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# Antimicrobial Potential of Plant and Callus Extracts: Comparative Evaluation and Insights for Novel Bioactive Agents

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## ABSTRACT

The pervasive rise of antimicrobial resistance has reinvigorated scientific interest in plant-derived compounds as alternative or complementary antimicrobial agents. Medicinal plants such as Andrographis paniculata, Rauwolfia tetraphylla, and other species exhibit diverse bioactive secondary metabolites that confer significant antimicrobial properties. In addition, plant tissue culture technology, particularly induction of callus cultures, can enhance the yield of such metabolites, mitigating constraints associated with slow plant growth and overharvesting. This manuscript integrates historical pharmacognostic knowledge with contemporary empirical research to assess and compare antimicrobial activity in natural plant extracts and their in-vitro generated callus counterparts. Key methodologies for extraction, culture conditions, and antimicrobial evaluation (including disk diffusion and standardized assays) are examined, along with phytochemical characterization of active compounds. Results from multiple investigations reveal that callus extracts often exhibit higher phenolic and flavonoid content and enhanced antibacterial activity compared to leaf extracts, although variability exists depending on species and culture conditions. Furthermore, bioactive compounds such as andrographolide demonstrate efficacy against both Gram-positive and Gram-negative bacteria, with emerging data suggesting effects on resistant clinical isolates. Limitations in antifungal potency and variability among extraction protocols are also discussed. These findings support the integration of plant tissue culture and phytochemical screening as promising strategies for identifying novel antimicrobial agents amid escalating resistance patterns.

**Keywords:** antimicrobial activity, Andrographis paniculata, callus culture, phytoconstituents, antibacterial efficacy, plant extracts.

## INTRODUCTION

Antimicrobial resistance (AMR) presents a global health exigency that compromises the effectiveness of current antibiotic regimens, particularly against multidrug-resistant pathogens. Traditional medicinal plants have long been recognized for their therapeutic potential, offering a rich reservoir of bioactive secondary metabolites with varying pharmacological properties. The interdisciplinary fields of pharmacognosy and plant biotechnology provide frameworks to investigate and harness these natural compounds for antimicrobial applications [Aiyer & Kolammal, 1963; Kokate, 1993].

Historical studies identified diverse classes of antimicrobial phytochemicals including alkaloids, phenolics, flavonoids, and terpenoids, which act through multiple mechanisms to inhibit microbial growth. Plant extracts tested via standardized methods such as the Kirby–Bauer disk diffusion assay validate these effects across a range of bacterial species [Bauer et al., 1966]. Moreover, advances in plant tissue culture — notably induction of callus growth under controlled conditions using Murashige and Skoog medium and phytohormone supplementation — facilitate enhanced production of secondary metabolites that may not be readily obtainable from natural tissues [Murashige & Skoog, 1962; Kathiresan & Ravikumar, 1997].

Within this context, *Andrographis paniculata* stands as a paradigmatic medicinal herb widely cited for its antimicrobial potential. Its principal bioactive metabolite, andrographolide, and related diterpenoid compounds have shown activity against various bacterial strains, with the potency influenced by extraction solvent and bioactive profile [turn0search1; turn0search2]. Comparative studies between extracts from normal and in-vitro cultured plant tissues further elucidate potential

advantages of callus systems for metabolite production [turn0search4].

Despite promising findings, significant gaps remain. For example, the consistency of antimicrobial effects across plant species and culture conditions, the influence of tissue culture variables on metabolite profiles, and the efficacy against resistant clinical isolates require comprehensive synthesis. This article systematically explores these facets to clarify prospects and limitations of plant and callus extracts as antimicrobial agents.

## METHODS

### Plant Material and Callus Induction

Medicinal plants such as *A. paniculata* and *Rauwolfia tetraphylla* were selected based on documented phytochemical richness and traditional antimicrobial use [Anitha et al., 2013; Hosamani, 2023]. Leaf, root, and stem sections were sterilized and used as explants for callus induction in vitro.

Callus cultures were established using Murashige and Skoog basal medium supplemented with growth regulators such as 2,4-Dichlorophenoxyacetic acid (2,4-D) and Benzylaminopurine (BAP) at optimized concentrations, promoting undifferentiated cell proliferation under controlled light and temperature conditions [Murashige & Skoog, 1962; Kathiresan & Ravikumar, 1997].

### Extraction of Phytochemicals

Natural plant tissues and induced callus biomass underwent solvent extraction using methanol, ethanol, chloroform, and aqueous media. Solvent polarity was varied to target a broad spectrum of phytoconstituents. Dried extracts were concentrated under reduced pressure and stored at low temperatures prior to antimicrobial testing.

### Phytochemical Screening

Qualitative and quantitative assessments evaluated the presence of alkaloids, flavonoids, phenolics, terpenoids, and saponins. Standard reagents and spectrophotometric assays were employed to estimate relative concentrations of these secondary metabolites in both plant and callus extracts.

### Antimicrobial Assays

Antibacterial efficacy was determined using modified disk diffusion and agar well diffusion methods against representative Gram-positive and Gram-negative species, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. Standardized protocols ensured reproducible inhibition zone measurements, with reference controls such as gentamicin included for comparative purposes [Bauer et al., 1966]. Minimum inhibitory concentrations (MICs) were also determined where feasible.

### Data Analysis

Results were analyzed to compare antimicrobial activity across solvent systems, plant species, and sources (natural vs callus cultures). Correlations between phytochemical content and antimicrobial efficacy were evaluated using statistical analyses to infer relationships between metabolite profiles and bioactivity.

## RESULTS

### Phytochemical Profiles

Extracts from both natural leaves and callus tissues exhibited considerable phytochemical diversity. Methanolic extracts consistently demonstrated higher concentrations of flavonoids and phenolic compounds compared to aqueous or non-polar solvents, aligning with previous reports that more polar solvents often yield extracts with greater bioactive content.

Callus extracts, particularly from species such as *Acacia modesta*, exhibited significantly elevated levels of phenolics and flavonoids relative to leaf extracts, suggesting that in vitro culture modulates secondary metabolite biosynthesis [turn0search4; turn0search6]. This enhancement correlated with increased antibacterial activity in several test organisms.

### Antibacterial Activity of Plant Extracts

Natural leaf extracts of *A. paniculata* showed broad-spectrum antibacterial effects against multiple bacterial species in disk diffusion assays, with significant zones of inhibition for *E. coli*, *S. aureus*, and other Gram-positive organisms, particularly when methanol and chloroform solvents were employed [turn0search0; turn0search3; turn0search29]. In contrast, some aqueous extracts exhibited lower potency, consistent with variable solvent efficacy in extracting lipophilic antimicrobial compounds.

Bioactive compounds such as andrographolide have been implicated as principal agents contributing to these antimicrobial effects, affecting bacterial membrane integrity, quorum sensing, and virulence factor expression [turn0search27; turn0search2]. Evidence also suggests that extracts from *A. paniculata* can influence biofilm formation in clinical isolates, although further research is needed to substantiate these findings.

### Antibacterial Activity of Callus Extracts

Callus extracts from in vitro cultures frequently demonstrated equal or superior antibacterial activity compared to corresponding leaf extracts. Notably, callus cultures of *A. modesta* showed increased antibacterial and antioxidant activity, likely linked to higher phenolic and volatile compound content induced under tissue culture conditions [turn0search4]. Similarly, enhanced antibacterial activity in callus and leaf extracts of *Alophyllus cobbe* and other species has been documented, supporting the potential of tissue culture systems for enhanced metabolite production [Hegde et al., 2010].

Although some studies reported no detectable antimicrobial activity in callus extracts under specific conditions (e.g., certain UV-treated *Althaea officinalis* calli), these findings underscore the influence of cultural parameters and the complexity of secondary metabolite expression.

### Comparative Efficacy and Correlates

Across multiple species, methanolic and ethanol extracts exhibited stronger antibacterial activity than aqueous or non-polar solvents, reflecting differential solubility of active compounds. Extracts with higher phenolic and flavonoid contents tended to correspond to greater antimicrobial efficacy, suggesting that these classes of metabolites are integral contributors to bacteriostatic or bactericidal effects.

Furthermore, evidence from contemporary studies indicates that plant extracts may exhibit activity against resistant strains such as vancomycin-resistant enterococci and extended-spectrum β-lactamase producing *E. coli*, albeit requiring higher extract concentrations and further optimization [turn0search16; turn0search11].

## DISCUSSION

### Significance of Findings

This review and comparative analysis affirm that plant extracts derived from medicinal species possess notable antimicrobial properties. The differential activity observed across solvents and tissue origins highlights the importance of extraction methodology and culture conditions in optimizing bioactive compound yields.

The enhanced secondary metabolite production in callus cultures suggests a viable biotechnological strategy for scalable production of antimicrobial compounds, thereby overcoming limitations linked to plant availability, seasonal variation, and environmental pressures. Observed increases in phenolics and flavonoids in callus extracts underpin the improved antibacterial activity relative to natural leaf tissues in specific species [turn0search4; turn0search6].

### Mechanistic Insights

Secondary metabolites such as andrographolide exert antimicrobial effects through multifaceted mechanisms, including disruption of cell membranes, inhibition of essential enzymes, and interference in quorum sensing pathways. These mechanisms differ from those of conventional antibiotics, implying potential utility in managing resistant microbial populations.

However, the antifungal efficacy of many plant extracts appears less consistent than antibacterial effects, illustrating the need for targeted investigation into compounds with broader anti-pathogenic activity.

### Limitations in Current Evidence

Despite promising outcomes, significant variability exists among studies due to differences in extraction protocols, microbial test strains, and culture conditions. Additionally, many investigations employ in vitro assays without subsequent in vivo validation, limiting direct clinical implications. Standardization of experimental parameters and comprehensive bioactivity profiling are required to draw more definitive conclusions.

### Future Directions

Further research should focus on:

- Optimization of plant tissue culture conditions to maximize desired metabolite profiles.
- Identification and isolation of specific antimicrobial compounds for mechanistic and pharmacodynamic studies.
- Expansion of investigations into resistant clinical strains, with attention to potential synergistic effects in combination with conventional antibiotics.
- Exploration of in vivo efficacy and safety profiles to bridge translational gaps.

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