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Harnessing Rubiaceae Plant Endophytic Fungi: A Comprehensive Review of Novel Antimicrobial Compounds for Drug Discovery

(Memanfaatkan Kulat Endofitik Tumbuhan Rubiaceae: Ulasan Komprehensif Sebatian Antimikrob Baharu untuk Penemuan Ubat)

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ABSTRACT

Antimicrobial resistance (AMR) continues to threaten the effectiveness of existing therapies, driving the urgent search for novel therapeutic leads from nature. Rubiaceae plant-associated endophytic fungi are increasingly recognized as valuable producers of structurally diverse secondary metabolites with antibacterial, antifungal, and antimycobacterial activities. Unlike previous reviews that primarily focus on bioactive compounds, this narrative-critical review integrates ecological factors influencing endophytic diversity with advanced strategies to awaken cryptic biosynthetic pathways. We systematically evaluate approaches including One Strain-Many Compounds (OSMAC), co-culture, chemical and epigenetic elicitation, genome-guided discovery, and heterologous expression. Furthermore, this review critically analyzes pharmacological metrics, specifically minimum inhibitory concentration (MIC), cytotoxicity, Selectivity Index (SI), mechanisms of action, and the potential of fungal metabolites as antibiotic adjuvants. Current evidence suggests that the clinical development of these natural products is constrained by methodological inconsistencies, a lack of animal model testing, insufficient pharmacokinetic profiling, and large-scale manufacturing hurdles. Future progress in drug discovery will heavily depend on adopting standardized evaluation criteria and comprehensive pharmacological screening to successfully transform these endophytic metabolites into viable clinical candidates.

Keywords: Antimicrobial secondary metabolites; drug discovery; endophytic fungi; OSMAC; Rubiaceae; selectivity index

ABSTRAK

Kerintangan antimikrob (AMR) terus mengancam keberkesanan terapi sedia ada, sekali gus mendorong pencarian segera untuk terapeutik baharu daripada alam semula jadi. Kulat endofit yang berkaitan dengan tumbuhan famili Rubiaceae semakin dikenali sebagai pengeluar pelbagai metabolit sekunder yang memiliki aktiviti antibakteria, antikulat dan antimikobakteria. Tidak seperti ulasan terdahulu yang sebahagian besarnya tertumpu kepada sebatian bioaktif, ulasan ini menggabungkan faktor ekologi yang mempengaruhi kepelbagaian endofit dengan strategi lanjutan untuk meningkatkan laluan biosintetik yang masih samar. Kami menilai pelbagai pendekatan secara sistematik termasuk Satu Strain-Banyak Sebatian (OSMAC), kultur bersama, elisitasi kimia dan epigenetik, penemuan berpandukan genom dan pengekspresan heterolog. Tambahan pula, ulasan ini menganalisis secara kritis metrik farmakologi, khususnya kepekatan perencatan minimum (MIC), kesitotoksikan, Indeks Kepilihan (SI), mekanisme tindakan dan potensi metabolit kulat sebagai adjuvan antibiotik. Bukti semasa mencadangkan bahawa pembangunan klinikal produk semula jadi ini masih dikekang oleh ketidakseragaman metodologi, kekurangan ujian model haiwan, pemprofilan farmakokinetik yang tidak mencukupi dan halangan pembuatan berskala besar. Kemajuan pada masa hadapan dalam penemuan ubat akan sangat bergantung kepada penerapan kriteria penilaian piawai dan penyaringan farmakologi yang komprehensif untuk berjaya mentransformasikan metabolit endofit ini menjadi calon klinikal yang berdaya maju.

Kata kunci: Indeks keselektifan; kulat endofit; metabolit sekunder antimikrob; OSMAC; penemuan ubat; Rubiaceae

INTRODUCTION

Antimicrobial resistance (AMR) is increasingly regarded as a critical challenge for contemporary healthcare, progressively reducing the efficacy of available antimicrobial therapies and intensifying the global public health burden (Prestinaci, Pezzotti & Pantosti 2015). Recent surveillance data indicate that antimicrobial resistance continues to increase globally, with a substantial proportion of bacterial infections exhibiting resistance to one or more antibiotic classes (WHO 2025), resulting in increased morbidity, mortality and healthcare expenditure (Bhavnani, Krause & Ambrose 2020; Petrosillo & Granata 2022). These trends highlight the urgent need for alternative antimicrobial-discovery strategies, with bioprospecting emerging as an important approach for identifying novel therapeutic compounds from underutilized biological resources (Atanasov et al. 2021).

Bioprospecting efforts have increasingly focused on underexplored ecological niches as sources of antibiotics and other bioactive metabolites (Gao et al. 2025; Santos-Aberturas & Vior 2022). Among these, plant-associated microorganisms, particularly endophytic fungi, are promising producers of structurally diverse antimicrobial metabolites and valuable models for understanding specialized metabolic pathways (Calvo-Gomez, Eshboev & Mullaiarova 2025; Chen et al. 2021; Hashem et al. 2023).

Because fungal biosynthetic capacity is influenced by host phytochemistry, chemically rich plants represent attractive targets for endophyte-based bioprospecting. The Rubiaceae family is well known for its abundance of alkaloids, terpenoids and phenolic compounds (Laforest et al. 2025; Ochoa et al. 2025), and this chemical diversity may promote equally diverse biosynthetic capabilities among associated endophytic fungi (Cruz, da Silva & Hamerski 2020; Martins, Nunez & Coordenation 2015). This concept is reflected in the proposition that 'microbial treasure follows chemical treasure', highlighting chemically rich host plants as reservoirs of metabolically diverse endophytes (Gouda et al. 2016; Pellissier et al. 2023). Figure 1 summarizes the general workflow used to isolate Rubiaceae plant-associated endophytic fungi and identify their bioactive secondary metabolites.

Despite this promise, current understanding of the diversity, cultivation optimization and antimicrobial metabolite production of Rubiaceae plant-associated endophytic fungi remains fragmented (Tiwari & Bae 2022). Moreover, the translational value of reported metabolites is limited by the lack of standardized antimicrobial evaluation, particularly consistent reporting of Selectivity Index (SI) and minimum inhibitory concentration (MIC) values (Gonzalez-Pastor et al. 2023; Tang et al. 2026). Although previous reviews have addressed endophytic fungi, antimicrobial natural products, or Rubiaceae phytochemistry separately, few have integrated fungal diversity, metabolite-production strategies and translational antimicrobial evaluation within a single Rubiaceae-focused framework. This review therefore provides an integrated framework for evaluating metabolites produced by endophytic fungi associated with Rubiaceae plants, from early discovery to translational assessment.

Accordingly, this review has three objectives: (i) to examine the ecological and biochemical factors influencing endophytic fungal diversity in Rubiaceae host plants; (ii) to evaluate cultivation optimization and biosynthetic activation strategies for enhancing secondary-metabolite production; and (iii) to synthesize current evidence on antimicrobial activities, with particular emphasis on MIC and SI as quantitative indicators relevant to antimicrobial drug development and clinical translation.

MATERIALS AND METHODS

A narrative-critical review was conducted to consolidate published evidence on Rubiaceae plant-associated endophytic fungi and their antimicrobial metabolites. Literature published between January 2010 and April 2026 was retrieved from Google Scholar, PubMed/MEDLINE, Web of Science and Scopus using the keywords ('endophytic fungi' OR 'endophytes') AND ('Rubiaceae' OR '*Cinchona*' OR '*Coffea*' OR '*Morinda*' OR '*Gardenia*' OR '*Ixora*') AND ('antimicrobial' OR 'bioactive compound'). Reference lists of selected publications were also screened for additional studies. The literature was synthesized according to fungal diversity, host-endophyte interactions, cultivation optimization, biosynthetic activation, antimicrobial activity, and translational relevance, with emphasis on MIC, CC_{50}, SI, mechanisms of action, *in vivo* efficacy, and ADME/Tox parameters. Studies were evaluated for their contribution to metabolite production, biosynthetic activation, efficacy, safety, and translational potential. Although publications from 2010-2026 were included, emphasis was placed on studies from 2016-2026. Table 1 summarizes reported compounds and identifies future research priorities.

ECOLOGICAL COMPLEXITY, CHEMICAL DIVERSITY AND BIOPROSPECTING PERSPECTIVES OF RUBIACEAE PLANT-ASSOCIATED ENDOPHYTIC FUNGI

The exploration of Rubiaceae plant-associated endophytic fungi offers important opportunities for antimicrobial bioprospecting but requires an understanding of host specificity, biochemical adaptation and methodological constraints associated with fungal isolation and cultivation (Dos Reis, Lorenzi & Vale 2022; Escudero-Leyva et al. 2023). Widely distributed in tropical ecosystems, Rubiaceae plants harbor metabolically diverse endophytic communities whose composition and biological activities are shaped by host metabolism and

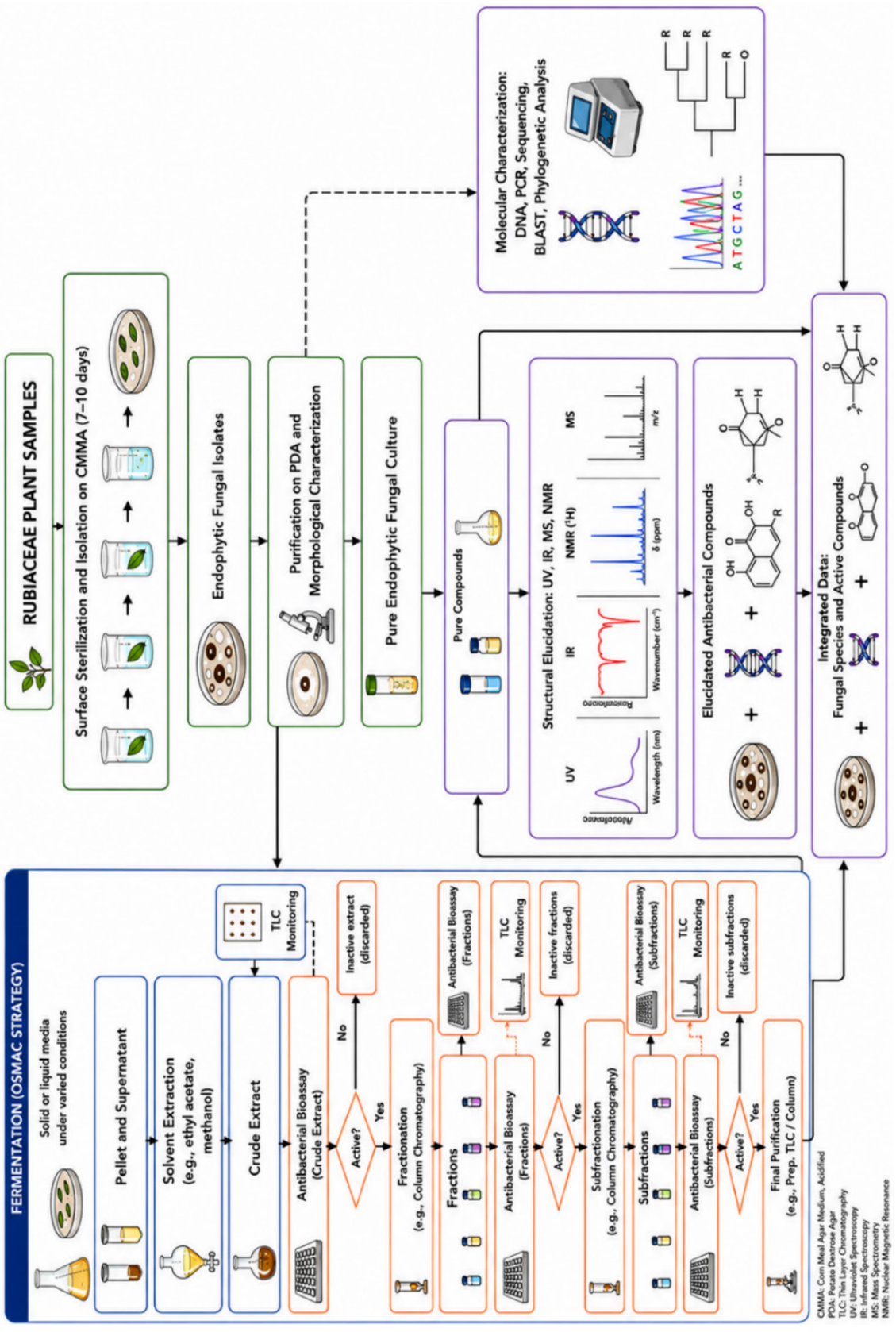

FIGURE 1. Schematic workflow for the isolation of Rubiaceae plant-associated endophytic fungi and the discovery of antimicrobial secondary metabolites. This workflow illustrates the integration of fungal isolation, molecular identification, cultivation-based optimization, bioassay-guided fractionation, and chemical characterization. Combined molecular and chemical analyses facilitate the correlation of bioactive metabolites with their corresponding endophytic fungal producers

TABLE 1. Bioactive compounds isolated from Rubiaceae endophytic fungi with antimicrobial activity (2016-2025)

Rubiaceae host species	Endophytic fungus	Isolated compound	Antimicrobial activity & quantification	Reference
Morinda officinalis	*Trichoderma koningiopsis* A729	Koninginol A	Antibacterial against *Bacillus subtilis* (MIC = 10 µg/mL)	(Chen et al. 2019)
Morinda officinalis	*Trichoderma koningiopsis* A729	Koninginol B	Antibacterial against *Bacillus subtilis* (MIC = 2 µg/mL)	(Chen et al. 2019)
Morinda citrifolia	*Guignardia mangiferae*	Sydowinol (S1)	Antibacterial against *Xanthomonas axonopodis* pv. *passiflorae* (active up to 3.125 µg/mL)	(de Oliveira et al. 2022)
Morinda citrifolia	*Guignardia mangiferae*	Sydowinin A (S2)	Antibacterial against *Xanthomonas axonopodis* pv. *passiflorae* (active up to 3.125 µg/mL)	(de Oliveira et al. 2022)
Mussaenda luteola	*Chaetomium cupreum*	6-Heptacosa-18′Z-enyl-2-(18′-hydroxy-1′E,19′-oxy)-3-hydroxybenzoquinone	Antimycobacterial against *Mycobacterium tuberculosis* H37Rv (MIC = 6.25 µg/mL)	(Shylaja & Sathiavelu 2019)
Mussaenda luteola	*Chaetomium cupreum*	Ergosterol derivative	Antimycobacterial against *Mycobacterium tuberculosis* H37Rv (MIC = 25 µg/mL)	(Shylaja & Sathiavelu 2019)
Mussaenda luteola	*Chaetomium cupreum*	(3β,5α)-3-hydroxy-4-(p-phenylacetoxy)-ergosta-7,22-diene	Antibacterial (MIC = 6.25-50 µg/mL)	(Shylaja, Sasikumar & Sathiavelu 2018)
Mussaenda luteola	*Chaetomium cupreum*	Resorcinol type lipid	Antimycobacterial against *Mycobacterium tuberculosis* H37Rv (MIC = 6.3 µg/mL)	(Shylaja, Sasikumar & Sathiavelu 2018)
Geophila repens	*Xylaria feejeensis*	Eremophilane-type sesquiterpenoid	Antibacterial against *Bacillus subtilis* (MIC = 16 µg/mL) and methicillin-resistant *Staphylococcus aureus* (MRSA; MIC = 64 µg/mL); non-haemolytic up to 45 µg/mL	(Rajendran et al. 2023)
Uncaria gambir	*Diaporthe* sp. GNBP-10	(+)-1,1’-bislunatin (Bis)	Antimycobacterial against *Mycobacterium tuberculosis* H37Rv (MIC = 0.422 µM)	(Oktavia et al. 2020)
Uncaria gambir	*Diaporthe* sp. GNBP-10	(+)-2,2′-Epicytoskyrin A	Antimycobacterial against *Mycobacterium tuberculosis* H37Rv (MIC = 0.844 µM)	(Oktavia et al. 2020)
Uncaria gambir	*Diaporthe* sp. GNBP-10	(+)-2,2’-Epicytoskyrin A	Antifungal against *Candida tropicalis*, *C. albicans*, *Aspergillus flavus*, *A. niger* (MIC = 16-128 µg/mL)	(Wulansari et al. 2016)

environmental factors, including altitude, climate and soil characteristics (Castillo-González & Slot 2025; Cruz, da Silva & Hamerski 2020). These endophytes contribute to host adaptation while serving as reservoirs of structurally diverse secondary metabolites with ecological and pharmaceutical significance (Caruso et al. 2022; Hashem et al. 2023).

Host-endophyte interactions strongly influence fungal community structure and metabolite production. Although generalist genera such as *Fusarium* and *Aspergillus* are common, the phytochemical characteristics of Rubiaceae chemotypes can promote greater diversity of bioactive metabolites (Christian et al. 2020; Cruz, da Silva & Hamerski 2020). Rubiaceae plant-associated endophytes produce four principal metabolite classes, terpenoids, polyketides, alkaloids and unusual amino acid or peptide-derived compounds (Chen et al. 2021; Cruz, da Silva & Hamerski 2020). Chemical overlap between host plants and their microbiota further suggests that metabolic interactions contribute to secondary-metabolite diversification and support the concept that 'microbial treasure follows chemical treasure' (Gouda et al. 2016; Pellissier et al. 2023).

The host biochemical environment may regulate fungal biosynthetic pathways and promote the production of structurally complex metabolites, including quinine-related alkaloids and indole diterpenoids (Maehara et al. 2019; Rutkowska et al. 2023). Reports describing quinine-like compounds produced by *Diaporthe* sp. associated with *Cinchona ledgeriana* support a close metabolic relationship between host and endophyte and illustrate their potential to generate clinically relevant metabolites (Maehara et al. 2019, 2011). Interactions among coexisting microbial populations may further stimulate the biosynthesis of previously unexpressed compounds (Bertrand et al. 2014; Wu et al. 2024).

Despite this promise, endophyte bioprospecting remains constrained by cultivation biases, unstable metabolite production and incomplete integration of genomic and chemical datasets (Dos Reis, Lorenzi & Vale 2022; Sagita, Quax & Haslinger 2021). The inconsistent production of Taxol by *Taxomyces andreanae* exemplifies these broader limitations (Subban & Kempken 2023). Consequently, integrating ecological, chemical and genomic approaches with strategies for activating silent biosynthetic gene clusters (BGCs) will be essential for expanding fungal chemical diversity and accelerating antimicrobial lead discovery (Zakariyah et al. 2024; Zheng et al. 2025).

OPTIMIZATION AND BIOSYNTHETIC PATHWAY ACTIVATION: A ROADMAP FOR HARNESSING RUBIACEAE FUNGAL ENDOPHYTES

The efficient production of secondary metabolites from Rubiaceae plant-associated endophytic fungi depends on integrating cultivation optimization with strategies that activate silent or dormant biosynthetic pathways (Pillay et al. 2022; Zheng et al. 2025). Figure 2 summarizes this framework by linking biosynthetic activation with metabolite production, bioactivity assessment and preclinical evaluation. Optimization of culture parameters, including temperature, pH, nutrient composition and incubation period, remains a fundamental approach for enhancing metabolite yield and unlocking silent biosynthetic potential (Hoyos et al. 2024). Nutritional manipulation, particularly adjustment of the carbon-to-nitrogen ratio, can redirect metabolic flux toward secondary-metabolite biosynthesis, as demonstrated by enhanced bis-anthraquinone production in *Diaporthe* sp. GNBP-10 from *Uncaria gambir* (Oktavia et al. 2023).

The One Strain-Many Compounds (OSMAC) strategy exploits fungal metabolic plasticity to generate distinct metabolite profiles under altered cultivation conditions, thereby expanding access to otherwise silent or low-abundance biosynthetic pathways (Gakuubi et al. 2022; Romano et al. 2018). This principle is illustrated by the production of new eremophilane-type sesquiterpenes by *Camarops* sp. isolated from *Alibertia macrophylla* (Gubiani et al. 2014). Endophytic fungi may also function as biological elicitors, stimulating host secondary metabolism, as demonstrated by *Colletotrichum dianesei* and *Xylaria* sp. in *Duroia saccifera* callus cultures (Lima et al. 2025).

Since cultivation optimization alone may not activate all cryptic pathways, co-cultivation, chemical elicitation and epigenetic modulation have emerged as complementary approaches for stimulating silent biosynthetic gene clusters (BGCs) (Gakuubi et al. 2022; Pillay et al. 2022). Fungal-fungal and fungal-bacterial interactions, including co-culture with *Streptomyces* spp., can activate silent BGCs through chromatin-level regulation and induce metabolites absent under monoculture conditions (Bertrand et al. 2014; Nicault et al. 2021; Wu et al. 2024). Such interaction-based systems highlight the latent biosynthetic capacity of endophytic fungi and provide phenotypic evidence for pathway activation (Lima et al. 2025).

Integration of cultivation-based approaches with genome mining has substantially expanded access to fungal biosynthetic diversity (Kenshole et al. 2021; Rutledge & Challis 2015). Whole-genome sequencing of *Talaromyces* sp. identified 76 BGCs, whereas genome mining of *Diaporthe* sp. BR109 showed multiple putative novel sesquiterpene synthases and evidence of horizontal gene transfer (de Sena Filho et al. 2016; Shenoy et al. 2024). Since the presence of a BGC does not necessarily indicate active metabolite production, genome mining should be integrated with transcriptomic, metabolomic and functional analyses to distinguish biosynthetic potential from actual metabolic expression (Palmer & Keller 2010; Wang et al. 2015).

Functional characterization can be further advanced through heterologous expression systems, epigenetic

elicitation and CRISPR/Cas-based genome editing (Geistodt-Kiener et al. 2023; Komal & Ye 2022; Xu, Huang & Yin 2021). Recent advances in fungal artificial chromosome technology and chromatin-remodeling approaches further facilitate activation of cryptic BGCs and metabolic rewiring (Mózsik et al. 2022; Wu et al. 2023). However, their practical value ultimately depends on maintaining metabolite production under scalable and economically viable fermentation conditions (Hashem et al. 2023).

Despite these advances, most studies remain limited to laboratory-scale fermentation, and metabolite production often changes during scale-up because of differences in culture conditions and fungal physiology (Ganeshan et al. 2021). Consequently, successful exploitation of Rubiaceae plant-associated endophytes will require integration of cultivation optimization, elicitation strategies, genome-guided pathway activation and scalable production systems. Such an integrated approach is expected to unlock cryptic biosynthetic potential and expand the chemical diversity of Rubiaceae plant-associated endophytic fungi.

ANTIMICROBIAL ACTIVITIES AND MECHANISMS OF ACTION OF SECONDARY METABOLITES FROM RUBIACEAE PLANT-ASSOCIATED ENDOPHYTIC FUNGI

The Rubiaceae plant family is an important source of structurally diverse secondary metabolites, and antimicrobial compounds identified from Rubiaceae plant-associated endophytic fungi during 2016-2026 are summarized in Table 1. Collectively, the available evidence indicates that Rubiaceae plant-associated endophytic fungi constitute a rich reservoir of natural products with significant potential for combating antimicrobial resistance (AMR).

Among the reported metabolites, antimycobacterial compounds exhibit particularly strong activity, with anthraquinone-derived metabolites from *Diaporthe* sp. GNBP-10 (*Uncaria gambir*) and quinone-, ergosterol-, and lipid-derived metabolites from *Chaetomium cupreum* (*Mussaenda luteola*) demonstrating potent activity against *Mycobacterium tuberculosis* H37Rv (Oktavia et al. 2020; Shylaja & Sathiavelu 2019; Shylaja, Sasikumar & Sathiavelu 2018). Notably, anthraquinone-derived metabolites from *Diaporthe* sp. GNBP-10 exhibited sub-micromolar activity, highlighting their promise as potential antimycobacterial leads (Oktavia et al. 2020). Antibacterial metabolites also demonstrate activity against Gram-positive bacteria and, in some cases, clinically important resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), highlighting the broad antimicrobial spectrum of Rubiaceae plant-associated endophytic fungi (Chen et al. 2019; de Oliveira et al. 2022; Rajendran et al. 2023). Although less extensively investigated than antibacterial and antimycobacterial metabolites, antifungal metabolites have shown promising activity against pathogenic yeasts and filamentous fungi. For example, (+)-2,2′-epicytoskyrin A produced by *Diaporthe* sp. GNBP-10 (*Uncaria gambir*) exhibited antifungal activity against multiple fungal pathogens (Wulansari et al. 2016). Collectively, these findings demonstrate the broad-spectrum antimicrobial potential of metabolites produced by Rubiaceae plant-associated endophytic fungi.

Metabolomic studies have highlighted the diversity of bioactive compounds produced by Rubiaceae plant-associated endophytic fungi, many of which exhibit activity against both drug-sensitive and multidrug-resistant (MDR) pathogens (Cruz, da Silva & Hamerski 2020; Prajapati et al. 2025). Many metabolites display greater activity against Gram-positive bacteria than against Gram-negative species through disruption of cell-envelope integrity, membrane function, and cellular homeostasis, whereas the outer membrane and multidrug efflux systems contribute to the intrinsic resistance of Gram-negative bacteria (Maher & Hassan 2023; Silva, Cardoso & Macedo 2022).

Beyond membrane-associated effects, metabolites produced by Rubiaceae plant-associated endophytic fungi may inhibit cell-wall biosynthesis, interfere with nucleic-acid and protein synthesis, disrupt biofilm formation, and affect microbial communication pathways, thereby reducing pathogen survival and virulence (Bansal et al. 2025; Eshboev et al. 2024). Antifungal metabolites may similarly act through multiple mechanisms, including membrane and cell-wall damage, altered energy metabolism, and modulation of efflux systems (Silva-Beltrán et al. 2024; Wulansari et al. 2016). Such mechanistic diversity may reduce resistance development and expand opportunities for combination therapy (Bansal et al. 2025; Nazir et al. 2024).

Endophytic fungal metabolites also have the potential to function as antibiotic adjuvants through synergistic interactions with existing antibiotics (Jha et al. 2023; Roy et al. 2026). Although isolated from non-Rubiaceae hosts, the methanolic extract of *Colletotrichum gloeosporioides* has been reported to enhance the activity of penicillin and vancomycin against methicillin-resistant *Staphylococcus aureus* (MRSA), whereas spiroaspertrione A produced by *Aspergillus* sp. TJ23 can restore sensitivity to oxacillin. These findings support the potential of fungal metabolites as adjuvants against multidrug-resistant pathogens (He et al. 2017). Although such synergistic effects have not yet been systematically evaluated in Rubiaceae plant-associated endophytic fungi, their diverse bioactive metabolites warrant further investigation as potential antibiotic adjuvants. Given that Rubiaceae plant-associated endophytes also produce various potent antimicrobial compounds, systematic evaluation of combination therapies could yield novel strategies for managing MDR infections (Cruz, da Silva & Hamerski 2020; Prajapati et al. 2025).

Overall, the structural diversity and distinct biological mechanisms summarized in Table 1 underscore the immense therapeutic potential of these

microorganisms. As illustrated in Figure 3, the diverse antimicrobial actions and antibiotic-potentiating effects discussed collectively position Rubiaceae plant-associated endophytic fungi as a highly promising frontier for the discovery of future antibacterial, antifungal, and antimycobacterial lead compounds.

QUANTITATIVE ASSESSMENT AND TRANSLATIONAL PERSPECTIVES OF RUBIACEAE PLANT-ASSOCIATED ENDOPHYTIC FUNGAL METABOLITES

Reliable quantitative evaluation is essential for advancing antimicrobial discoveries toward clinically relevant applications. Minimum inhibitory concentration (MIC) remains the principal parameter for assessing antimicrobial potency, although variations in inoculum density, culture medium composition, incubation conditions and assay formats can substantially influence results and complicate cross-study comparisons (Gonzalez-Pastor et al. 2023; Kavanagh et al. 2019). Methodological differences may generate substantial MIC discrepancies, emphasizing the need for standardized approaches to antimicrobial screening and resistance surveillance (Tang et al. 2026). Broader adoption of CLSI and EUCAST guidelines, together with transparent reporting of antimicrobial efficacy, cytotoxicity and experimental parameters, is essential for improving data comparability because therapeutic selectivity cannot be reliably assessed without accompanying CC_{50} and SI values (CLSI 2026; Cos et al. 2006; EUCAST 2026).

Assessment of antimicrobial efficacy should be accompanied by evaluation of compound safety. Determination of CC_{50} values and calculation of the Selectivity Index (SI = CC_{50}/MIC) provide important indicators of therapeutic selectivity, with SI values above 10 generally considered favorable during early-stage drug discovery (Cos et al. 2006; Majoumouo et al. 2020). Toxicity and cytotoxicity testing remain indispensable because potent antimicrobial activity alone does not

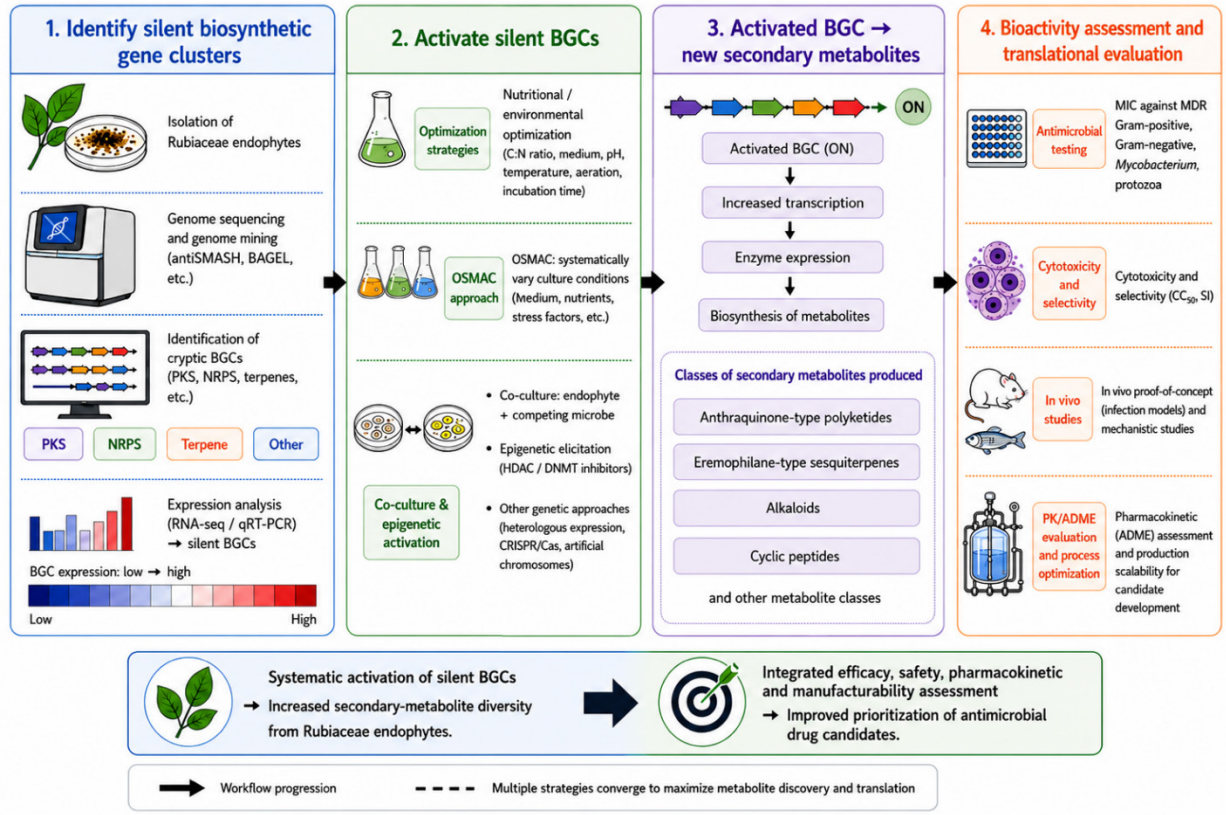

FIGURE 2. Conceptual framework illustrating the activation of silent biosynthetic gene clusters (BGCs) in Rubiaceae plant-associated endophytic fungi and their progression toward antimicrobial drug discovery. The framework integrates genome mining, cultivation-based optimization, OSMAC, co-culture, and epigenetic and genetic activation strategies, leading to the production of bioactive secondary metabolites. Subsequent evaluation encompasses antimicrobial efficacy, cytotoxicity and selectivity, *in vivo* validation, pharmacokinetic assessment, and process optimization to support the translational development of antimicrobial candidates

necessarily indicate therapeutic suitability, particularly for fungal metabolites with broad biological activities (Caruso et al. 2022; Nazir et al. 2024). Although integration of antimicrobial and cytotoxicity assays facilitates candidate prioritization, SI information remains unavailable for many metabolites because of resource limitations and methodological variability, reducing reproducibility and comparability among studies (Agustina et al. 2024; Riss et al. 2016).

Natural products remain an important source of therapeutic leads; however, most antimicrobial metabolites reported from Rubiaceae plant-associated endophytic fungi remain at the *in vitro* stage, with limited evidence regarding *in vivo* efficacy, pharmacokinetic behavior and scalable production (Atanasov et al. 2021; Chandra et al. 2024; Cruz, da Silva & Hamerski 2020; Nazir et al. 2024). Few antimicrobial metabolites produced by Rubiaceae plant-associated endophytic fungi have progressed beyond laboratory screening, and proof-of-concept studies using murine, zebrafish, or *Galleria mellonella* infection models may provide an important bridge toward preclinical development (Habjan et al. 2024; Piątek, Sheehan & Kavanagh 2021).

Although MIC and SI provide valuable information regarding efficacy and safety, successful drug development also requires favorable pharmacokinetic and toxicological properties. Pharmacokinetic and ADME/Tox characterization remain insufficient, limiting assessment of bioavailability, systemic exposure and drug-likeness, although integration of *in silico* ADME/Tox profiling with targeted *in vitro* assays may facilitate early candidate prioritization before resource-intensive preclinical studies are undertaken (Aniceto, Freitas & Ghafourian 2026; Durán-Iturbide, Díaz-Eufracio & Medina-Franco 2020; Tiwari & Bae 2022). Another important bottleneck concerns metabolite scalability and regulatory readiness because information regarding fermentation optimization, production stability, yield reproducibility and large-scale cultivation remains scarce, whereas successful drug development requires robust evidence for efficacy, safety, pharmacokinetics and manufacturing reproducibility (Atanasov et al. 2021; Ganeshan et al. 2021).

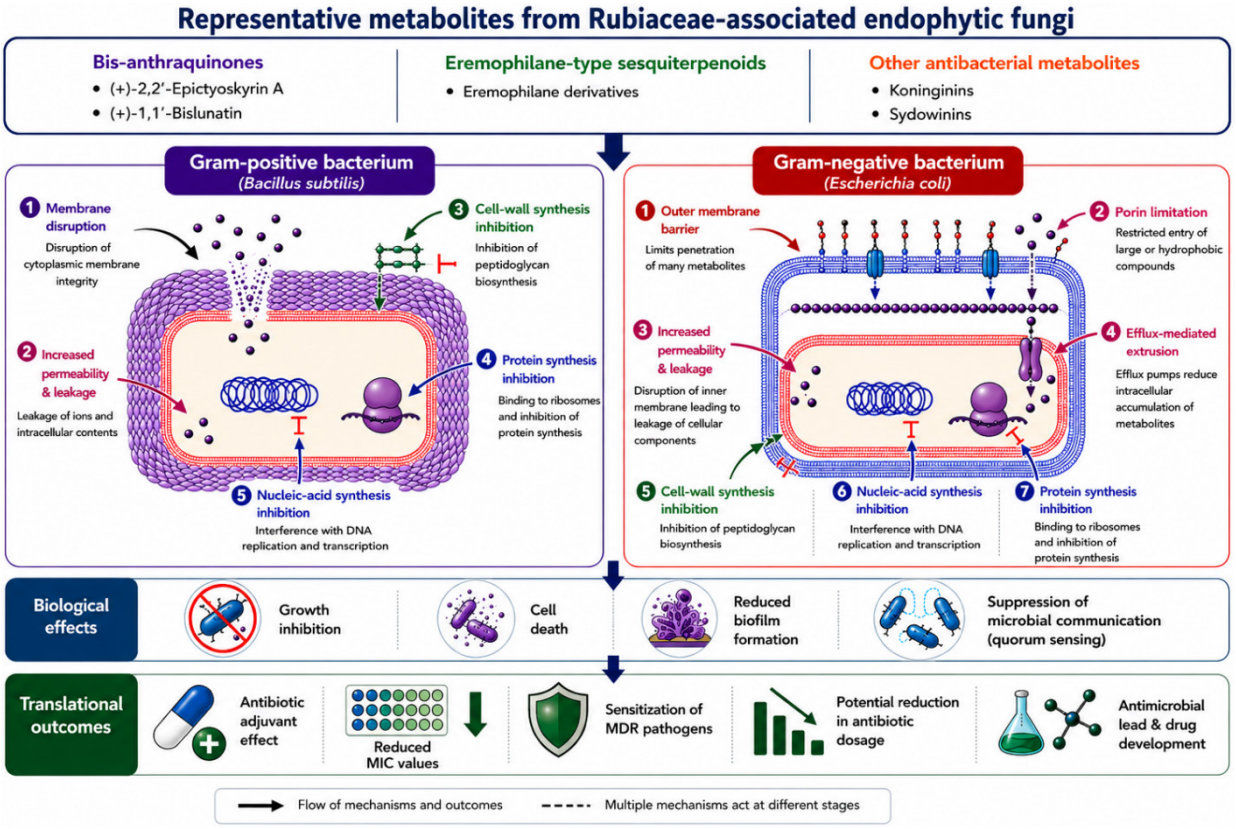

FIGURE 3. Proposed antimicrobial mechanisms and translational outcomes of metabolites from Rubiaceae plant-associated endophytic fungi. Representative metabolites target multiple cellular processes in Gram-positive and Gram-negative bacteria, including membrane integrity, cell-wall biosynthesis, nucleic-acid synthesis, protein synthesis, biofilm formation and microbial communication. These activities may result in growth inhibition, cell death, enhanced susceptibility of multidrug-resistant pathogens and the development of novel antimicrobial and antibiotic-adjuvant candidates

While discovering novel metabolites with low minimum inhibitory concentrations remains the fundamental first step in antimicrobial bioprospecting, relying solely on *in vitro* potency (Structure–Activity Relationship, SAR) is insufficient because antimicrobial potency alone cannot predict tissue exposure, therapeutic selectivity, or clinical efficacy. Up to 90% of clinical drug development fails when *in vivo* tissue exposure and selectivity are neglected, thus, quantitative assessments of Rubiaceae plant-associated endophytic fungal metabolites should evolve toward the Structure-Tissue Exposure/Selectivity-Activity Relationship (STAR) framework to more accurately predict clinical dose, efficacy, and toxicity (Sun et al. 2022).

CONCLUSION

Rubiaceae plant-associated endophytic fungi represent a valuable yet underexplored resource for antimicrobial drug discovery. Evidence synthesized in this review demonstrates that these microorganisms produce structurally diverse secondary metabolites with antibacterial, antifungal, and antimycobacterial activities, highlighting their potential as sources of future antimicrobial leads. The metabolic interplay between Rubiaceae hosts and their associated endophytes provides a strong biological basis for the generation of chemically diverse bioactive compounds. Nevertheless, the translation of these metabolites into clinically relevant candidates remains limited by insufficient selectivity data, inadequate *in vivo* validation, incomplete pharmacokinetic characterization, and challenges associated with large-scale production. Future progress will depend on integrating cultivation optimization, biosynthetic gene cluster activation, genome-guided discovery, and metabolomic approaches with standardized efficacy and safety assessment. Such an integrated strategy will improve candidate prioritization and accelerate the translation of Rubiaceae plant-associated endophytic fungal metabolites into novel antimicrobial agents and antibiotic adjuvants for combating antimicrobial resistance.

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