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Antibiotic Adjuvants from Natural Resources against Multi-Drug Resistance Bacteria

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ABSTRACT

The rise of multi-drug-resistant bacteria signals the end of the antibiotic era, a global threat confirmed by the World Health Organization. Bacteria have evolved sophisticated resistance mechanisms, such as target modification, enzymatic drug inactivation, efflux pumps, and biofilm formation, which render many first-line antibiotics ineffective. In response, combination therapy has been established as a critical strategy, proven effective in treating infections caused by pathogens like *Mycobacterium tuberculosis* and *Helicobacter pylori*. This review explores the potential of natural compounds as antibiotic adjuvants to enhance or restore the efficacy of existing antibiotics. The success of Clavulanic Acid, a β-lactamase inhibitor originally extracted from *Streptomyces clavuligerus,* combined with amoxicillin, serves as a prime example of this approach. Investigating the vast biodiversity of natural resources, such as those found in Indonesia, offers a promising avenue for discovering novel adjuvants for antibiotic. While numerous in vitro studies have identified promising antibiotic-adjuvant combinations, translating these findings into successful animal models and clinical therapies remains a significant challenge.

THE POST-ANTIBIOTIC ERA IS TODAY

A stark contrast in our hospitals tells the story of a medical revolution. Before World War II, hospital wards were filled with young patients battling infectious diseases. After the discovery of penicillin, those same wards became populated by older patients managing chronic, non-infectious conditions like diabetes and cancer (McDermott & Rogers, 1982). This dramatic shift marked the dawn of the antibiotic era, a golden age we have enjoyed for decades, but now approaching a critical juncture. The shadow of a "post-antibiotic era" was first raised by Alexander Fleming himself during his Nobel Prize Lecture in 1945 (NobelPrize.org., n.d.), later supported by clinical data in a seminal 1992 review(Cohen, 1992) and officially declared a global threat by the World Health Organization (WHO) in 2014 (Reardon, 2014).

So, is the post-antibiotic era a future threat, or is it today's reality? The evidence suggests we are already living in it. The 2024 WHO Bacterial Priority Pathogens List serves as a sobering confirmation. The "Critical" tier is dominated by bacteria resistant to our last-resort antibiotics, including carbapenem-resistant *Acinetobacter baumannii* and Enterobacterales, alongside rifampicin-resistant *Mycobacterium tuberculosis*. Just below them in the "High" priority tier are formidable threats like methicillin-resistant *Staphylococcus aureus* (MRSA) and fluoroquinolone-resistant *Salmonella* (*WHO Bacterial Priority Pathogens List*, 2024). These superbugs imply that entire classes of first-line drugs ineffective, a predictable outcome given that many antibiotics lose their efficacy within just a few years of widespread clinical use (Fair & Tor, 2014).

HOW BACTERIA DEFEAT ANTIBIOTICS

Bacteria outsmart antibiotics through rapid evolution. This evolution follows two primary pathways: vertical evolution through chromosomal mutation and horizontal evolution through the acquisition of resistance genes from other bacteria, often via plasmids (Estrada et al., 2016). The effectiveness of these evolutionary strategies is deeply connected to an antibiotic's mechanism of action, particularly whether it has a single or multiple targets.

TARGET MODIFICATION: A WEAKNESS IN SINGLE-TARGET DRUGS

Antibiotics that act on a single pharmacological target are the most susceptible to the rapid development of resistance. For example, synthetic sulfonamides inhibit just one specific enzyme in the folate synthesis pathway (Miert, 1994). Consequently, a single mutation in the gene for that enzyme, or the acquisition of a single gene that provides a bypass, can confer complete resistance, explaining why sulfonamides were quickly rendered ineffective after their introduction (Pato & Brown, 1963).

In contrast, natural antibiotics like β-lactams, aminoglycosides, and tetracyclines have remained clinically useful for longer because they are poly-pharmacological (Silver, 2007). β-lactams do not just attack one enzyme; they inhibit multiple enzymes involved in building the bacterial cell wall. Similarly, aminoglycosides and tetracyclines disrupt the ribosome by binding to multiple ribosomal protein and RNA components. Defeating these multi-target antibiotics would require a series of coordinated mutations, a much more challenging evolutionary path for bacteria.

EVOLVING COUNTER-ATTACKS AND DEFENSES

Even against multi-target drugs, bacteria have evolved sophisticated defensive strategies. Rather than simply altering the antibiotic's target, they can neutralize the threat directly or prevent it from reaching its target.

Drug Inactivation by Enzymes: Bacteria can produce enzymes that destroy the antibiotic molecule itself. The most famous example is β-lactamase, an enzyme that inactivates penicillin and related drugs by breaking a key chemical bond in their structure (Liebmann et al., 1944). Drug Efflux Pumps: Bacteria can acquire genes that code for protein channels, or pumps, embedded in their cell membranes. These efflux pumps actively expel antibiotic molecules from the cell before they can accumulate and cause damage, a mechanism that confers broad-spectrum resistance to drugs like tetracyclines (McMurry et al., 1980). Collective Defense via Biofilms: Bacteria can evolve to form slimy, aggregated communities called biofilms. This dense, protective matrix acts as a physical barrier, preventing antibiotics from penetrating the colony and reaching the cells within, making the infection incredibly difficult to treat (Costerton et al., 1999).

DRUG COMBINATION

In the late 1970s, Clavulanic Acid, a compound extracted from *Streptomyces clavuligerus*, found to be a potent inhibitor against β-lactamase (Neu & Fu, 1978). Soon, the idea to combine it with the almost retired amoxicillin evidentially effective (Matsuura et al., 1980) and remain the story of success for drug combination against bacterial infection to date (Tyers & Wright, 2019). Previously, drug combinations pursued as an idea to have broader spectrum against multi-species infections and to ensure superior efficacies, like penicillin-streptomycin and trimethoprim-sulfonamides in the 1950s-1960s (Bushby & Hitchings, 1968; Jawetz et al., 1950).

Yet antibiotics combination use against *Mycobacterium tuberculosis* is not an enhancement; it is the absolute, non-negotiable standard of care. The bacterium is a master of evolving resistance and attacking it with a single drug is a guaranteed failure that breeds stronger, more resilient strains. Consequently, the mandatory cocktail consists of rifampicin, isoniazid, pyrazinamide, and ethambutol was developed as a multi-pronged assault, designed to overwhelm the bacterium's ability to mutate its way to survival during the long months of treatment (Kerantzas & Jacobs, 2017). This multi-pronged strategy is also used to treat leprocy (Noordeen, 2016).

Although, the combination between β-lactam and aminoglycoside have been proposed in the 70s (Kluge et al., 1974). This combination got into the critical care wards of hospitals, where doctors relied on it as empirical combinations (without clinical trial or consistent dosages) to fight life-threatening sepsis as it has been professionally encouraged (Dellinger et al., 2008). Retrospective and meta analysis this practice believed to provide broad-spectrum coverage and a synergistic killing effect when time is of the essence (Micek et al., 2010; Vazquez-Grande & Kumar, 2015). Even the eradication of the stomach bacterium *Helicobacter pylori* requires a "triple therapy" of two antibiotics and an acid reducer (Hentschel et al., 1993). These examples clearly prove that the principle of combination therapy is a vital, time-tested strategy in the fight against bacteria.

Despite the clinical benefits of co-administered antibiotics, the successful development of new combination therapies remains limited. The development of new drug combinations encounters numerous obstacles. In some cases, the two compounds exhibit poor compatibility in vivo. Combining certain drugs may also result in adverse effects (Tyers & Wright, 2019). Furthermore, from the business perspective, the regulatory approval process for dual-drug regimens is both costly and complex. This made the pharmaceutical industry quickly lose their “appetite” particularly when the medications involved are older and inexpensive. As a result of these challenges, concepts such as targeting bacterial defenses through combination therapies often do not progress beyond laboratory research to clinical application.

In contrast to the challenges encountered in antibiotic development, the fight against viral infections particularly HIV has showcased the transformative success of combination therapies. The introduction of Highly Active Antiretroviral Therapy (HAART) revolutionized HIV treatment by simultaneously targeting multiple stages of the virus's lifecycle. This multi-drug approach proved so successful that it rapidly became the gold standard, dramatically improving patient outcomes and transforming HIV from a fatal diagnosis into a manageable chronic condition (Gulick et al., 1997; Hammer et al., 1997). Due to HIV's high rate of evolution and mutation, single-drug regimens were shown to be less effective. In contrast, using a combination of drugs, referred to as a "drug cocktail," demonstrated greater effectiveness. This showed how powerful combination therapy can be when science and regulations work together.

In bacterial infections, combination therapies remain largely empirical and are often reserved for severe or specific cases. Despite bacteria’s exceptional capacity to evolve, modern frameworks for developing new antibiotic combinations remain limited. It is increasingly clear that a broadened search for alternative solutions is needed. Importantly, a multi-pronged attack against bacterial infection is not limited to the use of multiple drugs—this strategy can also be powerfully executed through the adjuvant approach. By harnessing the diverse chemical compounds found in natural resources, we tap into a rich source of extracts and phytochemicals that have evolved over millennia as robust defenses against microbial threats. Therefore, searching and investigating antibiotic adjuvants from natural resources is a promising avenue.

NATURAL RESOURCES FOR ANTIBIOTIC ADJUVANTS

Clavulanic acid, a widely used antibiotic adjuvant, is a natural product extracted from the *Streptomyces* bacterium. Indonesian researchers are actively investigating their country's natural resources to identify novel compounds with potential applications in medicine and other fields. With a wide range of species including plants, algae, fungi, and other biological sources Indonesia is recognized as one of the centers of biodiversity (Von Rintelen et al., 2017). These resources suggest extensive diversity in natural compounds. While commonly studied groups such as alkaloids, flavonoids, terpenes, and tannins may exhibit limited direct antibiotic activity, they may have significant potential as adjuvants to enhance or restore the effectiveness of existing antibiotics.

If the purpose of combining drugs is to use two agents with different mechanisms and targets, applying a similar approach could be advantageous. As this phase is exploratory, the

primary aim is to identify adjuvant effects regardless of the specific mechanism of the compounds involved. Additionally, the mechanism or target of such compounds may be unknown and requires further investigation. Thus, having a standard method for determining the adjuvant efficacy of plant extracts or purified compounds is considered more important than methods focused on identifying the mechanism of action.

SYNERGYSM

Synergy, as opposed to antagonism, is a crucial concept in the field of drug combinations for antimicrobial activity. Understanding when and how synergy occurs is essential, though a comprehensive review of the mechanisms is beyond the scope of this section. In brief, synergy is established through the calculation of the fractional inhibitory concentration index (FICI).

The gold standard for testing a single compound's antimicrobial effect is the microdilution assay, which is used to determine the minimum inhibitory concentration (MIC). When evaluating combinations of two compounds, the checkerboard assay is commonly employed as the extension of the microdilution assay. This method involves systematically combining the two compounds in varying ratios to assess their interactions(Bellio et al., 2021).

To calculate the FICI, researchers first determine the MIC of compound A in the presence of compound B. This MIC is then divided by the MIC of compound A alone, resulting in the fractional inhibitory concentration for A (FIC A). The same process is applied to compound B. The FICI is obtained by summing FIC A and FIC B. A FICI of ≤ 0.5 signifies a synergistic interaction, indicating that the combination is substantially more effective than the individual compounds alone. Conversely, a FICI greater than 4.0 denotes antagonism, where the compounds hinder each other's efficacy. Values in between these thresholds suggest no significant interaction (Fatsis-Kavalopoulos et al., 2024).

Even so, in practice, indications of synergy often emerge even before the FICI is calculated, particularly when viability dyes such as resazurin or tetrazolium are employed to detect living cells. For instance, observing a two- or four-fold decrease in MIC when two compounds are combined provides strong evidence of a synergistic interaction. Typically, after such findings, researchers confirm synergism at the combined MIC using a kill-time assay to further validate the effect. The time-kill curve analysis then measures how quickly bacteria are eliminated over a 24-hour period, with a potent synergistic combination producing a significantly faster and deeper drop in bacterial counts than the antibiotic alone (Lady et al., 2023; Lim et al., 2015; Rabodoarivelo et al., 2025). Additionally, results are often analyzed graphically through isobologram plots to illustrate the interactions between the compounds (Huang et al., 2019).

EVIDENCE IN ACTION: PROMISING COMBINATIONS

Efforts to find the promising combination are still on the way. As the massive number of antibiotics available, phytochemicals to test, and bacterial model to work with, the combinatorial between the three is ginormous. For example, one study evaluated nine antibiotics, six purified phytochemicals, and three bacteria manage species to squeeze out gallic acids as adjuvant for ceftiofur against *Salmonella* and adjuvant for ampicillin against *E. coli* (Hossain et al., 2020). More recent studies maintain to try only one or two combinations, like Eugenol + Cefotaxime against *S. aureus* (Lady et al., 2023) and Rutin/Naringenin + Amikacin against *E. coli* (Yi, Bai, et al., 2024; Yi, Cao, et al., 2024). The common natural products Quercetin also shows adjuvant capabilities in combination with meropenem against *E. coli* and *Klebsiella pneumoniae* and with colistin or amikacin against *Acinetobacter baumannii* (Alnour et al., 2022; Odabaş Köse et al., 2023; Pal & Tripathi, 2020). Plant extracts with minimal and rough purification also attracted some test as adjuvant for tetracycline against *Acinetobacter baumannii* (H. M. Nasution et al., 2025; H. R. Nasution et al., 2024).

Numerous antibiotic-adjuvant combinations that pass through these checkerboard and time-kill assay as an effective combination *in vitro*. Some, carry the investigation few steps further

suggesting the mechanism of action for some of these "natural" adjuvants. Quercetin, for instance, proposed to disrupt bacterial enclosures, DNA synthesis, and biofilm formation (review in Nguyen & Bhattacharya, 2022), but the evidences provided were remain descriptive and correlative. Direct mechanistic evidence came from rutin (mainly extracted from celery and in synergistic mode with Colistin) disrupting the PmrA/PmrB for iron homeostasis of Gram negative bacteria (Luo et al., 2025). Despite the recent *in vivo* success with rutin against Salmonellosis (Luo et al., 2025), many "natural" adjuvant candidates failed to show effectiveness in animal models. Eight combination between β-lactam and β-lactamase inhibitors get into clinical trials and even commercially available (Bush & Bradford, 2016; Dhanda et al., 2023). Synthetic diarrhea medicine, Loperamide, discovered as adjuvant that potentiate tetracycline against *Salmonella enterica* serovar Thyphimurium in mouse mode of infectious colitis (Ejim et al., 2011). Even the classic example of berberine + various antibiotics (pioneered by (Yu et al., 2005)) maintained to be published as in vitro studies, until the most recent combination of berberine and sulbactam against *Acinetobacter baumannii* in murine thigh infection model (Li et al., 2021). The famous phytochemicals from tea extract, epigallocatechin gallate (EGCG) reported first with ampicillin against *S. aureus* in vitro (Zhao et al., 2001) and remain so until very recently reported with vancomycin in mice model for wound healing (Ni et al., 2025).

CONCLUSIONS

In summary, the pursuit of synergistic combinations between antibiotics and natural compounds remains an essential strategy in overcoming antimicrobial resistance. While in vitro studies consistently demonstrate promising adjuvant effects, translating these findings into effective therapies in animal models and clinical settings continues to present challenges. Standardized methods for assessing synergy, such as checkerboard and time-kill assays, are vital for advancing our understanding and guiding future research. Despite the complexities and setbacks, ongoing innovation and collaboration fuel optimism that new, effective antimicrobial combinations will emerge to address the evolving threat of resistant pathogens. With continued dedication and scientific rigor, there is hope that these efforts will ultimately lead to safer and more powerful treatments for patients worldwide.

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AUTHORS' CONTRIBUTIONS

Initiation, Conceptualization, and Manuscript Writing-First Draft by F. Editing and rewriting the manuscript was done by LH, RAH, and RGS, under supervision of F.

CONFLICT OF INTERESTS

The author declares no conflict of interest.

ETHICAL CONSIDERATION

We have used AI-assisted technologies in creating this article. AI tools (Google Gemini model 2.5 Pro as of 1st October 2025) were used between 1st October and 20th October 2025 only to improve the readability and language of the revised main text. All scientific content was written and verified by the author. We value the role of AI tools in reducing language barriers and promoting inclusivity in science.

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