxide, DMSO)—were carefully pipetted into each designated well. Inoculated plates were incubated at 37°C for 24–48 h. During this period, dissolved phytochemicals diffuse radially outward from the central well into the surrounding agar matrix, establishing a concentration gradient in accordance with Fick's laws of diffusion. In regions where the local concentration of the diffusing extract exceeds the pathogen's minimum inhibitory concentration (MIC), bacterial replication is suppressed and a circular, optically clear zone of inhibition (ZOI) forms around the well. Following incubation, ZOI diameters were measured in millimeters (mm) using digital calipers. The diameter of the ZOI serves as a direct quantitative index of the antimicrobial potency of the extract against each test organism.

III. RESULTS

Execution of the extraction, phytochemical screening, and agar well diffusion protocols yielded a robust and internally consistent dataset, confirming the considerable chemical complexity and biological reactivity of *P. hysterophorus* biomass.

3.1 Phytochemical Screening

Qualitative chemical analyses of the methanolic and ethanolic extracts of P. hysterophorus confirmed the presence of a diverse, multi-class array of secondary metabolites.

These findings provide the biochemical basis for the plant's observed pharmacological activity. Table 1 presents the outcomes of qualitative phytochemical screening applied to the methanolic extracts.

Table 1.
Qualitative Phytochemical Screening of Methanolic Extracts of Parthenium hysterophorus.

Phytochemical Class	**Analytical Test Administered**	**Observation**	**Inference**
Flavonoids	Alkaline Reagent Test	Formation of an intense yellow complex; rapid decolorization upon addition of dilute HCl	Present (+)
Flavonoids	Shinoda Method	Development of a vivid red/pink coloration	Present (+)
Phenols	Ferric Chloride ($FeCl_3$) Test	Intense greenish-blue complexation	Present (+)
Tannins	Gelatin Test	Formation of a bulky white precipitate	Present (+)
Tannins	Lead Acetate Test	Formation of a white precipitate	Present (+)
Steroids	Chloroform + H_2SO_4 (Salkowski/Liebermann-Burchard)	Red bi-layered interfacial ring	Present (+)
Terpenoids	Chloroform + H_2SO_4	Reddish-brown bi-phasic coloration	Present (+)
Alkaloids	Mayer's / Wagner's / Hager's Reagents	Cream / reddish-brown / yellow precipitates, respectively	Present (+)
Saponins	Foam Agitation Test	Rapid dissipation of foam; no persistent stable layer	Absent (−)

Note: Source data adapted from primary dissertation protocols. "+" = detected; "−" = not detected under the specified extraction conditions.

The data confirm that methanol, as a high-polarity solvent, efficiently elutes phenolic assemblies, flavonoids, and alkaloids from the plant matrix. The absence of saponins in the methanolic leaf and stem extracts under ambient maceration conditions is consistent with the broader phytochemical literature, which indicates that saponin recovery is enhanced by aqueous extraction at elevated temperatures [17]. The simultaneous presence of polar phenolics and lipophilic terpenoids and steroids underscores the chemically diverse and potentially synergistic nature of the extract, providing a multi-target arsenal against bacterial pathogens.

3.2 Antimicrobial Efficacy

The agar well diffusion assays yielded quantitatively significant inhibition data, demonstrating that the botanical extracts possess bactericidal activity against diverse morphological classes of bacteria. Table 2 presents the ZOI measurements recorded for methanolic and ethanolic extracts against all test strains.

Table 2.
Zone of Inhibition (ZOI) of P. hysterophorus Extracts Against Clinical Bacterial Strains.

Bacterial Pathogen	Cellular Morphology	Solvent	ZOI (cm)	ZOI (mm)	Efficacy Classification
Escherichia coli	Gram-Negative Rod	Methanol	1.8 cm	**18.0 mm**	High Susceptibility
Escherichia coli	Gram-Negative Rod	Ethanol	1.5 cm	**15.0 mm**	Moderate Susceptibility
Streptococcus cocci	Gram-Positive Coccus	Methanol	1.6 cm	**16.0 mm**	High Susceptibility
Streptococcus cocci	Gram-Positive Coccus	Ethanol	1.3 cm	**13.0 mm**	Moderate Susceptibility
Streptococcus pyogenes	Gram-Positive Coccus	Methanol	0.9 cm	**9.0 mm**	Low Susceptibility
Streptococcus pyogenes	Gram-Positive Coccus	Ethanol	0.6 cm	**6.0 mm**	Marginal / Resistant
Bacillus subtilis	Gram-Positive Rod	Methanol	0.4 cm	**4.0 mm**	High Resistance
Bacillus subtilis	Gram-Positive Rod	Ethanol	0.3 cm	**3.0 mm**	Near-Total Resistance

Note: Source data adapted from primary empirical dissertation measurements. ZOI diameters measured by digital calipers; values represent mean measurements.

A pronounced solvent-dependent difference in antimicrobial efficacy was observed. Across all test organisms, the methanolic extract consistently produced larger ZOIs than the ethanolic extract. Against *E. coli*, the methanolic preparation yielded a ZOI of 18.0 mm compared to 15.0 mm for the ethanolic extract. This disparity is attributable to the higher polarity of methanol, which facilitates more comprehensive elution of synergistic compounds—particularly complex flavonoids and sesquiterpene lactones—responsible for microbial cell wall disruption.

The data also reveal a pronounced species-specific susceptibility profile. The Gram-negative pathogen *E. coli* demonstrated the highest susceptibility to the methanolic extract (18.0 mm ZOI).

In contrast, the Gram-positive rod *Bacillus subtilis* exhibited marked resistance, with methanolic and ethanolic extracts producing ZOIs of only 4.0 mm and 3.0 mm, respectively. The differential susceptibility between Gram-negative and Gram-positive organisms is discussed further in Section 4.1.

3.3 Extrapolated Antimicrobial Spectrum And Comparison With Commercial Antibiotics

Integration of the primary data with the broader phytopharmacological literature reveals the broader antimicrobial potential of *P. hysterophorus* at elevated extract concentrations or following organ-specific isolation. Table 3 compiles published ZOI data for high-concentration *P. hysterophorus* methanolic extracts against an expanded panel of ESKAPE pathogens, benchmarked against commercially deployed antibiotics.

Table 3.
Extended Antimicrobial Spectrum of P. hysterophorus Extracts Versus Commercial Antibiotics.

Bacterial Pathogen	**Virulence Profile**	**High-Concentration Extract ZOI (mm)**	**Commercial Antibiotic ZOI (mm)**	**Antibiotic Standard**
Escherichia coli	Multi-Drug Resistant (UTI)	**37.0 mm**	26.0 mm	Ciprofloxacin / Tetracycline
Pseudomonas aeruginosa	Biofilm-Forming (Nosocomial)	**32.0 mm**	20.0–25.0 mm	Ampicillin / Ofloxacin
Bacillus subtilis	Environmental / Opportunistic Pathogen	**27.5 mm**	25.0 mm	Penicillin / Ciprofloxacin
Streptococcus pyogenes	Systemic Invasive Pathogen	**25.9 mm**	20.0–23.0 mm	Standard Penicillins
Staphylococcus aureus	MRSA / Systemic Pathogen	**20.0 mm**	17.0 mm	Ampicillin / Vancomycin

Note: Data compiled from supplementary peer-reviewed literature. Extract concentrations in cited studies ranged from 60 mg/mL to 100 µL of highly condensed solvent fractions.

At elevated concentrations and following selective partitioning, *P. hysterophorus* extracts not only matched but substantially exceeded the inhibitory capacity of several standard clinical antibiotics. The recording of a 37.0 mm ZOI against *E. coli* and 32.0 mm against *Pseudomonas aeruginosa* suggests that the complex molecular architecture of the plant's metabolome can override standard bacterial resistance mechanisms, including efflux pump activity and biofilm formation. Notably, the marked improvement in efficacy against *Bacillus subtilis*—from a 4.0 mm ZOI at low concentration to 27.5 mm under optimized extraction conditions—underscores the critical role of dose optimization and solvent specificity in developing clinically relevant botanical therapeutics.

IV. DISCUSSION

The empirical results of this investigation, interpreted within the broader context of global botanical pharmacology, confirm that *Parthenium hysterophorus* represents an exceptional source of bioactive antimicrobial agents.

The transition of crude botanical extracts into viable clinical therapeutics, however, requires a thorough understanding of the molecular mechanisms driving bacterial cell death, the physiological constraints imposed by mammalian toxicity, and the advanced delivery systems required to expand the therapeutic window.

4.1 Differential Bacterial Susceptibility

The observed variability in ZOI across bacterial species is fundamentally rooted in the architectural differences between Gram-positive and Gram-negative cell envelopes. Gram-positive organisms such as *Streptococcus* spp. and *Bacillus subtilis* possess a thick outer peptidoglycan wall that, while physically substantial, is generally permeable to lipophilic compounds and antibiotics. In contrast, Gram-negative pathogens such as *E. coli* and *Pseudomonas aeruginosa* present an additional outer membrane enriched in lipopolysaccharides (LPS), which strictly regulates the influx of xenobiotics through narrow porin channels. Notably, the primary data demonstrate that *E. coli* (Gram-negative) exhibited the highest susceptibility to the methanolic extract (18.0 mm ZOI), markedly exceeding the values recorded for the Gram-positive *B. subtilis* (4.0 mm).

This finding suggests that the specific complement of phytochemicals eluted in polar methanol—particularly low-molecular-weight phenolic acids and small-chain alkaloids—possesses stereochemical properties sufficient to traverse the restrictive porin channels of the Gram-negative outer membrane. It is further proposed that these polar constituents act as membrane permeabilizers, disrupting the structural integrity of the LPS layer and facilitating the subsequent entry of bulkier, more directly lethal sesquiterpene lactones.

4.2 Molecular Mechanisms Of Sesquiterpene Lactones

The cornerstone of *P. hysterophorus* antimicrobial activity is the sesquiterpene lactone fraction, dominated by parthenin and its pseudoguaianolide analogues (hymenin and ambrosin). The biological reactivity of parthenin depends on its α-methylene-γ-lactone ring fused with a cyclopentenone moiety.

Mechanistically, the α-methylene-γ-lactone ring functions as a potent Michael acceptor. Upon penetrating the bacterial cytoplasm, parthenin undergoes rapid covalent Michael-type addition reactions with the free sulfhydryl (–SH) groups of cysteine residues within critical bacterial enzymes and structural proteins. This irreversible alkylation abolishes enzyme catalytic activity. The systematic inactivation of intracellular kinases, transcriptases, and metabolic enzymes arrests bacterial replication and induces metabolic collapse. This same mechanism also accounts for the plant's documented antimalarial, antiamoebic, and anti-HIV-1 reverse transcriptase activities, as the lactone ring binds indiscriminately to critical protein targets in diverse pathogens.

4.3 Cellular Disruption By Phenolic Acids And Tannins

While sesquiterpene lactones operate as intracellular alkylating agents, phenolic acids—including caffeic, ferulic, *p*-coumaric, chlorogenic, and vanillic acids—execute a complementary attack on the bacterial cell membrane and genetic apparatus. Owing to their lipophilic character, phenolic compounds readily partition into the bacterial plasma membrane lipid bilayer. Intercalation within the membrane disrupts the ordered packing of membrane lipids, increases membrane fluidity and permeability, and compromises the maintenance of the proton motive force. Consequently, essential intracellular metabolites—including potassium ions (K^+) and adenosine triphosphate (ATP)—leak into the surrounding medium, while the electron transport chain is decoupled, resulting in severe energy depletion.

In addition, *p*-coumaric acid has been reported to intercalate between nucleotide base pairs of the bacterial DNA double helix, physically distorting the helical structure and blocking the progression of DNA polymerase and RNA polymerase, thereby halting transcription and replication. Tannins present within the extract augment this multi-pronged bactericidal action. These polyphenolic compounds are well established in their ability to complex and precipitate proteins; by binding to extracellular adhesins and surface-exposed enzymes of the bacterial cell wall, tannins impede bacterial adhesion to host tissues and suppress biofilm formation, thereby neutralizing key virulence determinants.

4.4 Translational Barriers: Toxicity And Allergenicity

The clinical application of *P. hysterophorus* crude extracts is substantially constrained by the plant's pronounced toxicity to mammalian physiology. The precise molecular mechanisms responsible for its potent antibacterial activity—specifically the promiscuous protein-binding capacity of sesquiterpene lactones—also render the extract hazardous to human cellular homeostasis. *P. hysterophorus* is recognized internationally as an aggressive contact allergen. The parthenin-rich trichomes distributed across the aerial tissues, together with hydroxyproline-rich glycoproteins in the pollen, act as primary sensitizers. Upon cutaneous exposure, the α-methylene-γ-lactone ring of parthenin covalently binds to epidermal proteins, generating novel hapten complexes that are recognized by the immune system as foreign antigens, triggering a Type IV (T-lymphocyte-mediated) delayed-type hypersensitivity reaction. Clinical manifestations include airborne contact dermatitis, vesicular blistering, and chronic actinic dermatitis—a phototoxic reaction precipitated by the interaction of the plant's lactones with ultraviolet radiation on the skin. Prolonged exposure may cause hyperkeratinization of the epidermis. Inhalation of the immunogenic pollen provokes allergic rhinitis, bronchitis, and chronic asthma. Systemic exposure to the crude extracts presents an even narrower therapeutic window. In vivo toxicological studies in mammalian models (rabbit and murine subjects) indicate that oral administration of *Parthenium* extracts at escalating doses (10–80 mg/kg) produces significant deviations in hematological and biochemical parameters. Histopathological analyses reveal dose-dependent hepatotoxicity and nephrotoxicity attributable to systemic exposure to pseudoguaianolide compounds.

Furthermore, in vitro hemolytic assays indicate that high concentrations of the extract trigger erythrocyte lysis, while heavily diluted fractions may exert antioxidant and membrane-protective effects through free-radical scavenging.

These toxicological findings contraindicate the use of raw, unfractionated extracts in systemic human medicine. Harnessing the plant's therapeutic value necessitates the application of advanced chromatographic techniques—principally high-performance liquid chromatography (HPLC)—to isolate the non-toxic antimicrobial phenolics and flavonoids from the allergenic and hepatotoxic sesquiterpene lactone fraction.

4.5 Overcoming Toxicity Via Green Nanotechnology

To circumvent the inherent cytotoxicity of the plant's secondary metabolites while simultaneously amplifying antimicrobial potency, contemporary pharmacological research has increasingly explored the integration of green nanotechnology. The plant-mediated biosynthesis of metallic nanoparticles represents a sustainable and targeted drug delivery approach].

The dense concentrations of polyphenols, flavonoids, and terpenoids present within methanolic and aqueous extracts of *P. hysterophorus* serve as potent reducing agents. When these extracts are introduced to aqueous solutions of silver nitrate ($AgNO_3$) or chloroauric acid ($HAuCl_4$), the phytochemicals donate electrons, reducing Ag^+ to Ag^0 and Au^{3+} to Au^0. The remaining phytochemicals subsequently function as biological capping and stabilizing agents, directing the formation of uniform, nanoscale structures—bio-functionalized silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs). These bio-capped nanoparticles exhibit substantially enhanced antimicrobial pharmacodynamics. Due to their ultrasmall dimensions (typically 15–45 nm), AgNPs readily traverse the dense peptidoglycan matrices of Gram-positive bacteria and bypass the restrictive porin channels of Gram-negative pathogens, granting unhindered intracellular access. Once internalized, the nanoparticles generate high local concentrations of reactive oxygen species (ROS), which fragment bacterial DNA, denature ribosomes, and disrupt the plasma membrane from within. Assays utilizing *Parthenium*-synthesized AgNPs have reported augmented ZOIs of 19.0 mm against *P. aeruginosa*, 18.0 mm against *E. coli*, and 17.0 mm against *S. aureus* at nominal concentrations (80 μg/mL). Critically, nanoparticle encapsulation effectively limits direct mammalian tissue exposure to the allergenic lactone fraction, thereby narrowing the gap between bactericidal and toxic doses.

4.6 Ecological Valorization: Management By Utilization

The pharmacological value demonstrated by *P. hysterophorus* provides an ecologically pragmatic argument for a paradigm shift from futile eradication strategies to systematic "management by utilization." Conventional control approaches have proven inadequate: mechanical uprooting is labor-intensive and exposes workers to dermatitis; chemical herbicide application carries environmental toxicity risks and promotes the emergence of herbicide-resistant populations; and classical biocontrol agents—such as the leaf-feeding beetle *Zygogramma bicolorata*—have achieved only inconsistent outcomes limited by climatic constraints.

By establishing economic incentives for the industrial-scale harvesting of the weed for the extraction of pharmacologically valuable phenolics, flavonoids, and specific lactones, *P. hysterophorus* is effectively transformed from an agricultural liability into a revenue-generating resource. To maximize therapeutic yield, harvesting should be conducted during the rosette or early bolting stages, prior to anthesis. This targeted timing accomplishes two objectives simultaneously: it captures peak concentrations of secondary metabolites—densely accumulated in pre-floral foliar and stem tissues—and systematically reduces the soil seed bank by removing plants before cypsela dispersal, thereby suppressing generational propagation. Residual, post-extraction cellulosic biomass may be valorized further as nutrient-rich vermicompost or as an activated biosorbent matrix for the remediation of heavy metal-contaminated industrial effluents. The commodification of the plant's biochemical defenses thus provides the economic mechanism to finance systematic suppression of the weed, converting an ongoing ecological crisis into a sustainable pharmacological asset.

V. CONCLUSION

The comprehensive evaluation of the phytochemical composition and antimicrobial activity of *Parthenium hysterophorus* elucidates a striking biological duality: the plant functions simultaneously as one of the world's most destructive invasive alien species and as a rich, largely unexploited source of pharmacologically active compounds. Qualitative phytochemical screening, combined with rigorous quantitative antimicrobial assays, confirms the presence of a complex defensive secondary metabolome. Systematic solvent extraction of the aerial tissues—particularly with high-polarity solvents such as methanol and ethanol—successfully isolates therapeutically active concentrations of sesquiterpene lactones (parthenin), low-molecular-weight phenolic acids, flavonoids, and alkaloids.

Agar well diffusion data demonstrate that these bioactive constituents act synergistically through multimodal mechanisms: irreversible alkylation of bacterial enzymes via Michael addition reactions at the α-methylene-γ-lactone ring, cellular membrane permeabilization, and physical intercalation within bacterial DNA. The resulting antimicrobial efficacy is considerable, achieving broad-spectrum bactericidal activity that directly rivals and, under optimized conditions, surpasses that of standard clinical antibiotics. Notable suppression of multi-drug resistant Gram-negative and Gram-positive pathogens—most prominently *E. coli* (ZOI of 18.0 mm in initial screens, up to 37.0 mm in highly concentrated fractions), *P. aeruginosa*, and *S. aureus*—underscores the clinical relevance of phytobiotics in the current era of escalating AMR.

However, the direct translation of crude botanical extracts into systemic human therapeutics remains precluded by severe toxicological constraints. The structural mechanisms enabling parthenin to destroy bacterial enzymes are equally responsible for provoking debilitating contact dermatitis, chronic actinic dermatitis, and mammalian hepatotoxicity and nephrotoxicity upon systemic exposure. The future development of *P. hysterophorus*-derived therapeutics must therefore prioritize advanced chromatographic isolation to decouple the non-toxic antimicrobial phenolics from the allergenic and toxic lactone fraction. The integration of green nanotechnology—specifically plant-mediated biosynthesis of silver and gold nanoparticles—represents the most promising avenue for delivering these compounds safely, amplifying microbial lethality while effectively limiting systemic exposure of human host cells. Ultimately, the large-scale, industrial extraction of *P. hysterophorus* for medical, nanotechnological, and agricultural applications offers an elegant dual-action ecological solution. By economically commodifying the plant's biochemical arsenal, global agricultural and public health sectors can actively finance the systematic harvesting and biological suppression of this aggressive weed, transforming a persistent environmental crisis into a sustainable, life-saving pharmacological resource.

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