One Health Microbiology & Infection

OPEN ACCESS

eISSN:3054-5161

Review article

Plant-derived antibiotic adjuvants against drug-resistant ESKAPE pathogens: a critical narrative review

Umesha Silva[1], Chamith Chandrakumara[2], Kasun Suraweera[3], Nekadage D. A. Wageesha[4], Laura-Mădălina Lixandru[5], and Dulmini N. Sapugahawatte[5]*

[1]Riga International School of Economics and Business Administration, Riga, Latvia
[2]Department of Management and Administration, EKA University of Applied Sciences, Riga, Latvia
[3]Riga Technical University, Ķīpsalas iela 6a, Kurzemes rajons, Rīga, Latvia
[4]Department of Biochemistry, Faculty of Medicine, Sabaragamuwa University of Sri Lanka, Ratnapura, Sri Lanka
[5]Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia

***Corresponding author**: Dulmini N. Sapugahawatte, dulmini.sapugahawatte@emu.ee
Article history: Received 3 September 2026; Accepted 5 November 2026; Available online 10 November 2026
Article information: Silva U et al., 2026. *One Health Microbiol. Infect*. Vol. 2, No. 1: 283-294. https://doi.org/10.66585/ohmi.2026.2.0037

Abstract

Antimicrobial resistance in ESKAPE pathogens has intensified interest in antibiotic-adjuvant strategies that restore the activity of existing drugs rather than relying exclusively on *de novo* antibiotics. Plant-derived molecules are attractive in this context because chemically defined alkaloids, polyphenols, lignans, terpenoids, and related natural products can interfere with bacterial resistance systems, including multidrug efflux, outer-membrane integrity, lipid A modification, metallo-β-lactamase activity, biofilm formation, and quorum-regulated virulence. This critical narrative review evaluates plant-derived compounds as antibiotic adjuvants against drug-resistant ESKAPE pathogens, emphasizing the distinction between mechanistic plausibility and translational readiness. The literature base comprises *in vitro* susceptibility and synergy studies, time-kill experiments, biochemical and genetic mechanism studies, invertebrate infection models, and mammalian models. High-priority claims were cross-checked against the cited primary literature, and a targeted update through August 2026 was used to capture recent developments. The most persuasive preclinical examples are not uniformly distributed across phytochemical classes; rather, they cluster around specific resistance mechanisms and antibiotic-rescue problems. These include berberine-sulbactam combinations against multidrug-resistant *Acinetobacter baumannii*, *mcr*-1-directed or membrane-sensitizing adjuvants that restore polymyxins, NDM-1 inhibitors that protect carbapenems, and alkaloids that reverse tmexCD1-toprJ1-mediated tigecycline resistance. However, most evidence remains preclinical, and apparently strong *in vitro* synergy may depend on phytochemical concentrations that are difficult to achieve systemically. Future development therefore requires exposure-aware pharmacokinetics/pharmacodynamics (PK/PD) studies, rigorous toxicology, standardized compound characterization, resistance-evolution experiments, and infection models designed around clinically plausible dosing. Plant-derived compounds should currently be regarded primarily as a source of antibiotic-adjuvant scaffolds and mechanistic probes, with a smaller subset meriting structured translational development.

Keywords: Antibiotic adjuvants, Antimicrobial resistance, ESKAPE pathogens, Plant-derived compounds, Review

Introduction

Antibiotic resistance is increasingly defined not only by the emergence of resistance to individual drugs, but also by the convergence of multiple resistance mechanisms within highly adapted clinical pathogens. The ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and Enterobacter spp.) includes several organisms that most effectively combine healthcare-associated transmission, virulence, and multidrug resistance (MDR) [1, 2, 3]. Throughout this review, "*Enterobacter* spp." refers specifically to the historical ESKAPE member, whereas "Enterobacterales" (and, in older source material, "*Enterobacteriaceae*") is used when the underlying evidence, such as most *mcr*- and NDM-mediated resistance studies, was generated across the broader order that includes *Escherichia coli*, *Klebsiella* and other genera and is not restricted to the ESKAPE species; the broader term is retained where narrowing it to *Enterobacter* spp. alone would misrepresent the organisms actually studied. The 2024 World Health Organization (WHO) Bacterial Priority Pathogens List does not use the ESKAPE acronym as its organizing framework, but it independently places carbapenem-resistant *A. baumannii* and carbapenem-resistant Enterobacterales in the critical tier, and vancomycin-resistant *E. faecium* (VRE), carbapenem-resistant *P. aeruginosa* and methicillin-resistant *S. aureus* (MRSA) among high-priority threats [4, 5].

Forecasts of the global bacterial AMR burden reinforce the need for strategies that preserve or recover the usefulness of existing drugs. Global research on antimicrobial resistance (AMR) estimates that by 2050, approximately 1.91 million deaths annually could be attributable to bacterial AMR and 8.22 million associated with bacterial AMR [6]. At the same time, the antibacterial pipeline may be narrowing, with WHO reporting 90 antibacterial products in clinical development as of 15 February 2025 (50 traditional and 40 non-traditional candidates), down from 97 in the previous reporting cycle [7]. Pipeline size alone does not indicate therapeutic adequacy, and the limited number of genuinely innovative

Infinity

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candidates active against critical-priority Gram-negative bacteria remains a central concern. A single-cycle reduction of this magnitude does not, on its own, establish a structural contraction of the pipeline, since year-to-year counts are also influenced by company mergers and portfolio decisions, regulatory milestones, and reporting-cycle timing (see [8], on economic barriers to antibacterial development); the concern is better supported by the sustained shortage of candidates with genuinely new mechanisms of action against critical-priority Gram-negative pathogens across successive WHO reporting cycles than by the single-year change alone. These development difficulties are one of the reasons this review focuses on adjuvant strategies: because they aim to restore or extend the useful life of antibiotics that already exist, they can, in principle, deliver benefit on a shorter timeline than *de novo* antibiotic discovery and can complement, rather than substitute for, efforts to expand the pipeline itself.

One approach is to treat resistance mechanisms themselves as drug targets. In this model, an adjuvant need not be strongly antibacterial on its own. Instead, it can increase intracellular antibiotic exposure, block a resistance enzyme, reverse an adaptive cell-envelope phenotype, or dismantle a biofilm-associated barrier. Plant secondary metabolites are particularly relevant because their structural diversity has yielded compounds that interact with membrane lipids, transport proteins, enzymes and regulatory systems [9, 10, 11]. Their value is therefore better judged by whether they restore antibiotic activity at experimentally and pharmacologically plausible concentrations than by whether a crude plant extract inhibits bacterial growth.

This distinction changes the scientific question. The relevant issue is not whether plants contain antibacterial molecules, a proposition supported by a large and heterogeneous literature, but which plant-derived molecules have sufficiently coherent evidence to justify advancement as antibiotic adjuvants. Accordingly, this review organizes the evidence around resistance mechanisms, antibiotic rescue, and translational depth rather than botanical taxonomy.

A caveat about scope should be stated explicitly rather than left implicit. Although the review is formally organized around all six ESKAPE genera, the underlying evidence is not evenly distributed across them: the plant-adjuvant literature A caveat about scope retrieved for this review is heavily concentrated on *A. baumannii*, *K. pneumoniae* and *P. aeruginosa*, with a smaller but still substantial body of work on *S. aureus* and *E. faecium*, and comparatively few dedicated plant-adjuvant studies in *Enterobacter* spp. specifically. This pattern most likely reflects the distribution of the available primary literature rather than a deliberate editorial choice, but it means the review should be read as most informative for the three most heavily studied genera; the pathogen(s) addressed by each strategy are indicated throughout the text.

Critical narrative review approach and appraisal framework

The review strategy searched PubMed/MEDLINE, Scopus, and Web of Science for literature published from January 2000 to August 2026, using combinations of ESKAPE pathogen names and resistance phenotypes with terms describing plant-derived antimicrobials, phytochemicals, essential oils, efflux-pump inhibition, synergy, biofilm, quorum sensing, *mcr*-1, NDM-1, and membrane permeabilization. Eligible evidence included quantitative antimicrobial and synergy studies, mechanistic studies, animal infection models, and computational analyses linked to experimentally relevant targets. The synthesis was organized around resistance mechanisms, and high-priority mechanistic and translational claims were independently cross-checked against primary articles and authoritative WHO sources. A targeted literature update through August 2026 was also conducted for recent adjuvant studies.

To make the process reproducible, the final search across all three databases was completed on 31 August 2026. Studies were included if they reported quantitative antimicrobial or synergy data, biochemical or genetic mechanistic evidence, or invertebrate/mammalian infection-model outcomes for a plant-derived compound tested against an ESKAPE pathogen or a resistance determinant relevant to this group (e.g., *mcr* genes, NDM-1, RND-family efflux systems, tmexCD1-toprJ1). Conference abstracts without a subsequent peer-reviewed publication, non-English records that could not be verified against a translated abstract, and studies without a resistance-relevant comparator (e.g., a compound tested only against fully susceptible reference strains) were excluded. As detailed below, no numerical weighting score was applied to individual studies; instead, each piece of evidence was assigned to one of seven qualitative evidence layers, and claims were required to be internally consistent with the layer of evidence actually available.

As this is a critical narrative review rather than a systematic review, the search supports a critical synthesis rather than estimating pooled effects or claiming exhaustive retrieval. Exact study counts are not used as evidence of completeness. Instead, the review emphasizes internal consistency between the reported phenotype, the proposed mechanism, the experimental model, and the cited source.

To prevent *in vitro* synergy from being conflated with therapeutic readiness, evidence is described along a translational continuum: (i) minimum inhibitory concentration (MIC) or growth-inhibition data; (ii) reproducible synergy by checkerboard or equivalent

assays; (iii) confirmation by time-kill or related kinetic methods; (iv) direct mechanistic evidence using biochemical, genetic or biophysical methods; (v) invertebrate infection models; (vi) mammalian efficacy; and (vii) exposure, pharmacokinetics/pharmacodynamics (PK/PD) and toxicology data sufficient to judge whether active concentrations are clinically plausible. Computational docking is treated as hypothesis-generating unless experimental target validation supports it.

Because it is used throughout this review, the term "synergy" is applied only where a study demonstrates a reproducible, defined statistical or microbiological interaction between the compound and the antibiotic, most commonly a checkerboard fractional inhibitory concentration index (FICI) ≤ 0.5 [12] or a ≥ 2-$\log_{10}$ improvement over the most active single agent in a time-kill assay at a stated comparator and time point [13]. "Potentiation" or "antibiotic rescue" is used for the broader phenomenon in which a compound restores or enhances antibiotic activity without necessarily meeting a formal synergy threshold or without the interaction having been tested by checkerboard or time-kill methods, for example, when only an MIC shift or improved *in vivo* outcome is reported. "Restoration of susceptibility" is reserved for cases in which a resistant isolate is shown to cross a recognized clinical or epidemiological breakpoint in the presence of the adjuvant. These terms are not interchangeable: a reduction in MIC or an increase in the proportion of susceptible isolates does not, by itself, establish synergy, and checkerboard and time-kill assays test related but distinct aspects of an interaction and are not treated as equivalent evidence in this review. Table 1 summarizes the evidence levels used throughout this review and clarifies the conclusions that can and cannot reasonably be drawn from each experimental stage.

Table 1: Translational evidence framework used in this critical narrative review to appraise plant-derived antibiotic adjuvants against drug-resistant ESKAPE pathogens.

Evidence layer	What it establishes	What it does not establish
In vitro MIC/checkerboard	Activity and possible interaction at fixed concentrations	Mechanism, durability, exposure feasibility or *in vivo* benefit
Time-kill / dynamic assays	Kinetic confirmation of enhanced killing	Human-relevant PK/PD or tissue efficacy
Mechanistic validation	A plausible or demonstrated target/pathway	Therapeutic feasibility by itself
Galleria mellonella or other invertebrate models	Whole-organism proof of concept	Mammalian PK, metabolism or immune context
Mouse infection model	Preclinical efficacy under defined dosing	Human exposure, safety or clinical effectiveness
PK/PD and toxicology	Whether effective exposure and safety may coexist	Clinical benefit without human trials
Human studies	Clinical safety/efficacy in the studied context	Generalizability beyond the studied population

MIC = minimum inhibitory concentration; PK = pharmacokinetics; PD = pharmacodynamics.

Plant-derived compounds as antibiotic resistance modulators

Before turning to individual mechanisms, it is useful to outline the main chemical classes from which the adjuvant candidates discussed in this review are drawn. Alkaloids, including the isoquinoline berberine, the steroidal alkaloid conessine, the indole alkaloid gramine, and the β-carboline harmaline, are of interest mainly as efflux pump inhibitors and membrane-perturbing agents [14, 15, 16, 17]. Flavonoids, including quercetin, kaempferol, myricetin and luteolin, are chemically diverse; in the studies discussed here, they have been linked to altered iron homeostasis [18], membrane injury and reduced expression of resistance genes [19]. The neolignans honokiol and magnolol, together with the bibenzyl gigantol, have been linked to more specific effects on *mcr*-1 or PmrA/B signaling [20, 21, 22]. Phenylpropanoids and terpenoids, such as eugenol, carvacrol, thymol and celastrol, are generally lipophilic; eugenol potentiates colistin largely by increasing membrane permeability [23], whereas celastrol acts on a defined target, the cell-division protein FtsZ [24]. Coumarin and the stilbene resveratrol illustrate a different situation: resveratrol has been tested against a broad panel of colistin-resistant strains, but its mechanism remains unresolved [25], and coumarin has so far been supported mainly by phenotypic and *in silico* data [26]. Essential oils and crude extracts, such as thyme and clove oils, are complex mixtures that complicate attribution of activity to any one molecule [27]. This classification is provided as orientation only; as the sections below illustrate, therapeutic relevance tracks the resistance mechanism engaged rather than chemical class membership.

Plant-derived compounds are chemically heterogeneous, and grouping them by class (alkaloids, phenolics, terpenoids, organosulfur compounds or lignans) is useful for chemistry but less useful for therapeutic prioritization. Two compounds from the same class may act on different bacterial targets, while structurally unrelated compounds may converge on the same resistance phenotype. A resistance-centered framework therefore provides a more coherent route from observation to development.

For Gram-negative bacteria in the ESKAPE group, key recurring problems include restricted entry across the outer membrane, active multidrug efflux, β-lactamase-

mediated drug destruction, lipid A remodeling that reduces polymyxin binding, and biofilm-associated tolerance. In Gram-positive pathogens in the same group, target alteration, cell-envelope remodeling and multidrug efflux dominate several clinically important phenotypes. Plant-derived molecules can, in principle, intervene at each level, but the strength of evidence varies sharply.

An important practical implication is that direct antibacterial potency should not be used as the sole screening criterion. Berberine, for example, has weak intrinsic activity against many MDR *A. baumannii* isolates, yet it can markedly reduce antibiotic MICs and enhance sulbactam activity in a murine thigh infection model [28]. Conversely, a compound with a low MIC may still be a poor adjuvant if its effect is nonspecific, cytotoxic or achievable only at implausible systemic concentrations. Figure 1 summarizes these principal mechanisms.

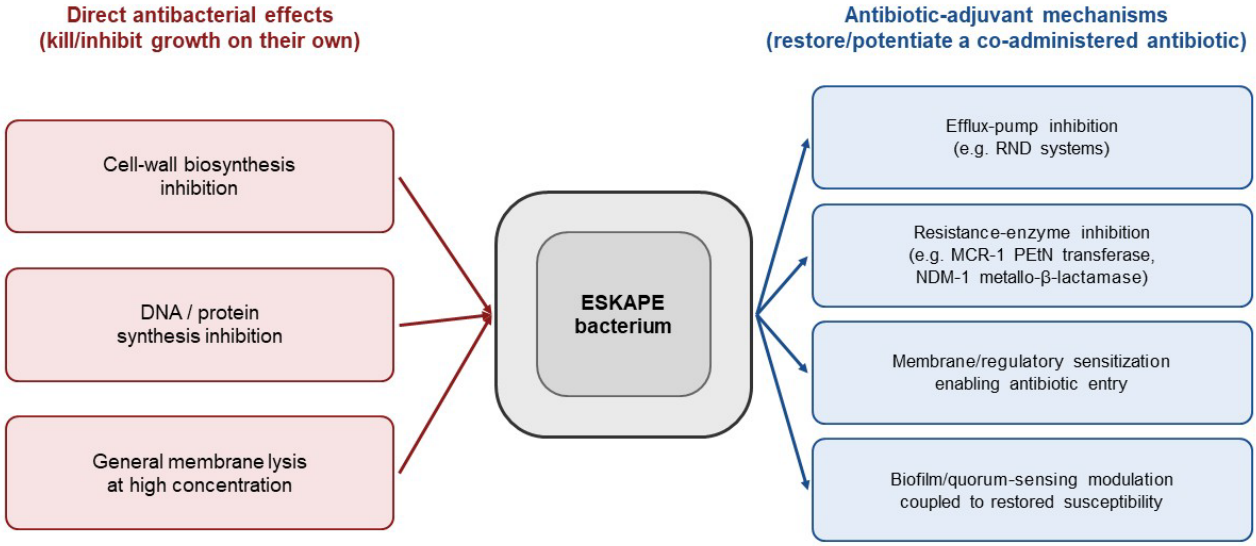


Figure 1: Mechanistic landscape of plant-derived compounds investigated against drug-resistant ESKAPE pathogens. The schematic separates mechanisms that are directly antibacterial (cell-wall biosynthesis inhibition, DNA/protein synthesis inhibition, non-specific membrane lysis at high concentration), shown on the left, from mechanisms that are relevant to this review because they restore or potentiate a co-administered antibiotic (efflux-pump inhibition, resistance-enzyme inhibition, membrane/regulatory sensitization, and biofilm/quorum-sensing modulation coupled to restored susceptibility), shown on the right. Panels are conceptual and do not imply that every phytochemical class acts uniformly through the indicated mechanism. For *mcr*-1, the relevant resistance mechanism is phosphoethanolamine modification of lipid A; adjuvants may inhibit *mcr*-1 activity or otherwise alter polymyxin-resistance pathways. Abbreviations: *mcr*-1 = mobilized colistin resistance protein 1; NDM-1 = New Delhi metallo-β-lactamase 1; PEtN = phosphoethanolamine; RND = resistance-nodulation-division.

Resistance mechanisms targeted by plant-derived antibiotic adjuvants

Multidrug efflux: a tractable target with important caveats

Efflux pumps are attractive adjuvant targets because inhibiting them can increase the intracellular concentration of multiple antibiotic classes simultaneously. RND-family systems are especially important in Gram-negative ESKAPE pathogens. However, many compounds described as efflux-pump inhibitors also perturb membranes or the proton motive force, making target specificity difficult to establish. Claims of pump inhibition should therefore be strongest when supported by transporter mutants, accumulation assays, or direct interaction data rather than by MIC reduction alone [29].

Berberine illustrates both the opportunity and the mechanistic complexity. In *P. aeruginosa*, Morita et al. showed that berberine attenuates MexXY-mediated aminoglycoside resistance [14]. In MDR *A. baumannii*, Li et al. found broad antibiotic sensitization and an interaction with the *ade*ABC system; the study also demonstrated enhanced sulbactam efficacy in a neutropenic murine thigh model [28]. The latter result provides stronger translational support than checkerboard synergy alone, although the exposure used was parenteral and cannot be extrapolated directly to oral berberine products [14, 28].

Conessine provides a second well-characterized example. In *A. baumannii*, conessine potentiated novobiocin and rifampicin against extensively drug-resistant (XDR) isolates. In *P. aeruginosa*, studies using MexAB-OprM-overexpressing and mexB-deletion strains, together with dye-accumulation and membrane-permeability assays, supported inhibition of MexAB-OprM and possibly additional efflux systems, without evidence that conessine simply acted as an outer-

membrane permeabilizer. In a *Galleria mellonella* model, conessine-containing regimens improved antibiotic activity against MDR *P. aeruginosa* [15, 30, 31]. These data justify describing conessine as a mechanistically supported preclinical efflux modulator, but not as a clinically validated efflux pump inhibitor.

Restoring polymyxins: mcr inhibition, lipid A biology and membrane sensitization

Polymyxin resistance has produced one of the most extensive plant-adjuvant evidence clusters in the literature. Colistin initially binds the negatively charged lipid A component of lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria, and resistance can arise through chromosomally encoded regulatory pathways or plasmid-mediated *mcr* genes that alter lipid A charge and reduce polymyxin binding [32]. *mcr*-1 is a phosphoethanolamine (PEtN) transferase that catalyzes the transfer of PEtN from phosphatidylethanolamine to lipid A, thereby decreasing the net negative charge of LPS and reducing its affinity for polymyxins [33]. This mechanistic diversity offers several potential adjuvant strategies, including direct inhibition of *mcr* activity, modulation of resistance-gene expression or regulatory pathways, and enhancement of membrane susceptibility to polymyxins.

Honokiol is among the best mechanistically characterized examples. Guo et al. reported that honokiol restored polymyxin susceptibility in *mcr*-1-positive Enterobacterales, reduced polymyxin MICs, interacted with the *mcr*-1 active site in mechanistic analyses, and improved treatment outcomes in infected mice. The study supports direct inhibition of *mcr*-1 activity rather than suppression of *mcr*-1 expression [20].

A 2025 study showed that magnolol interferes with PmrA/B signaling by disrupting PmrA's binding to target DNA, thereby limiting protective LPS modifications and increasing colistin binding. Hence, magnolol acts differently, and this effect was demonstrated in both *in vitro* and *in vivo* experiments [22]. Gigantol provides another route: it restored colistin activity against bacteria carrying *mcr*-1, *mcr*-3, or *mcr*-8, reduced *mcr*-1 expression and activity, and showed efficacy in animal infection models [21].

Other phenolics potentiate colistin without a single defined enzyme target. Quercetin combined with colistin showed checkerboard and time-kill synergy against colistin-resistant *E. coli* and *K. pneumoniae* clinical isolates. This combination was associated with reduced *mcr*-1 or *mgr*B transcript levels in the relevant organisms, increased membrane injury, and improved outcomes *in vivo* [19]. Natural flavonoids, including myricetin, luteolin, and 7,8-dihydroxyflavone, have also been shown to disrupt bacterial iron homeostasis, thereby increasing colistin efficacy [18]. The naphthoquinone β,β-dimethylacrylalkannin has likewise been reported to restore colistin efficacy against *mcr*-mediated and two-component-system-mediated resistance in Gram-negative bacteria through membrane disturbance [34]. These mechanisms should not be collapsed into a generic statement that all flavonoids 'inhibit *mcr*-1'.

Resveratrol is a useful example of strong *in vitro* evidence that has not yet translated into clinical application. Cannatelli et al. tested 21 Gram-negative strains with diverse colistin-resistance backgrounds. Resveratrol itself had little intrinsic antibacterial activity, but its combination with colistin showed broad checkerboard synergy and bactericidal activity in time-kill experiments against many resistant strains. The mechanism was not established, and the effective resveratrol concentrations were high. It is therefore best regarded as a robust *in vitro* potentiator rather than a near-clinical candidate [25].

Eugenol similarly potentiated colistin against resistant *P. aeruginosa* and *K. pneumoniae*, with checkerboard and time-kill confirmation, increased membrane permeability, antibiofilm effects, limited cytotoxicity in RAW 264.7 cells at the tested concentrations, and improved survival in *Galleria mellonella*. This whole-organism evidence is valuable, but the invertebrate model is not a substitute for mammalian PK/PD [23, 24].

Protecting carbapenems from NDM-1

NDM-1 is a zinc-dependent metallo-β-lactamase that hydrolyses carbapenems and many other β-lactams. The most persuasive natural-product adjuvants in this domain are those that demonstrate both enzyme inhibition and restoration of antibiotic activity in NDM-producing bacteria.

Embelin is a relatively well-supported *in vitro* example within this mechanistic category. Ning et al. reported direct NDM-1 inhibition, with biochemical potency in the low micromolar range, molecular modeling consistent with an interaction near the zinc-containing active site, and marked restoration of carbapenem activity across NDM-1-positive *E. coli, K. pneumoniae,* and *A. baumannii*. The study did not establish efficacy in an infection model, so embelin should be described as a mechanistically supported *in vitro* carbapenem adjuvant rather than an *in vivo*-validated therapy [35].

Isoliquiritin enhanced meropenem activity against NDM-1-positive *Enterobacteriaceae in vitro*, supporting its role as a biochemical and microbiological lead rather than an *in vivo*-validated adjuvant [36]. Morin, by contrast, was evaluated with meropenem in NDM-1-producing *E. coli* using checkerboard and time-kill assays as well as a mouse infection model, where combination treatment reduced bacterial burden and inflammatory/tissue injury measures [37].

Coumarin has been investigated as a carbapenemase inhibitor against carbapenem-resistant *K. pneumoniae* using phenotypic, molecular, and *in silico* approaches. Such studies can identify useful leads, but computational binding should not be presented as equivalent to validated *in vivo* inhibition [26].

Biofilms, quorum sensing and anti-virulence effects

Biofilm disruption and quorum-sensing interference are frequently invoked as plant-antimicrobial mechanisms, but this area is especially vulnerable to overgeneralization. Reduced crystal-violet staining, lower expression of a regulatory gene, and improved antibiotic killing represent different levels of evidence and should not be treated as interchangeable.

Because these distinctions are often blurred in the primary literature, it is useful to clarify what different assay outcomes do and do not demonstrate. At least four distinct effects are reported under the umbrella of "anti-biofilm" or "anti-quorum-sensing" activity, and they are not equivalent: (i) inhibition of biofilm formation, typically measured by reduced initial attachment or crystal-violet biomass in a prophylactic assay; (ii) reduction of an already-established, mature biofilm, a materially harder outcome that requires the compound to penetrate an existing matrix; (iii) increased antibiotic susceptibility of biofilm-embedded bacteria, which is the outcome most directly relevant to antibiotic adjuvancy because it demonstrates restored drug activity in the biofilm state rather than biofilm disruption per se; and (iv) attenuation of quorum-sensing-regulated virulence factors (e.g., pyocyanin, proteases, motility) that can reduce pathogenicity independently of any change in antibiotic sensitivity. A compound that inhibits biofilm formation or suppresses virulence-factor expression is not, by that evidence alone, an antibiotic adjuvant; it becomes relevant to this review's central question only when it directly demonstrates increased susceptibility of biofilm-associated bacteria to a co-administered antibiotic. Where the studies discussed below report only formation-inhibition, mature-biofilm reduction, or quorum-sensing/virulence endpoints without a paired antibiotic-susceptibility readout, they are presented as complementary infection-control strategies rather than as validated antibiotic-adjuvant evidence.

Chlorogenic acid has been evaluated against carbapenem-resistant, hypervirulent *K. pneumoniae* using biofilm, virulence, and quorum-sensing-related transcriptional assays, as well as a *Galleria mellonella* model. The findings support antivirulence and anti-biofilm effects in *K. pneumoniae*, while organism-specific quorum-sensing mechanisms should not be generalized to unrelated pathogens [38].

Celastrol provides a distinct Gram-positive example. As an FtsZ inhibitor, it demonstrated antibacterial and antibiofilm activity against enterococci and synergy with vancomycin against VRE *in vitro* and in *vivo* [24]. This illustrates the importance of matching mechanistic claims to the specific organism-antibiotic pairing tested experimentally.

Antibiotic restoration against drug-resistant ESKAPE pathogens

Polymyxin rescue

Previously, we described the individual mechanisms by which plant-derived compounds restore polymyxin activity; this section compares the same compounds in terms of evidence strength, resistance determinants studied, and translational maturity, rather than re-describing how each works. Among plant-derived adjuvant strategies, polymyxin rescue has the broadest cluster of mechanistically diverse preclinical evidence. Several independent approaches converge on the same phenotype, such as increased colistin binding or intracellular damage in otherwise resistant Gram-negative bacteria. Direct or functionally supported *mcr*-related strategies include honokiol, osthole, and gigantol; PmrA/B-directed modulation is represented by magnolol; and membrane/regulatory sensitization is represented by quercetin, kaempferol, eugenol, resveratrol, and other phenolics [19, 20, 22, 21, 39, 40].

The evidence should nevertheless be stratified. Honokiol, magnolol, gigantol, and quercetin have mammalian *in vivo* support alongside mechanistic work. Eugenol has *Galleria mellonella* evidence. Capsaicin synergized with colistin against colistin-resistant *A. baumannii in vitro* and in an *in vivo* model [41]. Resveratrol has unusually broad and careful *in vitro* testing but no mammalian validation in the cited study. Essential oils of thyme and clove increased susceptibility of *A. baumannii* and *K. pneumoniae* to colistin, but the primary study evaluated the oils rather than establishing that isolated thymol and carvacrol alone accounted for all observed fold changes; claims should therefore preserve the tested material [27].

Carbapenem rescue

Carbapenem rescue can arise through two distinct mechanisms: protection of the β-lactam from enzymatic hydrolysis or increased antibiotic uptake in organisms whose resistance is driven by permeability/efflux. The first is exemplified by embelin, isoliquiritin and morin against NDM-1-producing organisms. The second is more heterogeneous and should not be described as 'carbapenemase inhibition' without direct evidence.

Curcumin has been reported to synergize with meropenem against carbapenem-resistant *K. pneumoniae*

in vitro [42], but the study does not support a specific NDM-directed mechanism. Plumbagin has been studied as a gentamicin potentiator against gentamicin-resistant, carbapenem-resistant *K. pneumoniae*; it enhanced gentamicin uptake by altering bacterial energetics and proton motive force [43]. This is therefore better interpreted as aminoglycoside potentiation in carbapenem-resistant *K. pneumoniae* rather than as direct carbapenemase inhibition.

Tigecycline rescue

The plasmid-mediated tmexCD1-toprJ1 RND efflux system has emerged as a particularly compelling target for adjuvant discovery. Gramine, an indole alkaloid, sensitized tmexCD1-toprJ1-positive *K. pneumoniae* to tigecycline, increased intracellular tigecycline accumulation and restored activity *in vivo* [16]. Harmaline has an even more detailed mechanistic dataset: it increased tigecycline activity by 4- to 32-fold, disrupted TMexCD1-TOprJ1 function through effects on the pump and proton motive force, altered membrane and metabolic homeostasis, and improved outcomes in both *Galleria mellonella* and mouse infection models [17].

These studies are important because they link a defined mobile resistance determinant to a defined antibiotic-rescue phenotype. They also show that evidence quality should be assessed at the level of a compound-resistance mechanism-antibiotic combination rather than by phytochemical class.

Restoring activity against Gram-positive ESKAPE pathogens

Plant-derived adjuvant evidence against Gram-positive ESKAPE pathogens is substantial but less dominated by a single resistance pathway. The classic work of Stermitz et al. demonstrated that 5′-methoxyhydnocarpin from Berberis potentiated berberine against *S. aureus* by inhibiting a multidrug efflux pump, providing an early mechanistic demonstration that a plant can contain both an antibacterial compound and a resistance-modifying partner [44].

For enterococci, celastrol provides a more recent example with direct relevance to VRE. It inhibits *FtsZ*, shows antibacterial and antibiofilm activity, and synergizes with vancomycin *in vitro* and *in vivo* experiments [24]. Gambogic acid has also been reported as a vancomycin adjuvant against VRE *in vitro* and *in vivo* [45]. Owen et al. developed a vancomycin-carvacrol-cuminaldehyde combination against *E. faecium* through formulation and an *in vivo* model, illustrating the value of moving beyond checkerboard testing to formulation-aware validation [46].

Synergy criteria and the validation pathway

As defined in previous sections, antibiotic synergy is operationally identified by a fractional inhibitory concentration index (FICI) ≤ 0.5 from checkerboard assays or by a ≥ 2-$\log_{10}$ reduction in bacterial counts beyond the most active agent in time-kill kinetics [12, 13]. Beyond providing reproducible thresholds, these assays help address the mechanistic question of how and why combinations of plant compounds and antibiotics can be more effective against MDR ESKAPE pathogens than either agent alone. Mechanistically, phytochemicals have been reported to potentiate antibiotics by increasing intracellular drug concentration, permeabilizing membranes, inhibiting resistance enzymes, disrupting biofilms, or acting on complementary pathways (Figure 1) [47]. Figure 2 summarizes the sequence of assays used to establish and validate these interactions.

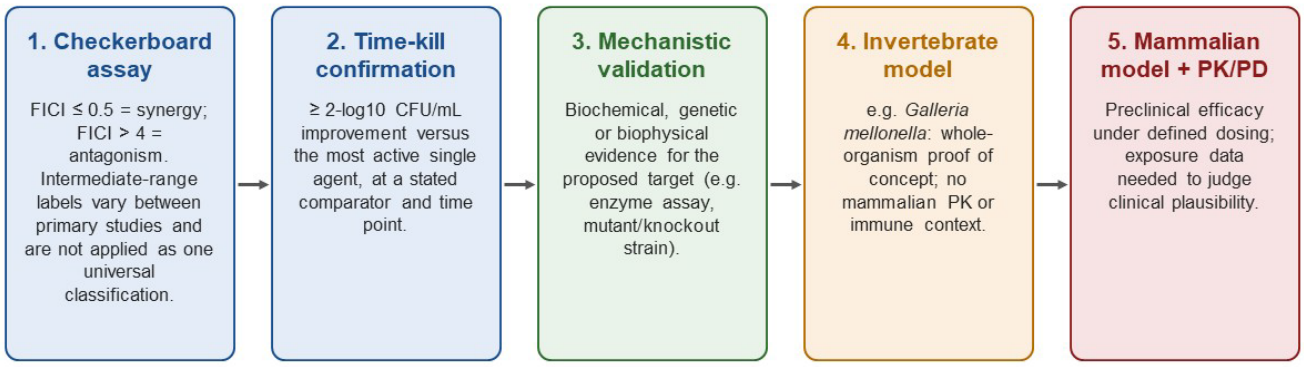


Figure 2: Stepwise synergy-testing and translational-validation pathway for plant-derived antibiotic adjuvants. Evidence tiers are shown as successive, non-equivalent steps: (1) checkerboard assay, in which synergy is defined as a fractional inhibitory concentration index (FICI) ≤ 0.5 and antagonism as FICI > 4 [12]. Intermediate-range labels such as "additive" or "indifferent" are not applied uniformly across primary studies and are therefore not shown as a single classification; (2) time-kill confirmation, in which synergy is defined as a ≥ 2-$\log_{10}$ CFU/mL improvement of the combination relative to the most active single agent at a stated comparator and time point; (3) mechanistic validation; (4) invertebrate infection model (e.g., *Galleria mellonella*); and (5) mammalian infection model with pharmacokinetic/pharmacodynamic (PK/PD) analysis. Reaching an earlier tier does not imply that a later tier has been met, and none of these stages establishes clinical efficacy in humans. CFU, colony-forming units.

Translational evidence and challenges

Priority candidates: summary of preclinical evidence

The compound- antibiotic combinations with the most developed preclinical evidence, discussed in the previous sections of this review, are shown in Table 2 and appraised against the framework in Table 1. To make the "Critical appraisal" column comparable across candidates, the qualitative labels used throughout Table 2 follow a single, explicit, three- tier rule applied uniformly: candidates are assigned "high preclinical support" only if they combine a defined mechanistic target with demonstrated efficacy in a mammalian infection model; "promising" or "mechanistically strong, translationally intermediate" if they have mechanistic evidence plus, at most, invertebrate (e. g., *Galleria mellonella*) *in vivo* data, or mammalian data without a fully resolved mechanism; and "strong *in vitro* signal" or "useful intermediate evidence" if the evidence is limited to *in vitro* checkerboard, time- kill, or biochemical data without any whole- organism model. Consistent with this rule, the "Highest model" column indicates only the most advanced experimental system reached for a given candidate; it marks model tier, not an independent judgment of overall evidence quality. Thus, a compound with mammalian-model data but an unresolved mechanism is not automatically ranked above a compound with a well-resolved mechanism and only invertebrate-model support. Where known, the specific resistance determinant engaged by each candidate (e. g., *mcr*-1, NDM-1, tmexCD 1- toprJ 1) is named in the "Target problem" column rather than described only as generic MDR, and the main factor currently limiting clinical translation for each candidate is stated in the "Critical appraisal" column. Table 2 is restricted to single, chemically defined compounds or a defined compound-antibiotic pair; complex mixtures such as essential oils and crude extracts (e. g., the thyme and clove oils) are deliberately excluded from this standardized ranking and are instead discussed narratively, so that variability inherent to a mixture is not collapsed into a single-compound evidence tier.

Translational challenges and limitations

Three recurring problems limit interpretation of phytochemical-antibiotic studies, and the first is concentration. A combination can be statistically and microbiologically synergistic but require phytochemical exposures unlikely to be achieved safely at the infection site. Resveratrol is illustrative: the *in vitro* synergy is real, but concentrations used in time-kill experiments are much higher than typical systemic exposure after conventional oral dosing. Similar exposure questions apply to poorly soluble compounds such as honokiol and poorly bioavailable compounds such as curcumin.

The second problem is chemical definition, as crude extracts and essential oils vary with plant chemotype, geography, harvest conditions, and processing and extraction. A result obtained with a characterized essential oil should not automatically be generalized to a presumed major constituent. Conversely, purified compounds offer better chemical reproducibility but may lose multi-component interactions present in the original extract. Both types of evidence are useful, but they address different questions.

The third problem is model translation. Checkerboard assays are static and concentration-ratio-dependent. Time-kill assays improve kinetic interpretation but still omit absorption, distribution, metabolism, elimination, and host immunity. *Galleria mellonella* provides a useful whole-organism bridge but does not model human pharmacokinetics. Mammalian models provide stronger evidence, yet many use high parenteral doses or treatment schedules that have not been linked to clinically achievable human exposure.

Safety must therefore be evaluated as an exposure problem rather than by citing that a compound is 'natural' or has prior human use. Berberine, curcumin, resveratrol, and piperine have human exposure histories, but the dose, formulation, and route required for antibacterial adjuvant activity may differ radically from those used in nutraceuticals. Drug-drug interactions are also relevant because an adjuvant intended to accompany a last-line antibiotic will often be used in critically ill patients receiving multiple medications.

Nanocarriers, liposomes and other delivery systems can improve solubility, stability and tissue exposure, but formulation should not be treated as a generic solution to weak pharmacology. A delivery system is valuable only if it increases the probability that the experimentally active concentration ratio can be achieved at the infected tissue without unacceptable toxicity.

Research priorities for a more translational field

First, synergy studies should report full concentration matrices, replicate variability, clinically relevant antibiotic breakpoints where appropriate, and time-kill confirmation for priority combinations. FICI values are useful screening tools but should not be the sole basis for claims of therapeutic rescue.

Second, mechanism claims should be experimentally testable. Efflux inhibition should be supported by accumulation assays and, preferably, transporter genetics; resistance-enzyme inhibition should include biochemical target assays; membrane mechanisms should distinguish generalized lysis from selective sensitization; and docking

Table 2: Priority plant-derived antibiotic adjuvant candidates against drug-resistant ESKAPE pathogens, with the resistance problem addressed, the highest experimental model reached and a critical appraisal of the current evidence.

Compound/ combination	Target problem	Key evidence	Highest model	Critical appraisal	Representative source
Berberine + sulbactam	MDR *A. baumannii* / efflux-associated resistance	Checkerboard; mechanistic *Ade*ABC work; bacterial burden reduction	Mouse thigh infection	High preclinical support; exposure feasibility still unresolved	[28]
Honokiol + polymyxin	*mcr*-1-mediated polymyxin resistance	MIC restoration; target-focused mechanism; *in vivo* efficacy	Mouse infection	High preclinical support for *mcr*-1-positive Enterobacterales	[20]
Magnolol + colistin	PmrA/B-dependent LPS modification	Regulatory mechanism; enhanced colistin binding; *in vivo* efficacy	Mammalian infection model	High preclinical support; pathway-specific	[22]
Gigantol + colistin	*mcr*-1/*mcr*-3/*mcr*-8 resistance	Checkerboard/time-kill; expression and target studies; *in vivo* efficacy	*Galleria mellonella* + mouse	High preclinical support across several *mcr* contexts	[21]
Quercetin + colistin	Colistin-resistant *E. coli* / *K. pneumoniae*	Checkerboard/time-kill; transcript and membrane effects; *in vivo* efficacy	Mouse infection	Promising but mechanism may be multi-factorial	[19]
Harmaline + tigecycline	tmexCD1-toprJ1-positive *K. pneumoniae*	Synergy; pump, PMF, membrane, metabolic mechanism; *in vivo* efficacy	*Galleria mellonella* + mouse	High preclinical support for a defined resistance determinant	[17]
Gramine + tigecycline	Tigecycline-resistant *K. pneumoniae*	Tigecycline accumulation; synergy; *in vivo* restoration	*Galleria mellonella* + mouse	Promising defined antibiotic-rescue strategy	[16]
Morin + meropenem	NDM-1-producing *E. coli*	Checkerboard/time-kill; docking/MD; mouse bacterial burden/tissue endpoints	Mouse infection	Promising NDM-focused lead requiring deeper enzyme/PK validation	[37]
Conessine + antibiotics	RND efflux in *A. baumannii* / *P. aeruginosa*	Synergy; efflux assays; pump-genetic evidence	*Galleria mellonella* model	Mechanistically strong, translationally intermediate	[15, 30, 31]
Resveratrol + colistin	Diverse colistin-resistant Gram-negative bacteria	Broad checkerboard and time-kill testing	*In vitro*	Strong *in vitro* signal; mechanism and exposure remain limiting	[25]
Eugenol + colistin	Colistin-resistant *P. aeruginosa* / *K. pneumoniae*	Checkerboard/time-kill; membrane; biofilm; cytotoxicity	*Galleria mellonella* model	Useful intermediate evidence, not mammalian validation	[23]
Embelin + meropenem	NDM-1-positive Gram-negative bacteria	Enzyme inhibition; MIC rescue across clinical isolates	*In vitro*	Mechanistically supported lead; lacks infection-model validation in cited study	[35]

Note: "Highest model" denotes the most advanced experimental system reported in the representative source(s) and is not a measure of the overall quality of the evidence. Appraisal labels follow the three-tier rule described above and refer to preclinical evidence only; none of the candidates listed has sufficient evidence from controlled human treatment studies to justify routine clinical use for MDR ESKAPE infections. Abbreviations: AdeABC = an RND-type efflux system of *A. baumannii*; LPS = lipopolysaccharide; *mcr* = mobilized colistin resistance; MD = molecular dynamics; MDR = multidrug-resistant; NDM-1 = New Delhi metallo-β-lactamase 1; PMF = proton motive force; RND = resistance-nodulation-division.

should be used to generate testable hypotheses rather than as proof of binding.

Third, candidate ranking should incorporate exposure from the beginning. Before extensive animal testing, investigators should assess whether the active free concentration is chemically and toxicologically plausible. PK/PD experiments should quantify both the antibiotic and the adjuvant at the infection site and test whether the synergy-associated ratio is maintained over time.

Fourth, resistance evolution deserves more attention. An adjuvant that initially restores an antibiotic but rapidly selects for resistance to the adjuvant mechanism may have limited value. Serial passage, mutation-prevention, and genomic analyses should therefore become routine for leading combinations.

Fifth, future studies should prioritize defined clinical problems rather than broad phytochemical screening. Examples include *mcr*-positive Enterobacterales, tmexCD1-toprJ1-positive *K. pneumoniae*, NDM-producing Enterobacterales and VRE. Targeted screening against a known resistance determinant makes mechanistic validation and patient stratification more realistic.

Finally, the field would benefit from reporting standards that separate botanical identity, chemical purity, vehicle, MIC methodology, synergy definition, evidence of mechanism, host model, toxicity, and exposure. Such standardization would make it easier to distinguish reproducible adjuvant biology from isolated screening results.

Conclusions

Plant-derived compounds are a credible source of antibiotic-adjuvant scaffolds against drug-resistant ESKAPE pathogens, but the evidence is much narrower than the size of the phytochemical literature suggests. The strongest preclinical cases arise when a defined compound addresses a specific resistance problem and the evidence progresses beyond a single checkerboard experiment to kinetic, mechanistic, and *in vivo* validation.

Polymyxin rescue currently provides the densest cluster of such examples, including *mcr*-1-directed and membrane-regulatory strategies. Efflux modulation by berberine and conessine, NDM-focused carbapenem protection by compounds such as embelin and morin, and reversal of tmexCD1-toprJ1-mediated tigecycline resistance by gramine and harmaline illustrate other mechanistically coherent routes. These examples support continued development, but they do not yet justify describing plant-derived adjuvants as clinically established solutions for MDR ESKAPE infections.

The most important next step is not to discover more compounds with measurable in vitro activity. It is to determine which existing leads can achieve the required unbound exposure in the required tissue for the required duration, without unacceptable toxicity or drug interactions. A smaller, rigorously prioritized set of candidates with reproducible chemistry, exposure-aware PK/PD, and mammalian efficacy will be more valuable than an expanding catalog of poorly comparable synergy claims.

Declarations and supplementary information

Acknowledgments: Not applicable.

Author contributions: US, CC and AW: data extraction and initial manuscript drafting. KS: figure development. LML: critical revision. DNS: conceptualization, critical revision.

Funding source: Not applicable.

Conflict of interest statement: The authors declare no conflict of interest.

Ethics statement: Not applicable.

Data availability: No specific data were generated in this study.

Supplementary materials: Not applicable.

Declaration regarding the use of generative AI and AI-assisted technologies in the writing process: AI-based tools were used during manuscript preparation to support language editing and structural reorganization; the authors specified, checked, and edited the output. The authors checked scientific claims against cited primary sources and authoritative reports, and remain responsible for the accuracy, interpretation, and final content of the manuscript.

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References

1. Santajit S, Indrawattana N. Mechanisms of antimicrobial resistance in ESKAPE pathogens. Biomed Res Int. 2016;2016:2475067. https://doi.org/10.1155/2016/2475067
2. Miller WR, Arias CA. ESKAPE pathogens: Antimicrobial resistance, epidemiology, clinical impact and therapeutics. Nat Rev Microbiol. 2024;22(10):598-616. https://doi.org/10.1038/s41579-024-01054-w
3. Kelly JB, Nolan AC, Zeden MS. How can we escape the ESKAPEs: Antimicrobial resistance mechanisms and what lies ahead? PLoS Pathog. 2024;20(6):e1012270. https://doi.org/10.1371/journal.ppat.1012270
4. World Health Organization. WHO bacterial priority pathogens list, 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. Geneva: World Health Organization; 2024. Available from: https://www.who.int/publications/i/item/9789240093461
5. Sati H, Carrara E, Savoldi A, Hansen P, Garlasco J, Campagnaro E, et al. The WHO Bacterial Priority Pathogens List 2024: A prioritisation study to guide research, development, and public health strategies against antimicrobial resistance. Lancet Infect Dis. 2025;25(9):1033-1043. https://doi.org/10.1016/S1473-3099(25)00118-5
6. GBD 2021 Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance 1990–2021: A systematic analysis with forecasts to 2050. Lancet. 2024;404(10459):1199-1226. https://doi.org/10.1016/S0140-6736(24)01867-1
7. World Health Organization. Analysis of antibacterial agents in clinical and preclinical development: Overview and analysis 2025. Geneva: World Health Organization; 2025. Available from: https://www.who.int/publications/i/item/9789240113091
8. Årdal C, Røttingen JA, Opalska A, Van Hengel AJ, Larsen J. Pull incentives for antibacterial drug development: An analysis by the Transatlantic Task Force on Antimicrobial Resistance. Clin Infect Dis. 2017;65(8):1378-1382. https://doi.org/10.1093/cid/cix526
9. Ayaz M, Ullah F, Sadiq A, Ullah F, Ovais M, Ahmed J, et al. Synergistic interactions of phytochemicals with antimicrobial agents: Potential strategy to counteract drug resistance. Chem Biol Interact. 2019;308:294-303. https://doi.org/10.1016/j.cbi.2019.05.050
10. Angelini P. Plant-derived antimicrobials and their crucial role in combating antimicrobial resistance. Antibiotics. 2024;13(8):746. https://doi.org/10.3390/antibiotics13080746
11. Berida TI, Adekunle YA, Dada-Adegbola H, Kdimy A, Roy S, Sarker SD. Plant antibacterials: The challenges and opportunities. Heliyon. 2024;10(10):e31145. https://doi.org/10.1016/j.heliyon.2024.e31145
12. Odds FC. Synergy, antagonism, and what the chequerboard puts between them. J Antimicrob Chemother. 2003;52(1):1. https://doi.org/10.1093/jac/dkg301
13. Pillai SK, Moellering RC Jr, Eliopoulos GM. Antimicrobial combinations. In: Lorian V, editor. Antibiotics in Laboratory Medicine. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 365-440.
14. Morita Y, Nakashima K, Nishino K, Kotani K, Tomida J, Inoue M, et al. Berberine is a novel type efflux inhibitor which attenuates the MexXY-mediated aminoglycoside resistance in *Pseudomonas aeruginosa*. Front Microbiol. 2016;7:1223. https://doi.org/10.3389/fmicb.2016.01223
15. Siriyong T, Srimanote P, Chusri S, Yingyongnarongkul BE, Suaisom C, Tipmanee V, et al. Conessine as a novel inhibitor of multidrug efflux pump systems in *Pseudomonas aeruginosa*. BMC Complement Altern Med. 2017;17(1):405. https://doi.org/10.1186/s12906-017-1913-y

16. Xiao X, Huan Q, Huang Y, Liu Z, Liu Y, Li R, et al. Gramine sensitizes *Klebsiella pneumoniae* to tigecycline killing. Phytomedicine. 2024;126:155421. https://doi.org/10.1016/j.phymed.2024.155421
17. Yang J, Xu L, Zhou Y, Cui M, Liu D, Wang J, et al. Repurposing harmaline as a novel approach to reverse tmexCD1-toprJ1-mediated tigecycline resistance against *Klebsiella pneumoniae* infections. Microb Cell Fact. 2024;23(1):152. https://doi.org/10.1186/s12934-024-02410-4
18. Zhong Z-X, Zhou S, Liang Y-J, Wei Y-Y, Li Y, Long T-F, et al. Natural flavonoids disrupt bacterial iron homeostasis to potentiate colistin efficacy. Sci Adv. 2023;9(23):eadg4205. https://doi.org/10.1126/sciadv.adg4205
19. Lin Y, Zhang Y, Liu S, Ye D, Chen L, Huang N, et al. Quercetin rejuvenates sensitization of colistin-resistant *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates to colistin. Front Chem. 2021;9:795150. https://doi.org/10.3389/fchem.2021.795150
20. Guo Y, Lv X, Wang Y, Zhou Y, Lu N, Deng X, et al. Honokiol restores polymyxin susceptibility to *mcr*-1-positive pathogens both in vitro and in vivo. Appl Environ Microbiol. 2020;86(5):e02346-19. https://doi.org/10.1128/AEM.02346-19
21. Huang Y, Wang Z, Liu Z, Huan Q, Liu Y, Li R, et al. Gigantol restores the sensitivity of *mcr* carrying multidrug-resistant bacteria to colistin. Phytomedicine. 2023;117:154886. https://doi.org/10.1016/j.phymed.2023.154886
22. Diao Q, Zhong Z, Zhong Q, Cao Y, Fan X, Liang Y, et al. A lignan compound regulates LPS modifications via PmrA/B signaling cascades to potentiate colistin efficacy in vivo. PLoS Pathog. 2025;21(12):e1013843. https://doi.org/10.1371/journal.ppat.1013843
23. Kong J, Wang Y, Yao Z, Lin Y, Zhang Y, Han Y, et al. Eugenol works synergistically with colistin against colistin-resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolates by enhancing membrane permeability. Microbiol Spectr. 2023;11(5):e03666-22. https://doi.org/10.1128/spectrum.03666-22
24. Lu X, Wang Y, Guo W, Zhang Z, Hu X, Nie T, et al. Antibacterial activity of an FtsZ inhibitor celastrol and its synergistic effect with vancomycin against enterococci in vitro and in vivo. Microbiol Spectr. 2023;11(1):e03699-22. https://doi.org/10.1128/spectrum.03699-22
25. Cannatelli A, Principato S, Colavecchio OL, Pallecchi L, Rossolini GM. Synergistic activity of colistin in combination with resveratrol against colistin-resistant Gram-negative pathogens. Front Microbiol. 2018;9:1808. https://doi.org/10.3389/fmicb.2018.01808
26. Abdel-Halim MS, El-Ganiny AM, Mansour B, Yahya G, Latif HKA, Askoura M. Phenotypic, molecular, and in silico characterization of coumarin as carbapenemase inhibitor to fight carbapenem-resistant *Klebsiella pneumoniae*. BMC Microbiol. 2024;24(1):67. https://doi.org/10.1186/s12866-024-03214-7
27. Vázquez-Ucha JC, Martínez-Guitián M, Lasarte-Monterrubio C, Conde-Pérez K, Arca-Suárez J, Álvarez-Fraga L, et al. *Syzygium aromaticum* (clove) and *Thymus zygis* (thyme) essential oils increase susceptibility to colistin in the nosocomial pathogens *Acinetobacter baumannii* and *Klebsiella pneumoniae*. Biomed Pharmacother. 2020;130:110606. https://doi.org/10.1016/j.biopha.2020.110606
28. Li X, Song Y, Wang L, Kang G, Wang P, Yin H, et al. A potential combination therapy of berberine hydrochloride with antibiotics against multidrug-resistant *Acinetobacter baumannii*. Front Cell Infect Microbiol. 2021;11:660431. https://doi.org/10.3389/fcimb.2021.660431
29. Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. J Pharm Anal. 2020;10(4):277-290. https://doi.org/10.1016/j.jpha.2019.11.002
30. Siriyong T, Chusri S, Srimanote P, Tipmanee V, Voravuthikunchai SP. *Holarrhena antidysenterica* extract and its steroidal alkaloid, conessine, as resistance-modifying agents against extensively drug-resistant *Acinetobacter baumannii*. Microb Drug Resist. 2016;22(4):273-282. https://doi.org/10.1089/mdr.2015.0194
31. Siriyong T, Voravuthikunchai SP, Coote PJ. Steroidal alkaloids and conessine from the medicinal plant *Holarrhena antidysenterica* restore antibiotic efficacy in a *Galleria mellonella* model of multidrug-resistant *Pseudomonas aeruginosa* infection. BMC Complement Altern Med. 2018;18(1):285. https://doi.org/10.1186/s12906-018-2348-9
32. Sabnis A, Hagart KLH, Klöckner A, Becce M, Evans LE, Furniss RCD, et al. Colistin kills bacteria by targeting lipopolysaccharide in the cytoplasmic membrane. eLife. 2021;10:e65836. https://doi.org/10.7554/eLife.65836
33. Stojanoski V, Sankaran B, Prasad BVV, Poirel L, Nordmann P, Palzkill T. Structure of the catalytic domain of the colistin resistance enzyme MCR-1. BMC Biol. 2016;14(1):81. https://doi.org/10.1186/s12915-016-0303-0
34. Liu Y, Song H, Zhang M, Jiang J, Zhang Y, Xu J, et al. β,β-Dimethylacrylalkannin restores colistin efficacy against *mcr*- and TCS-mediated resistant Gram-negative bacteria via membrane disturbance. Antibiotics. 2026;15(1):3. https://doi.org/10.3390/antibiotics15010003
35. Ning N-Z, Liu X, Chen F, Zhou P, Hu L, Huang J, et al. Embelin restores carbapenem efficacy against NDM-1-positive pathogens. Front Microbiol. 2018;9:71. https://doi.org/10.3389/fmicb.2018.00071
36. Wang Y, Sun X, Kong F, Xia L, Deng X, Wang D, et al. Specific NDM-1 inhibitor of isoliquiritin enhances the activity of meropenem against NDM-1-positive Enterobacteriaceae in vitro. Int J Environ Res Public Health. 2020;17(6):2162. https://doi.org/10.3390/ijerph17062162
37. Ren Q, Zhang B, Wang M, Wang T, Zhang W, Du L, et al. Morin combined with meropenem is a potent inhibitor of NDM-1 against NDM-1-producing *E. coli*. Sci Rep. 2025;15(1):24169. https://doi.org/10.1038/s41598-025-08532-y
38. Wang L, Zhang Y, Liu Y, Xu M, Yao Z, Zhang X, et al. Effects of chlorogenic acid on antimicrobial, antivirulence, and anti-quorum sensing of carbapenem-resistant *Klebsiella pneumoniae*. Front Microbiol. 2022;13:997310. https://doi.org/10.3389/fmicb.2022.997310
39. Zhou Y, Wang J, Guo Y, Liu X, Liu S, Niu X, et al. Discovery of a potential MCR-1 inhibitor that reverses polymyxin activity against clinical *mcr*-1-positive Enterobacteriaceae. J Infect. 2019;78(5):364-372. https://doi.org/10.1016/j.jinf.2019.03.004
40. Zhou H, Xu M, Guo W, Yao Z, Du X, Chen L, et al. The antibacterial activity of kaempferol combined with colistin against colistin-resistant Gram-negative bacteria. Microbiol Spectr. 2022;10(6):e02265-22. https://doi.org/10.1128/spectrum.02265-22
41. Guo T, Li M, Sun X, Wang Y, Yang L, Jiao H, et al. Synergistic activity of capsaicin and colistin against colistin-resistant *Acinetobacter baumannii*: In vitro/in vivo efficacy and mode of action. Front Pharmacol. 2021;12:744494. https://doi.org/10.3389/fphar.2021.744494
42. Gülen D, Şafak B, Erdal B, Günaydın B. Curcumin-meropenem synergy in carbapenem-resistant *Klebsiella pneumoniae*. Iran J Microbiol. 2021;13(3):345-351. https://doi.org/10.18502/ijm.v13i3.6397
43. Chen X, Yin L, Peng L, Liang Y, Lv H, Ma T. Synergistic effect and mechanism of plumbagin with gentamicin against carbapenem-resistant *Klebsiella pneumoniae*. Infect Drug Resist. 2020;13:2751-2759. https://doi.org/10.2147/IDR.S265753
44. Stermitz FR, Lorenz P, Tawara JN, Zenewicz LA, Lewis K. Synergy in a medicinal plant: Antimicrobial action of berberine potentiated by 5′-methoxyhydnocarpin, a multidrug pump inhibitor. Proc Natl

Acad Sci U S A. 2000;97(4):1433-1437. https://doi.org/10.1073/pnas.030540597

45. Pang J, Guo X, Zhang Z, Guo W, Yuan M, Li Z, et al. Discovery of gambogic acid as an antibacterial adjuvant against vancomycin-resistant enterococci in vitro and in vivo. Phytomedicine. 2024;128:155400. https://doi.org/10.1016/j.phymed.2024.155400
46. Owen L, Webb JP, Green J, Smith LJ, Laird K. From formulation to in vivo model: A comprehensive study of a synergistic relationship between vancomycin, carvacrol, and cuminaldehyde against *Enterococcus faecium*. Phytother Res. 2020;34(7):1638-1649. https://doi.org/10.1002/ptr.6631
47. Buchmann D, Schultze N, Borchardt J, Böttcher I, Schaufler K, Guenther S. Synergistic antimicrobial activities of epigallocatechin gallate, myricetin, daidzein, gallic acid, epicatechin, 3-hydroxy-6-methoxyflavone and genistein combined with antibiotics against ESKAPE pathogens. J Appl Microbiol. 2022;132(2):949-963. https://doi.org/10.1111/jam.15253