

REVIEW

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Synergistic relationship between plant extracts, essential oils, phytocompounds and antibiotics in controlling drug resistant bacterial species

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Abstract

Background Antimicrobial resistance (AMR) has emerged as a global threat to global public health, requiring innovative strategies to address antibiotic-resistant bacterial pathogens. The synergy between plant extracts, essential oils, phytocompounds, and antibiotics promises to be a viable solution to this grave issue.

Main body In addition to the synergistic effects of plant extracts, essential oils, and phytocompounds, the current review described the main groups of phytochemicals that function as anti-bacterial agents. The mechanical and toxicological issues are also highlighted in the current review.

Conclusion The most popular techniques examined in this review are checkerboard and time-kill tests. The synergistic actions of plant extracts, phytocompounds, and essential oils effectively reduced the drug's minimal inhibitory concentration against both standard and clinical bacterial isolates. Each of the three complementary strategies-plant extracts, phytocompounds, and essential oils-was equally significant.

Keywords Antimicrobial resistance, Drug dose, ESKAPE pathogens, Plant-derived compounds, Toxicity

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1 Introduction

Bacterial pathogens cause severe infections leading to life-threatening diseases, posing a significant risk to human life [1]. To overcome this challenge, researchers have employed various antibiotics and antibacterial agents that selectively target different components of the bacterial cells, including the cell wall, cell membrane, cellular enzymes, nucleic acids, and ribosomes as depicted in Fig. 1. The critical concern of significant importance in bacterial pathogenesis is the declining efficacy of antibacterial treatment [1, 2]. It is worth noting that the development of resistance to a single anti-bacterial treatment often leads to the emergence of multidrug resistance (MDR) in the subsequent bacterial variants [3]. The presence of microorganisms resistant to many drugs poses a significant challenge in the treatment of infections [4, 5]. The situation has been further aggravated by the COVID-19 pandemic, leading to widespread prescribing of empirical antibiotics despite a very low prevalence of bacterial coinfection among hospitalized COVID-19 patients [6]. This overuse of antibiotics has played a crucial role in the worldwide emergence of anti-bacterial resistance [6]. The mechanisms underlying anti-bacterial antibiotic resistance include processes, such as impeding anti-bacterial agents from entering the bacterial cells, bacterial membranes containing active export systems, biofilm formation, enzymatic breakdown of anti-bacterial agents, protection of bacterial active sites against

anti-bacterial, and modification of anti-bacterial targets (Fig. 2) [1].

Combination treatments are thought to be a potentially effective way to fight bacteria that are resistant to antibiotics [7, 8]. Trimethoprim and sulfamethoxazole are two examples of antibiotic combinations that have been utilized in clinical settings and shown to be successful [9]. The disadvantage of this combination, however, is that it may increase exposure to unnecessary antibiotics throughout use, thereby increasing bacterial resistance [10]. Combinations with antibiotics and non-antibiotics are therefore considered the most promising approach [8]. The benefits of active monomeric chemicals derived from natural plants include lower toxicity, fewer adverse effects, improved targeting, and reduced resistance. Compared to antibiotics, its anti-bacterial action is quite mild. However, a growing body of research has demonstrated that the active components of natural plants can enhance the effectiveness of antibiotics through several mechanisms, including disruption of cell membranes, inhibition of efflux pumps, inhibition of DNA, protein, and lipid synthesis, and inhibition of biofilm formation. In addition to exhibiting multi-targeted anti-bacterial activity, the combination of essential oils (EOs) with antibiotics effectively reduces or reverses microbial resistance, which may be a useful tactic in the fight against microbial resistance [11, 12].

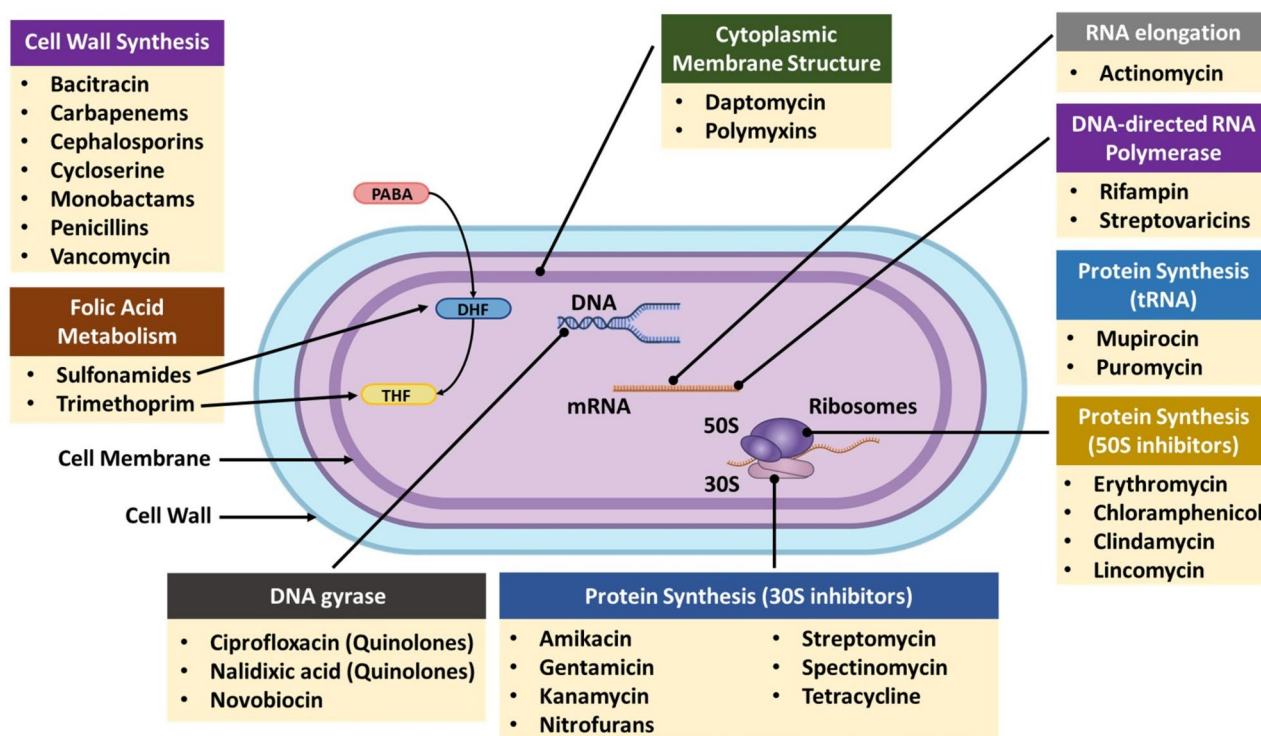


Fig. 1 Diagrammatic illustration of different categories of antibiotics along with their mode of action

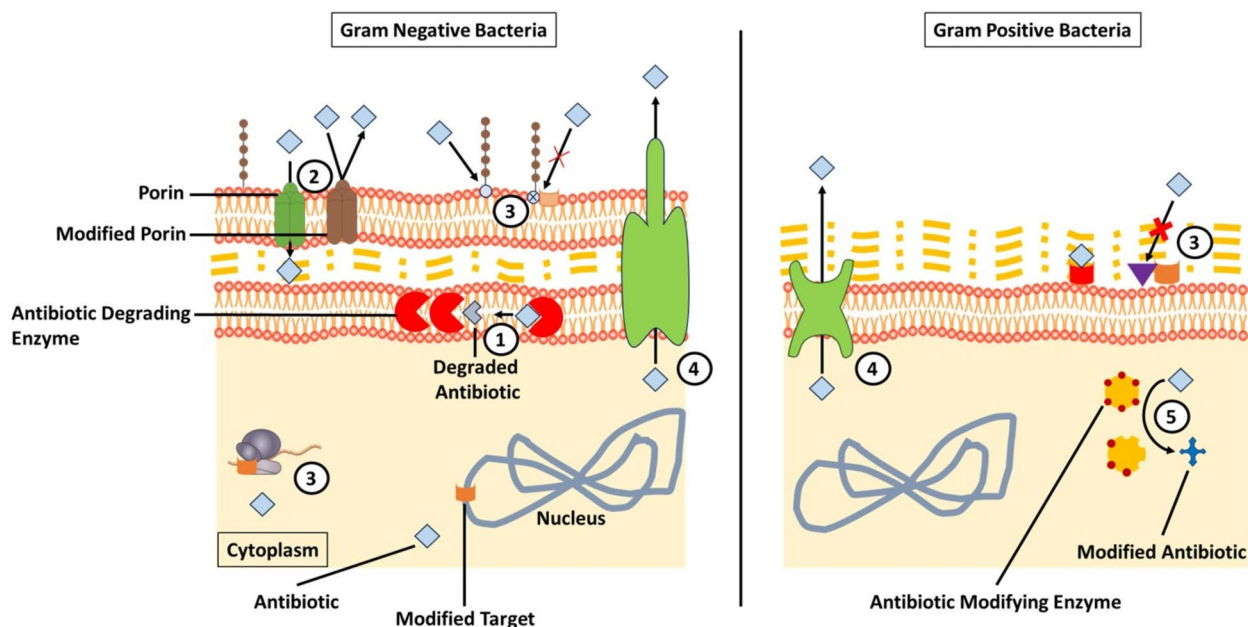


Fig. 2 Resistance mechanism adopted by bacteria against antimicrobial agents. The general antibiotic resistance mechanism in bacteria is (1) enzymatic hydrolysis, (2) modification of porins resulting in reduced permeability of antibiotics, (3) antibiotic targets modifications, (4) active antibiotic extrusion by efflux pumps in the membrane and (5) modification of antibiotics by enzymatic reaction through via group transfer and redox process

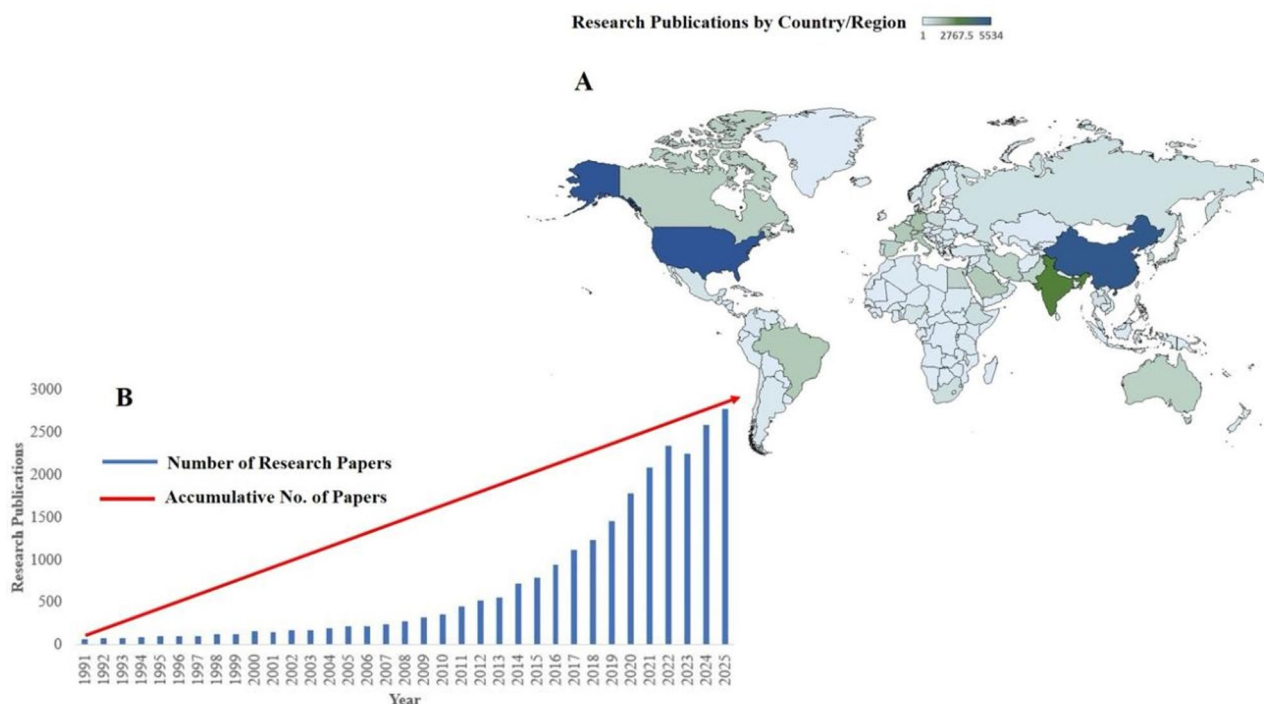


Fig. 3 **A** heatmap showing the number of research publication around the world. **B** The annual number of research publications (only in English language) and cumulative number of articles indexed in Web of Science Core Collection (WOSCC) from 1st January 1991 to 31st December 2025

Research publications using the keyword “bacterial antibiotic resistance” showed that the USA, China, India and England were leading, as shown in Fig. 3A. Whereas Fig. 3B presented the annual number of research publications and the accumulative number of articles related

to bacterial antibiotic resistance. Based on the above comprehensive information, this review aims to highlight the prospects for the utilization of plant-based or derived anti-bacterial agents to address the growing concern of drug resistance in pathogenic bacteria. Different

methods, such as time-kill analysis, checkerboard assay and the Epsilon meter (E-test), have also been discussed and widely used to assess the synergistic potential of these natural materials with anti-bacterial agents. Moreover, the mechanism underlying the synergistic action of plant extracts, phytochemicals, and essential oils in combination with antibiotics against pathogenic bacteria has also been discussed. Furthermore, the potential toxicity of essential oils and plant extracts as anti-bacterial agents has also been discussed.

2 Harnessing plant phytochemicals as anti-bacterial agents to combat antibiotic resistance: an overview

Plants are a rich source of phytochemicals, offering considerable opportunities to discover and develop new drugs. Today, there has been increased recognition of the significance of herbal and Ayurvedic medicines in the treatment of various health conditions, such as pediatric seizures, chronic gastritis, contagiosa, tuberculosis, and urinary tract infections within modern society [13]. Phytochemicals are organic molecules synthesized intracellularly in plants as a natural defense against predators and pathogens. Nevertheless, it is noteworthy that only a partial proportion of these plants have undergone comprehensive exploration and investigation [14]. Significantly, the biologically active components are first extracted from plants and their specific parts to further assess their efficacy against various disorders and diseases. The significance of this undertaking has been heightened by the ongoing development of antibiotic-resistant bacteria, which poses a substantial societal hazard in the present

context. Various medicinal plants attribute their therapeutic effectiveness mainly to the presence of several phytochemicals, such as flavonoids, tannins, carotenoids, phenolic acids, alkaloids, stilbenoids, indoles, terpenoids, isothiocyanates, and monoterpenes [15, 16]. These substances have exhibited the capability to impede crucial elements involved in the emergence of resistance, like cellular microstructure, efflux pumps, membrane permeability, replication machinery, and integrity, as well as other virulence mechanisms like biofilm formation and quorum sensing (QS), which ought to be crucial in the survival and resistance of pathogenic bacteria (Fig. 4).

2.1 Alkaloids

Alkaloids constitute a diverse group of heterocyclic nitrogen-containing compounds, well known for their broad-spectrum of anti-bacterial properties [13]. Even the scientific literature has regularly provided evidence of the potent anti-bacterial potential of specific classes of alkaloids, such as aporphines, phenanthrenes, Isoquinolines, and quinolones. Additionally, the efficacy of these alkaloid subclasses has been extensively established against a broad range of bacterial infections involving notable species such as *Bacillus cereus*, *Klebsiella pneumoniae* and *Staphylococcus aureus* [17]. The alkaloids exhibit anti-bacterial potential, ascribed to their ability to inhibit the type II topoisomerase enzyme, which is pivotal in DNA replication. The inhibition disrupts bacterial DNA replication, thereby impeding bacterial growth [17]. Furthermore, certain alkaloids, including diterpenoid alkaloids, are usually extracted from plants in the *Ranunculaceae* family and have exhibited significant anti-bacterial

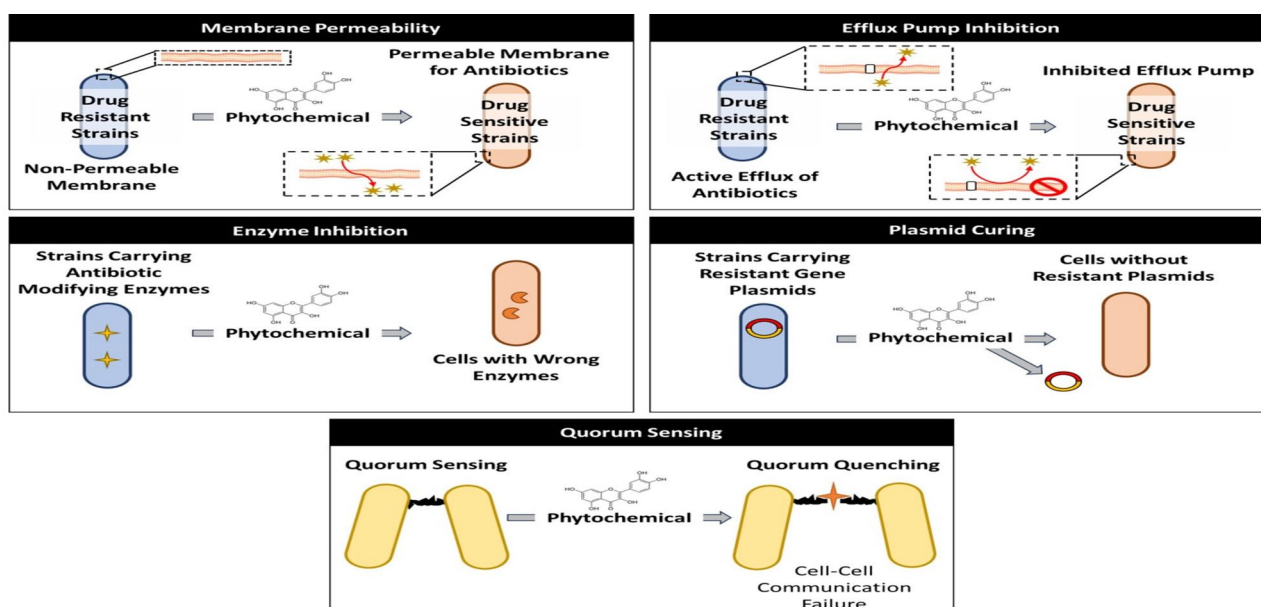


Fig. 4 Diagrammatic illustration of diverse resistance factors impeded by phytochemicals

properties. Notably, quaternary alkaloids, such as berberine and harmaline, exhibit anti-bacterial activity by intercalating into bacterial DNA. This interaction results in substantial impairment of cellular division, eventually leading to bacterial cell death [18].

2.2 Flavonoids

Flavonoids produced by plants are well known for their potent anti-bacterial properties. These compounds are distinguished by their distinctive structure, which consists of a 2-phenylbenzo- γ -pyrane core and dual benzene rings. The anti-bacterial potential of various flavonoids subgroups, including chalcones, dihydrochalcones, flavonols, flavanones, and isoflavonoids, has been extensively studied and documented [19]. Notably, catechin, a well-known flavonoid, has been shown to disrupt the MRSA (methicillin-resistant *Staphylococcus aureus*) membrane. This disruption of the bacterial cell membrane leads to the release of potassium ions. In addition, the mechanism of membrane disruption mediated by 3-arylidene-flavanone has been reported by Budzyńska et al. [20]. This disruption leads to the accumulation of bacterial cells, primarily through alterations in membrane integrity and permeability, particularly in pathogenic strains of *Enterococcus faecalis* and *S. aureus* isolated from clinical specimens. Flavonoids have also been reported to interfere with DNA synthesis. Noteworthy examples, such as chrysin, morin, myricetin, and kaempferol, have been reported to interfere with DNA synthesis [21, 22]. Additionally, studies by Ouyang et al. [23] have reported quercetin's QS and biofilm inhibitory capabilities in *Pseudomonas aeruginosa* PAO1 bacterial strain. The QS potential of quercetin can be associated with downregulation of critical genes, namely *rhII*, *rhIR*, *lasI*, and *lasR*, leading to decreased secretion of elastase, pyocyanin, protease, and virulence factors.

2.3 Phenolic acids

Phenolic acids, categorized as secondary metabolites, are essential constituents of hydrophilic compounds found in plant tissues [24]. These chemicals are generally found in low concentrations and rarely in their free state. In contrast, they are predominantly found in conjugated forms such as esters (depsides and depsidones) and phenolic glycosides [24]. Phenolic acids have been shown to form compounds with various natural constituents, such as cell wall polymers, fatty acids, flavonoids, and sterols in plant tissues [24]. Several phenolic acids, including caffeic acid, have exhibited notable anti-bacterial properties [25]. For example, in comparison to ampicillin, which had minimum inhibitory concentrations (MICs) of 3.2 $\mu\text{g/mL}$ and 0.1 $\mu\text{g/mL}$ against *Escherichia coli* and *S. aureus* respectively, caffeic acid showed markedly higher minimum inhibitory concentration of 1600 $\mu\text{g/}$

mL against both bacteria [26]. Furthermore, the triphenolic compound, gallic acid, has demonstrated significant anti-bacterial activity against *Enterococcus faecalis* [27]. Moreover, it has been found to exhibit strong anti-bacterial activity against a variety of bacterial strains, including *E. coli*, *Moraxella catarrhalis*, *P. aeruginosa*, *Streptococcus agalactiae*, *S. aureus*, and *Streptococcus pneumoniae* [28]. Notably, another study reported that gallic acid has significant anti-bacterial activity against *Campylobacter* spp. and exhibits bactericidal activity against two *Campylobacter coli* strains, with MIC values ranging between 61.5 and 125 $\mu\text{g/mL}$ [29]. Anti-bacterial potential against different strains of bacteria has been demonstrated by gallic and ferulic acid. The ferulic acid component displayed an MIC of 100 $\mu\text{g/mL}$ against both *E. coli* and *P. aeruginosa* while demonstrating MICs of 1250 $\mu\text{g/mL}$ and 1100 $\mu\text{g/mL}$ against *Listeria monocytogenes* and *S. aureus*, respectively. The MIC values of gallic acid were 500 $\mu\text{g/mL}$, 1500 $\mu\text{g/mL}$, 1750 $\mu\text{g/mL}$ and 2000 $\mu\text{g/mL}$ against *P. aeruginosa*, *E. coli*, *S. aureus* and *L. monocytogenes* respectively. Borges and coworkers [30] documented that both gallic and ferulic acid could disrupt the bacterial cell wall in *S. aureus*, *P. aeruginosa*, and *E. coli*, leading to localized damage and the release of cellular constituents. In addition, Sundaramoorthy et al. [31] have identified two ferulic acid derivatives, namely (E)-methyl 3-(4-((p-tolylcarbonyl) methoxy)-3-methoxyphenyl) acrylate and 4-((E)-2-(diethylcarbonyl) vinyl)-2-methoxyphenyl acetate, which exhibited promising potential as inhibitors of efflux pumps (EPs). These compounds have shown efficacy in impeding the NorA efflux pump growth in MRSA. According to Nowacka et al. [32], numerous naturally occurring acids, namely -hydroxybenzoic acid, 4p-coumaric acid or p-hydroxybenzoic acid, ferulic acid, 4-hydroxy-3-methoxybenzoic acid, also known as vanillic acid, protocatechuic acid, syringic acid and caffeic acid isolated from wild varieties of Polish mushrooms exhibit moderate anti-bacterial action towards a broad spectrum of bacteria, especially Gram-positive and Gram-negative. The MICs of these acids were determined, and they ranged from 156 to 5000 $\mu\text{g/mL}$ [32].

2.4 Stilbenoids

Stilbenoids are a class of plant-derived compounds that function as both phytoalexins and woody constitutive metabolites. These phytochemicals are synthesized by plants to protect against bacterial infections and various stressors [33]. Resveratrol, a member of the stilbenoid family, has attracted considerable attention for its potential health benefits [34]. However, it is essential to acknowledge that resveratrol-derived oligomers, dimers, and monomers also have diverse multidimensional biological functions [35]. Vestergaard and Ingmer [36]

provided a comprehensive analysis of the potential targets involved in resveratrol-mediated inhibition of bacterial growth. Resveratrol has been observed to exhibit the ability to reversibly bind with adenosine triphosphate (ATP) synthase in aerobic *E. coli* ($IC_{50} = 94\mu M$) and *Mycobacterium smegmatis* ($IC_{50} = 50\mu M$). This interaction reduces cellular energy production and impairs proliferation. The inhibition of oxidative phosphorylation by resveratrol was observed in the pathogenic bacterium *E. coli*. In the *E. coli* mutans strain (which lacks ATP synthase), resveratrol inhibited cell division at $228\mu g/mL$. This obstruction is believed to result from FtsZ protein suppression, a protein crucial in the septum development during cell division. Additionally, *E. coli* cells treated with $182\mu g/mL$ resveratrol showed propidium uptake, indicating membrane damage and potassium leakage. Conversely, *S. aureus* showed no indication of membrane damage in response to resveratrol treatment [36]. Additionally, it has been reported that resveratrol can induce DNA cleavage, leading to the formation of a copper (II) peroxide complex. The thus formed adducts bind to bacterial DNA, forming a ternary composite comprising DNA, resveratrol, and Cu (II). DNA cleavage initiates when Cu (II) is reduced to Cu (I). The involvement of the OH group at the 4th position in copper reduction is significant owing to its pro-oxidant activity. According to Bao et al. [37], resveratrol has been identified as a potentially effective agent in combating infections caused by the anaerobic Gram-negative bacterium *Porphyromonas gingivalis*, which is primarily involved in periodontitis (a chronic inflammatory dental disease). The deterioration of bone and connective tissue characterizes this chronic inflammatory dental disease. Previously published literature on resveratrol has unveiled that it demonstrates both bacteriostatic and bactericidal properties against several strains of *P. gingivalis*, possessing a minimum bactericidal concentration (MBC) and MIC of $312\mu g/mL$ and $156\mu g/mL$, respectively. However, some strains exhibited lower MBC and MIC values of $156\mu g/mL$ and $78\mu g/mL$, respectively. Furthermore, it has been reported that resveratrol inhibits biofilm formation at sub-minimum inhibitory concentrations, which is attributed to the downregulation of virulence factor genes, such as *kgp* and *fimA* (type II and type IV) [38]. Paulo et al. [39] demonstrated the inhibitory potential of resveratrol against *Helicobacter pylori* urease, thereby preventing the hydrolysis of urea into ammonia and the subsequent formation of an alkaline microenvironment, which is essential for the survival of the bacterium in the acidic stomach environment.

2.5 Tannins

Tannins are a class of molecules that can be further subdivided into two subcategories: proanthocyanidins,

known as condensed tannins, gallotannins, and ellagitannins, falling under the subcategory of hydrolyzable tannins [40]. Proanthocyanidins are commonly found in berries and have attracted considerable interest for their ability to inhibit the proliferation of various pathogenic bacteria, including cariogenic *Streptococcus mutans*, oxacillin-resistant *S. aureus* and uropathogenic *E. coli* [41]. Cranberry-derived proanthocyanidins primarily consist of tetrameric and pentameric epicatechin units, each with at least one A-type linkage, and exhibit antibacterial activity against various pathogenic microorganisms. The inhibitory action of A-type proanthocyanidins on bacterial growth may be attributed to multiple mechanisms. These mechanisms involve the augmentation of cell membrane permeability, destabilization of the cytoplasmic membrane, direct impact on microbial metabolism, suppression of microbial enzymes found outside cells, and deprivation of microbes of growth essential substrates, specifically micronutrients like zinc and iron. The ability of proanthocyanidins to form chelates with various metals has also been documented in the literature, leading to the sequestration of these metals and subsequently hindering bacterial growth [42, 43]. On the other hand, gallotannins exhibited antibacterial activity by virtue of their high iron affinity and their ability to deactivate proteins confined to membranes.

3 Essential oils (EOs) as potential anti-bacterial agents

EOs are a complex mixture of low-molecular-weight phytochemicals derived from natural products [25]. They are known for their robust anti-bacterial properties and are commonly employed in ethnic therapeutics [44]. The extraction of volatile and lipophilic compounds from various plant parts, particularly fruits and flowers, is possible; however, their occurrence is restricted to specific plant families. EOs are generally composed of several chemical compounds like aromatic and aliphatic aldehydes, terpenoids, terpenes, phenols, and derivatives of short-chain aliphatic hydrocarbons [45]. Various studies have acknowledged that EOs possess anti-bacterial potential and can act as reservoirs of anti-bacterial agents [46, 47]. The effectiveness of these agents in targeting and interfering with crucial mechanisms of microbial drug resistance, such as drug efflux pumps, cell wall and membrane integrity, QS, mobile genetic elements, and biofilm formation, has proven valuable [44]. The primary function of bacterial cell membranes is to provide initial defense against anti-bacterial drugs. To interact effectively with their intended targets, antibiotics must first overcome this barrier [48]. Remarkably, owing to their hydrophobic properties, EOs can interact with mitochondria and lipid membranes, disrupting cellular structures and ultimately increasing membrane permeability.

Furthermore, EOs can influence the quantity and composition of the bacterial cell membrane unsaturated fatty acids. Thus, leading to disruption of bacterial cells ability to effectively govern the escape of essential cellular molecules and ions when exposed to essential oils [44]. Topical studies on EOs have substantiated their considerable efficacy in specifically targeting key bacterial resistance factors. For instance, *Melaleuca alternifolia*-derived EOs have been found to improve the permeability of bacterial cell membranes [49]. *Dodartia orientalis*-derived EOs were found to significantly impact the cellular structures of *E. coli*, *Salmonella enteritidis* and *S. aureus*, primarily by suppressing biofilm formation [50]. Cinnamon EOs have also been documented to exhibit significant bactericidal activity against *Staphylococcus* and *E. coli* strains by altering cell microstructure, membrane integrity, and permeability [51]. *Ocimum gratissimum* derived EOs have been reported as permeabilizers and exhibit substantial anti-bacterial activity against *S. aureus* and *P. aeruginosa* [52]. The EOs derived from *Coriandrum sativum* have been documented to alter the porosity of bacterial cell membranes, thereby hindering the growth of multidrug-resistant uropathogenic *E. coli* [53]. These alterations result in apparent impairments to cellular integrity and functionality. Essential oils have been recognized as potent inhibitors of drug efflux pumps in bacteria, also referred to as EPIs [44]. *Chenopodium ambrosioides*-derived EOs have been documented as effective EPIs for *S. aureus* [54]. According to Chovanová et al. [55], EOs derived from *Salvia sclarea*, *Salvia officinalis*, and *Salvia fruticosa* reduced tet(K) gene expression and inhibited drug efflux in tetracycline-resistant *Staphylococcus epidermidis*. Furthermore, EOs derived from several other plants in conjugation with pure turpentine oil showed potent anti-bacterial activity and plasmid-curing potential against *S. epidermidis* and *E. coli* strains [56].

4 Investigating approaches to assess synergistic interactions among ligands and antibiotics

The evaluation of interactions among anti-bacterial drugs involves determining whether their combined effects are additive, synergistic, or antagonistic. Additivity is a phenomenon in which the total effect equals the sum of the effects produced by two or more drugs in combination, often referred to as indifference [57, 58]. Whereas synergy is defined as the phenomenon in which the collective influence of multiple elements exceeds the sum of their individual effects. On the other hand, antagonism arises when the collective impact is lower than the sum of the individual factor impact [58]. Different approaches are employed to precisely evaluate these interactions, as reviewed by White et al. [57], to ascertain whether they are additive, synergistic, or antagonistic. Thus, using

these scientific methodologies enables the accurate assessment of the interplay between anti-bacterial drugs, thereby providing insights into their collective efficacy.

4.1 Checkerboard method

The checkerboard technique is the predominant in-vitro method for assessing the synergistic effects of anti-bacterial drugs. The name of this method is derived from the practice of altering the concentration of one substance along the horizontal axis and the other substance along the vertical axis within a microtiter plate. The prepared dilutions used could typically be determined based on the MIC of the drugs, ranging from slightly lower than the anticipated MIC (e.g., $\frac{1}{2}$ X MIC) to twice the predicted MIC (2X MIC). The interpretation of checkerboard test outcomes entails either computing the fractional inhibitory concentration index (FICI) or plotting coordinates in an isobologram for two anti-bacterial agents [57, 58]. The calculations for the FICI determination for a two-drug (A and B) combination are carried out using the subsequent formula:

$$\text{FICI} = \text{FIC (A)} + \text{FIC (B)}$$

$\text{FIC (A)} = \text{MIC of agent A in combination with antibiotic} / \text{MIC of agent A alone.}$

$\text{FIC (B)} = \text{MIC of agent B in combination with antibiotic} / \text{MIC of agent B alone.}$

The calculated FICI can also be used to determine the nature of anti-bacterial interactions. If the FICI value is ≤ 0.5 , the combination can be considered synergistic. Conversely, if the FICI value falls between 0.5 and 1, the combination is classified as additive [59]. Moreover, to establish a more rigorous differentiation between synergistic and additive effects, it is deemed appropriate to classify an impact as true synergism only when the FICI is < 0.5 [59]. Furthermore, it is noteworthy that the checkerboard assay, which is frequently employed to determine FICI, provides a static representation of anti-bacterial interactions, as measurements are typically obtained at a single time point [60].

4.2 Time-kill analysis

The time-kill method uses a curve assay to provide valuable insights into the dynamic interactions between microorganisms and anti-bacterial agents, thereby elucidating their mechanisms of action over time [61]. This assay involves the quantification of bacteria (viable) in a liquid medium when exposed to specific combinations of anti-bacterial antibiotics at different time intervals [61]. Nevertheless, it is important to acknowledge that this approach is labour-intensive because it requires counting microbial colonies at multiple time intervals. This limitation restricts the feasible examination of concentrations

and the number of combinations [57]. Synergistic effects are evident when a colony count reduction of at least 100-fold is detected at the 24-hour mark, compared to the colony count reduction achieved by the most potent individual antibiotic. In contrast, an additive or indifferent impact is indicated when the alteration in colony count for the combination is lower than a 10-fold disparity after 24 h. The presence of antagonism is determined by observing a colony count at 24 h that is at least 100 times higher when the combination of components is used than when the individual components are used separately [57].

4.3 Epsilometer or E-test

The E-test approach presents a more efficient alternative in terms of labor-intensive requirements compared to the time-kill and checkerboard approaches. Additionally, it is advantageous because commercially developed standard test strips for antibiotics are readily available [57]. This approach includes the utilization of two strips of plastic, each coated with a uniform gradient of distinct antibacterial agents. These strips are arranged orthogonally (i.e., at 90° to one another) on a microorganism-coated agar plate. The point at which the two zones of inhibition overlap indicate the combined MIC of the antibacterial antibiotics [57]. Moreover, the determination of the nature of pharmacological interactions, whether they exhibit additivity, antagonism, or synergy, relies on the calculation of the FICI, which is determined using a methodology like the one outlined in the checkerboard approach [57].

5 Synergy potential of plant extracts with antibiotics against pathogenic bacteria

Several studies have documented enhanced anti-bacterial activity of clinical antibiotics when administered concomitantly with extracts from various medicinal plants. The investigations were conducted on several bacterial species, such as *Acinetobacter*, *Escherichia*, *Klebsiella*, *Pseudomonas*, *Salmonella*, and *Staphylococcus*. The subsequent findings have been thoroughly reviewed and concisely comprehended in Table 1. Farooqui et al. [62] conducted research to estimate the anti-bacterial activity of extracts from *Juglans regia* and *Camellia sinensis* when combined with various anti-bacterial agents. In this study, the E-test/agar incorporation assay, checkerboard titration and time-kill kinetics methods were used to assess the interaction. The investigation's results showed that *Camellia sinensis* extract enhanced anti-bacterial activity against MDR *S. typhi*, both alone and in combination with nalidixic acid. Whereas the *Juglans regia* extract combined with oxacillin displayed synergism, restoring oxacillin sensitivity in the methicillin-resistant MRSA strain in-vitro.

Another study conducted by Dey and his teammates [76] assessed the effectiveness of pomegranate fruit pericarp (*Punica granatum*) methanolic extract (PGME) in combination with the antibiotic ciprofloxacin against extended-spectrum β -lactamase (ESBL) producing strains of *E. coli* and *K. pneumoniae*, as well as metallo- β -lactamase (MBL) producing strains of *P. aeruginosa*. In this study, the individual MICs of PGME and ciprofloxacin were determined, and the potential synergy between the two agents was assessed using checkerboard assays and FIC calculations. This assessment identified 19 of 49 strains that exhibited synergy with ciprofloxacin (FIC ranging from 0.125 to 0.5), a finding further confirmed by agar-well assays. The observed synergy was attributed to the activity of the polyphenolic constituents present in PGME as bacterial EPIs. A study conducted by Olajuyigbe and Afolayan [77] reported that the methanolic extract of *Acacia mearnsii* enhances the bactericidal activity of various antibiotics, including amoxicillin, chloramphenicol, ciprofloxacin, erythromycin, kanamycin, metronidazole, nalidixic acid and tetracycline, against various bacterial strains. Fernandes and his co-workers [78] examined the synergistic effects of an aqueous extract of *Psidium guineense* (Swartz) leaves with various anti-bacterial antibiotics, including ampicillin, amoxicillin/clavulanic acid, ciprofloxacin, cefoxitin, and meropenem. The study revealed an 8-fold reduction in MIC values of these anti-bacterial agents when combined with the aqueous extract. The study suggested a synergistic interaction against MRSA strains, as revealed by FICI values ranging from 0.125 to 0.5.

Furthermore, a mango (*Mangifera indica*) peel-derived ethanol extract was examined against various *S. aureus* strains that possess efflux-mediated resistance to erythromycin, norfloxacin, and tetracycline. The co-administration of the extract with erythromycin and tetracycline at 512 $\mu\text{g/mL}$ resulted in a 4-fold decrease in the MIC values of the antibiotics [79]. Studies conducted by Dickson and his research group [80] examined various extracts (chloroform, ethanol, and petroleum spirit) of *Microglossa pyrifolia*, *Securinega virosa*, and *Mezoneuron benthamianum* for their resistance-modifying properties. The key goal of the research was to assess the impact of various extracts on the efficacy of standard anti-bacterial antibiotics against pathogenic *S. aureus* strains with efflux-mediated resistance mechanisms. The study reported a 4-fold increase in norfloxacin effectiveness with the ethanolic extract of *M. benthamianum* and the chloroform extract of *S. virosa*. Whereas, in the case of petroleum spirit extract, a dual increase in potency, as demonstrated by MIC values ranging from 8 to 16 $\mu\text{g/mL}$, was observed.

Table 1 From 2017–2025 various studies of synergetic combination of plant extracts and antibiotics against bacterial pathogens and their possible mechanism

Study country	Bacterial pathogen	Synergistic combination (Plant extracts + Antibiotics)	Synergy assay type	Possible phytochemicals	Possible mechanism of synergism	Outcomes	References
India	<i>Escherichia coli</i> ATCC 25,922, and <i>Staphylococcus aureus</i> ATCC 29,213	<i>Potentilla nepalensis</i> + Ampicillin, and chloramphenicol	Checkerboard	Gallic acid, rutin, quercetin, and ellagic acid	Binding interactions of ellagic acid to bacterial efflux pumps and multidrug resistance proteins	Bioactivity enhancements ranging from 5–32.26 folds with ampicillin and 8–62.5 folds with chloramphenicol	[63]
Ghana	Fluoroquinolone-resistant <i>Escherichia coli</i> ATCC 25,922, Fluoroquinolone-resistant <i>Klebsiella pneumoniae</i> ATCC 700,603, methicillin-resistant <i>Staphylococcus aureus</i> ATCC 43,300, and susceptible <i>Escherichia coli</i> (clinical), <i>Klebsiella pneumoniae</i> (clinical), and <i>Staphylococcus aureus</i> (clinical)	<i>Azadirachta indica</i> (AcE), <i>Bidens pilosa</i> (BpE), <i>Psidium guajava</i> (PgE), and <i>Adenia cissampeloides</i> (AcE) + Ciprofloxacin, cefixime, fluconazole	ND	ND	Alteration of membrane permeability and inhibit efflux mechanisms	Extracts also significantly enhanced the activity of ciprofloxacin against <i>E. coli</i> , reducing its MIC from 0.9 µg/ml to 0.12 µg/ml and 0.18 µg/ml, corresponding to 86% and 80% reductions, respectively	[64]
Australia	<i>Escherichia coli</i> , ESBL <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA), <i>Klebsiella pneumoniae</i> , and ESBL <i>Klebsiella pneumoniae</i>	<i>Terminalia canescens</i> DC. Radlk. + Ciprofloxacin	ND	Vitexin, quercetin, orientin, kaempferol, as well as the tannins ellagic acid, and pyrogallol	Inhibit the DNA gyrase, and affect the replication process; block the resistance mechanism of ciprofloxacin	The <i>Terminalia canescens</i> methanol extract (TCAM) and ciprofloxacin combination exhibited synergy against <i>E. coli</i> ; <i>Terminalia canescens</i> water extract (TCAW) and ciprofloxacin combination produced synergistic interactions against <i>K. pneumoniae</i>	[65]
India	<i>Escherichia coli</i> ATCC29213, <i>Bacillus subtilis</i> MTCC441, <i>Klebsiella pneumoniae</i> MTCC39, <i>Staphylococcus aureus</i> ATCC25922 and <i>Pseudomonas aeruginosa</i> MTCC424	<i>Phyllanthus emblica</i> L. + Ampicillin	Checkerboard	Ellagic acid, gallic acid, rutin, and quercetin	Bind to bacterial PBP-3 (penicillin binding protein-3)	Ellagic acid and quercetin interactions with ampicillin resulted in maximum bioactivity enhancement of 32–128 folds and 16–277 folds, respectively	[66]
Pakistan	<i>Acinetobacter</i> (clinical), <i>Klebsiella pneumoniae</i> (clinical), <i>Escherichia coli</i> (clinical), <i>Pseudomonas aeruginosa</i> (Clinical), Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) (clinical), and <i>Staphylococcus haemolyticus</i> (clinical)	<i>Gentian lutea</i> , <i>Mentha longifolia</i> , <i>Nigella sativa</i> , <i>Chamaecrista nictitans</i> , <i>Terminalia arjuna</i> , <i>Murraya koenigii</i> , <i>Momordica charantia</i> , <i>Terminalia chebula</i> and <i>Curcuma amada</i> + Cefixime	Checkerboard and Time-kill	Cinnamic acid, gallic acid, and quercetin	Internal components leak out of bacteria due to irreversible alterations in the permeability of their membranes caused by hydrophobicity variations	Time-kill kinetic studies showed that synergism was both time and concentration-dependent (2–8-fold decrease in concentration); Bacterial isolates treated with combinations at FICI showed significantly reduced bacterial growth, as well as protein content (5–62%) as compared to extracts/cefixime alone treated isolates	[67]

Table 1 (continued)

Study country	Bacterial pathogen	Synergistic combination (Plant extracts + Antibiotics)	Synergy assay type	Possible phytochemicals	Possible mechanism of synergism	Outcomes	References
India	<i>Staphylococcus aureus</i> ATCC 902 and <i>Escherichia coli</i> ATCC 443	<i>Andrographis paniculate</i> , and <i>Berberis aristate</i> + Ampicillin (Amp)	Checkerboard	Andrographolide, cis-resvetrol, dihydroxy methoxy flavone, dihydroquercetin, epicatechin, eserine trigonelline, glabridin, lupanine, myricetin, quercetin, syringic acid, and vanillic acid,	Inhibit the DNA gyrase, and affect the replication process	A combination of <i>Andrographis paniculata</i> extract (APE) with Amp showed synergistic interaction against <i>E. coli</i> and indifference was obtained against <i>S. aureus</i> ; While the combination of <i>Berberis aristata</i> extract (BAE) with Amp showed synergistic interaction against both the strains <i>E. coli</i> and <i>S. aureus</i>	[68]
Romania	Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) (clinical)	<i>Polyalthia longifolia</i> + Penicillin	Checkerboard	Apigenin, caffeic acid, catechin, epicatechin, gallic acid derivatives, harpagoside, loganin, quercetin-3-arabinoside, and, rosmarinic acid	Deterioration of cell lysis and membrane integrity	<i>P. longifolia</i> leaf extracts expressed synergistic antibacterial activity in combination with penicillin against a MRSA clinical isolate, inhibiting biofilm formation	[69]
Indonesia	Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) (clinical)	<i>Camelia sinensis</i> (Green tea) + Amoxicillin	Checkerboard	Epigallocatechin gallate (EGCG)	Disassemble the cytoplasmic membrane's structure	Green tea extract could potentially increase the antibacterial activity of amoxicillin in vitro with FICI of 0.28	[70]
Egypt	<i>Pseudomonas aeruginosa</i> (clinical)	<i>Rosmarinus officinalis</i> , and <i>Punica granatum</i> + Piperacillin, ceftazidime, imipenem, gentamycin, levofloxacin	Checkerboard	Benzoic acid, catechol, ellagic acid, gallic acid, pyrogallol, and rosmarinic acid	Inhibit the functions cytoplasmic membrane, cell wall, nucleic acids, and energy transport	Synergism between both plant extracts and antibiotics has significantly reduced the MICs of used antibiotics at the level lower than the susceptibility breakpoints; Pomegranate/rosemary/antibiotic combinations achieved the highest biofilm eradication, which ranging from 90.0 to 99.6%	[71]
Saudi Arabia	<i>Bacillus cereus</i> EMCC 1080, <i>Escherichia coli</i> O157:H7 ATCC51659, <i>Klebsiella pneumoniae</i> LMD 7726, <i>Pseudomonas aeruginosa</i> NRRLB-272, <i>Staphylococcus aureus</i> ATCC 13,565, and <i>Salmonella typhi</i> ATCC25566	<i>Ficus nitida</i> + Tetracycline	Checkerboard	Catechin, chlorogenic acid, procyanidin dimer I, and quinic acid,	Membrane fluidity is reduced as a result of flavonoid interactions with the cell membrane's hydrophilic side.	Synergistic interaction between the acetone extract and tetracycline was investigated against <i>B. cereus</i> , <i>S. aureus</i> , <i>E. coli</i> , and <i>P. aeruginosa</i> with FICI values of 0.43, 0.50, 0.38, and 0.375, respectively	[72]

Table 1 (continued)

Study country	Bacterial pathogen	Synergistic combination (Plant extracts + Antibiotics)	Synergy assay type	Possible phytochemicals	Possible mechanism of synergism	Outcomes	References
Brazil	<i>Staphylococcus aureus</i> ATCC29213, 25,923, and Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) (clinical)	<i>Vernonia condensata</i> Baker + Ampicillin, and chloramphenicol	Checkerboard and Time-kill	1,5-di-O-cafeoylquinic, apigenin, chlorogenic acid, and luteolin	The cell membrane is harmed (becoming unstable).	Ethyl acetate fraction from <i>V. condensata</i> leaves (Vc-EAF) plus ampicillin revealed a total synergic effect on MRSA 1,485,279, and Vc-EAF combined with chloramphenicol, a partial synergic action against <i>S. aureus</i> (ATCC 29213) and (ATCC 25923); The time kill data agreed with checkerboard results, and the treated cells number was reduced with release of bacterial content	[73]
Argentina	<i>Enterococcus faecalis</i> ATCC 29,212, <i>Enterobacter cloacae</i> (clinical), <i>Escherichia coli</i> ATCC 35,218, <i>Staphylococcus aureus</i> ATCC 29,213, <i>Morganella morganii</i> (clinical), <i>Klebsiella pneumoniae</i> (clinical), <i>Pseudomonas aeruginosa</i> ATCC 27,853, <i>Pseudomonas aeruginosa</i> (clinical), <i>Proteus mirabilis</i> (clinical), and Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) (clinical)	<i>Adenocalymma marginatum</i> , <i>Amphilophium vauthieri</i> , <i>Cuspidaria convolute</i> , and <i>Fridericia caudigera</i> + Ampicillin, gentamicin, and oxacillin	Checkerboard	Apigenin, chrysin, and luteolin	ND	A synergistic effect was observed in all extracts; The <i>F. caudigera</i> leaf extracts were most active	[74]
Chile	<i>Staphylococcus aureus</i> (clinical), and <i>Escherichia coli</i> (clinical)	<i>Vitis vinifera</i> pomace + Nalidixic acid, ciprofloxacin, norfloxacin, levofloxacin, oxacillin, tetracycline, and chloramphenicol	Checkerboard	Quercetin, gallic acid, protocatechuic acid, luteolin, (+)-catechin, (-)-epicatechin, vanillic acid, p-Coumaric acid, kaempferol, syringic acid, and ellagic acid	The cytoplasmic membrane's structure disintegrates, leading to integrity loss and cell death ultimately	Results showed that the grape pomace extract combined with representatives of different classes of antibiotics as β -lactam, quinolone, fluoroquinolone, tetracycline act in synergy in all <i>S. aureus</i> and <i>E. coli</i> strains tested with FICI values varying from 0.031 to 0.155; The MIC was reduced 4 to 75 times	[75]

ND = not defined, ESBL= extended-spectrum β -lactamase, FICI= fractional inhibitory concentration index; MIC= minimal inhibitory concentration

6 Synergy potential exhibited by phytocompounds with antibiotics against pathogenic bacteria

The discovery of novel, effective anti-bacterial agents targeting MDR bacteria is of utmost importance for addressing global health concerns [81]. In this context, certain phytocompounds have demonstrated the ability to either weaken or inactivate antibiotic resistance

mechanisms, thereby increasing bacteria's vulnerability to antibiotics [81]. This phenomenon results in a synergistic interplay among distinct phytocompounds, as illustrated in Fig. 5. Only a few phytocompounds demonstrated limited activity when used alone but exhibited significant efficacy when co-administered with antibiotics. On the other hand, other compounds exhibit synergistic activity through different mechanisms, along with their inherent

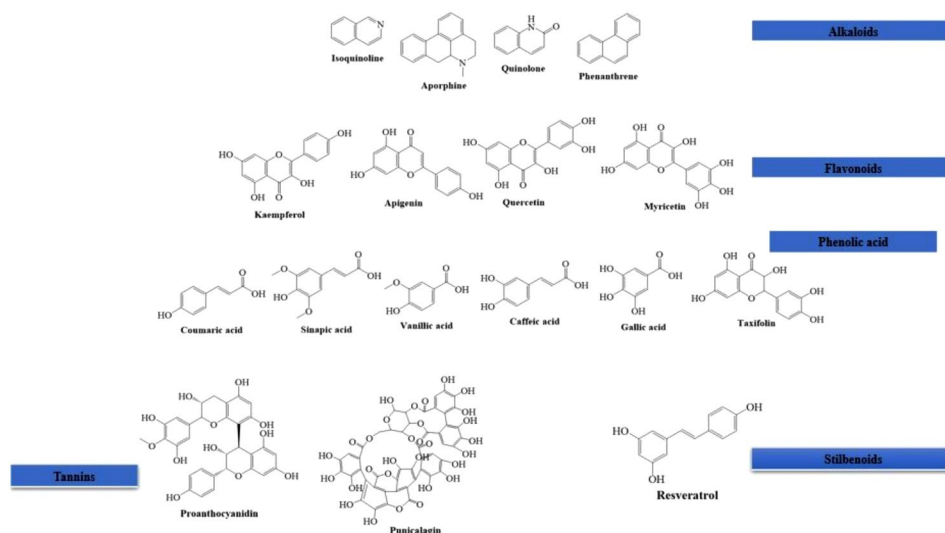


Fig. 5 Diagrammatic illustration of different phytochemicals showed synergistic action

anti-bacterial abilities attributable to pleiotropic effects [81]. Table 2 provides a comprehensive overview of pure compounds with synergistic mechanisms in conjunction with clinical antibiotics.

Tomás-Menor et al. [93] conducted a study in which the researchers employed high-performance liquid chromatography coupled with electrospray ionization mass spectrometry/mass spectrometry (HPLC-ESIMS/MS) to characterize key polyphenolic compounds in an extract of *Cistus salviifolius*. For this experiment, researchers employed the isobole approach and ascertained the FICI. In this study, the researchers investigated the pairwise combinations of certain ellagitannins and flavonoids isolated from the *C. salviifolius* extract on the in-vitro growth of *S. aureus*. The results obtained from the study showed synergistic effects in a few combinations, leading to a noteworthy decrease in the MIC₅₀ value, i.e., 20 times lower compared to the concentrations of individual antibiotics. Some of these combinations of antibiotics showed MIC₅₀ values in proximity to the potency range of antibiotics in a range of 0.5–1 µg/mL. Significantly, it has been found that punicalagin and myricetin play a considerable role in these combinations. In another study, Lin et al. [94] examined the effects of antibiotic and plant phenol combinations on 20 MRSA clinical isolates. In this study, checkerboard assays were used, which involved the co-administration of the antibiotic rifampicin with quercetin or kaempferol, demonstrating synergistic or partially synergistic activity against clinical MRSA isolates.

Furthermore, the combinations also showed significant inhibitory effects on β -lactamase, particularly when rifampicin was combined with kaempferol or quercetin. Oh, and Jeon [95] conducted an accumulation assay

to assess ciprofloxacin accumulation in *Campylobacter jejuni*. A total of six phenolic compounds were investigated in the study, including coumaric acid, gallic acid, caffeic acid, taxifolin, vanillic acid, and sinapic acid. Supplementation with these compounds increased susceptibility to erythromycin and ciprofloxacin in several poultry and human isolates of *C. jejuni*, even in cases where resistance to these antibiotics was conferred. These results unveiled that phenolic compounds present in *C. jejuni* enhanced membrane permeability and antibiotic accumulation. Moreover, certain compounds, such as taxifolin and gallic acid were found to reduce CmeABC multidrug efflux pump expression. Khan et al. [96] reported that piperine, a prominent plant alkaloid present in the Piperaceae family, which encompasses black pepper and long pepper, exhibited the ability to enhance the accumulation of ciprofloxacin in *S. aureus*. Akilandeswari and Ruckmani [97] conducted a study to assess the anti-MRSA effectiveness and synergistic potential of apigenin (AP) alone and in combination with ampicillin (AM) and ceftriaxone (CEF) against MRSA infections. The checkerboard assay revealed that apigenin addition led to a significant decline in the MIC values for ceftriaxone (from 58 µg/mL to 2.6 µg/mL) and ampicillin (from 800 µg/mL to 107 µg/mL) when used in combination against MRSA. The synergistic potential of these antibiotics and apigenin blends was examined using FICI, which ranged from 0.18 to 0.47. These antibiotic combinations were further assessed for their inhibitory effects and confirmed by Time-kill assays, which showed approximately 99% reduction in colony counts after 8 h. These results indicate that a combination of ampicillin and ceftriaxone, in conjunction with apigenin, disrupts the cytoplasmic membrane of MRSA, leading to the release of intracellular constituents.

Table 2 From 2016–2025, various studies of the synergetic combination of phytochemicals along with antibiotics against pathogenic bacteria and their plausible mechanism

Study country	Bacterial pathogen	Source of phytochemicals	Synergistic combination (Phytochemicals + Antibiotics)	Synergy assay type	Possible mechanism of synergism	Outcomes	References
Türkiye	Multidrug-resistant <i>Acinetobacter baumannii</i> (clinical)	Commercial	Curcumin, punicalagin, geraniol (GER), and linalool (LIN) + colistin (COL), meropenem, and ciprofloxacin	Checkerboard	Suppressed biofilm production	COL with GER (83.87%) and with LIN (77.42%) exhibited strong synergistic interactions, with FICI values between 0.12 and 0.5; Where synergism was observed, antibiotic MICs were reduced by 2- to 128-fold, indicating substantial enhancement in bacterial efficacy	[82]
Spain	<i>Staphylococcus aureus</i> ATCC9144, <i>Streptococcus agalactiae</i> ATCC 12,386, <i>Listeria monocytogenes</i> ATCC7644, <i>Enterococcus faecalis</i> ATCC19433, <i>Bacillus subtilis</i> ATCC 6633, <i>Escherichia coli</i> ATCC25922, <i>Salmonella typhimurium</i> ATCC13311, <i>Pseudomonas aeruginosa</i> ATCC27853, <i>Acinetobacter baumannii</i> ATCC19606, <i>Klebsiella aerogenes</i> ATCC13048, <i>Pasteurella aerogenes</i> ATCC27883, <i>Klebsiella pneumoniae</i> C6, <i>Proteus mirabilis</i> ATCC35659, and <i>Serratia marcescens</i> ATCC 13,880	Commercial	Nerol (NE), tannic acid (TA) + Gentamycin, streptomycin, chloramphenicol, amoxicillin, ampicillin, penicillin G, erythromycin, and tetracycline	Checkerboard	Affecting the permeability and integrity of the outer membrane, cell lysis	For NE, combinations with streptomycin and gentamicin were effective against <i>Salmonella enterica</i> , <i>Bacillus subtilis</i> , and <i>Streptococcus agalactiae</i> , with antibiotic MIC reductions between 75.0 and 87.5%; TA showed six synergies with chloramphenicol, ampicillin, erythromycin, and streptomycin against <i>Acinetobacter baumannii</i> , <i>Streptococcus agalactiae</i> , and <i>Pasteurella aerogenes</i> , with MIC reductions between 75.0 and 93.7%	[83]

Table 2 (continued)

Study country	Bacterial pathogen	Source of phytochemicals	Synergistic combination (Phytochemicals + Antibiotics)	Synergy assay type	Possible mechanism of synergism	Outcomes	References
Saudi Arabia	<i>Bacillus cereus</i> , <i>Klebsiella pneumoniae</i> , <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Salmonella typhi</i> , and <i>Pseudomonas aeruginosa</i>	<i>Ficus retusa</i> (bark)	Catechin, chlorogenic acid + Tetracycline	Checkerboard and Time-kill	Alters the membrane permeability	The synergistic interaction of catechin and chlorogenic acid as the most major compounds with tetracycline was evaluated by checkerboard assay followed by time-kill assay, against <i>B. cereus</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , and <i>S. typhi</i> with FICI of 0.38, 0.43, 0.38, 0.38, 0.38, and 0.75 for catechin and 0.38, 0.65, 0.38, 0.63, 0.38, and 0.75 for chlorogenic acid; The combination of catechin and chlorogenic acid with tetracycline significantly enhanced antibacterial action against Gram-positive and Gram-negative microorganisms	[84]
Germany	<i>Acinetobacter baumannii</i> ATCC19606, <i>Acinetobacter baumannii</i> DSM 9308, <i>Acinetobacter baumannii</i> PBIO721 (clinical), <i>Pseudomonas aeruginosa</i> ATCC 27,853, <i>Escherichia coli</i> PBIO730, PBIO1442 (clinical), <i>Escherichia coli</i> ATCC 25,922, <i>Klebsiella pneumoniae</i> ATCC 700,603, <i>Klebsiella pneumoniae</i> PBIO1455, PBIO1990 (clinical), <i>Klebsiella pneumoniae</i> PBIO712, PBIO2208 (clinical), <i>Staphylococcus aureus</i> PBIO483 (clinical) and <i>Staphylococcus aureus</i> ATCC 29,213	Commercial	Epigallocatechin gallate, epicatechin, 3-hydroxy-6-methoxyflavone, gallic acid, myricetin, daidzein, genistein + Ampicillin, gentamicin, cefotaxime, chloramphenicol, ciprofloxacin and tetracycline	Checkerboard and Time-kill	Cell wall disruption caused by beta lactams, suppression of trans-peptidation combined with possible suppression of efflux systems, namely the efflux pump NorA	Time-kill curves for combinations with possible synergistic effects confirmed the results against <i>Acinetobacter baumannii</i> as the one with the greatest need for research	[85]

Table 2 (continued)

Study country	Bacterial pathogen	Source of phytochemicals	Synergistic combination (Phytochemicals + Antibiotics)	Synergy assay type	Possible mechanism of synergism	Outcomes	References
South Korea	<i>Salmonella enterica</i> serovar Typhimurium ATCC 14,028, <i>Salmonella enterica</i> serovar Typhimurium V15-S-HA-02-210, V08-S-HA-06-170, SAL 202, SAL 109, and SAL 224 (clinical)	Commercial	Epicatechin, gallic acid (GA), epicatechin gallate, hamamelitannin, and epigallocatechin + Amoxicillin, ampicillin, ceftiofur, cefotaxime, florfenicol, erythromycin, norfloxacin, marbofloxacin, thiamphenicol and penicillin G	Checkerboard	SOS operon expression was induced by breaking DNA or stopping DNA replication process, and/or by inhibiting or mutating PBP (penicillin-binding proteins)	Synergistic antibacterial effect was obtained from the combination of erythromycin-epicatechin gallate (FICI: 0.50) against <i>S. Typhimurium</i> ; The time-kill assay showed that GA-ceftiofur combination more efficiently inhibited the growth of <i>S. Typhimurium</i> compared to individual antimicrobials	[86]
South Korea	<i>Escherichia coli</i> ATCC 25,922, <i>Escherichia coli</i> V17-13-S02-002-009, V10-13-A02-002-008, V03-13-A03-002-009, V03-13-A01-002-003, and V16-13-S01-002-002 (clinical)	Commercial	Gallic acid (GA), epigallocatechin, hamamelitannin, epicatechin gallate, and epicatechin + Penicillin G, thiamphenicol, norfloxacin, marbofloxacin, erythromycin, florfenicol, ceftiofur, amoxicillin, cefotaxime and ampicillin	Checkerboard	Changes in septum development and a lack of separation of daughter cells during cell division	The GA-ampicillin and hamamelitannin-erythromycin combinations more effectively prohibited the growth, biofilm viability, and swim and swarm motilities of <i>E. coli</i> than individual antibiotics	[87]
China	<i>Escherichia coli</i> (clinical), <i>Proteus mirabilis</i> (clinical), <i>Staphylococcus aureus</i> (clinical), <i>Streptococcus iniae</i> (clinical)	Commercial	Protocatechuic acid, chlorogenic acid + Fosfomycin, azithromycin, sulfamonomethoxine, amoxicillin, ceftiofur sodium, cefotaxime sodium, ceftriaxone sodium, florfenicol, clindamycin, and mequindox	Checkerboard	Block protein kinase expression and eliminate the protein synthesis inhibitor efflux pump.	Combined effects of protocatechuic acid and chlorogenic acid with antibiotics against Gram-negative bacteria were better than that of Gram-positive bacteria	[88]
Argentina	<i>Escherichia coli</i> (clinical), <i>Escherichia coli</i> ATCC 35,218, <i>Pseudomonas aeruginosa</i> (clinical), <i>Pseudomonas aeruginosa</i> ATCC 27,853, <i>Staphylococcus aureus</i> ATCC 29,213, ATCC 25,923, <i>Staphylococcus aureus</i> 5357, 5289, 5632, 5627, 5377, 5621, 5246, 5307, 5637, 5722 (clinical),	<i>Cuspidaria convolute</i> (Leaves)	Coumaric acid, catechin/epicatechin, and luteolin + Ampicillin, gentamicin, and Oxacillin	Checkerboard	Hinders the cytoplasmic membrane's stability, inhibits β -lactamase, and blocks the enzymes responsible for folic acid synthesis.	The combination of luteolin and ampicillin had the most potent antibacterial activities	[89]

Table 2 (continued)

Study country	Bacterial pathogen	Source of phytochemicals	Synergistic combination (Phytochemicals + Antibiotics)	Synergy assay type	Possible mechanism of synergism	Outcomes	References
India	<i>Staphylococcus aureus</i> ATCC 25,923, Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) (clinical)	Commercial	Rosmarinic acid (RA) + Vancomycin, ofloxacin, and amoxicillin	Checkerboard and Time-kill	MSCRAMMs (micro-bial surface components recognising adhesive matrix molecules) should be suppressed.	RA was synergistic with vancomycin, ofloxacin, and amoxicillin against <i>S. aureus</i> and only with vancomycin against MRSA; The time-kill analysis revealed that synergistic combinations were a more effective than individual antibiotics	[90]
Brazil	<i>Escherichia coli</i> (clinical), <i>Pseudomonas aeruginosa</i> (clinical), and <i>Staphylococcus aureus</i> (clinical),	Commercial	Gallic acid, caffeic acid, and pyrogallol + Imipenem, gentamicin and norfloxacin	ND	Inhibition of the bacterial RNA polymerase enzyme	Caffeic acid was the substance that presented with the greatest number of antibiotics and with the greatest number of bacteria	[91]
India	<i>Klebsiella pneumoniae</i> ATCC 700,603, and <i>Klebsiella pneumoniae</i> (Clinical)	Commercial	Ellagic acid, caffeic acid, quercetin, and epigallocatechin-3-gallate (EGCG) + Ciprofloxacin, gentamicin, and tetracycline	Checkerboard	Efflux pump inhibitor	EGCG and caffeic acid could synergize the activity of tested antibiotics within a major population of β -lactamase producing <i>K. pneumoniae</i>	[92]

ND = not defined, FICI= fractional inhibitory concentration index

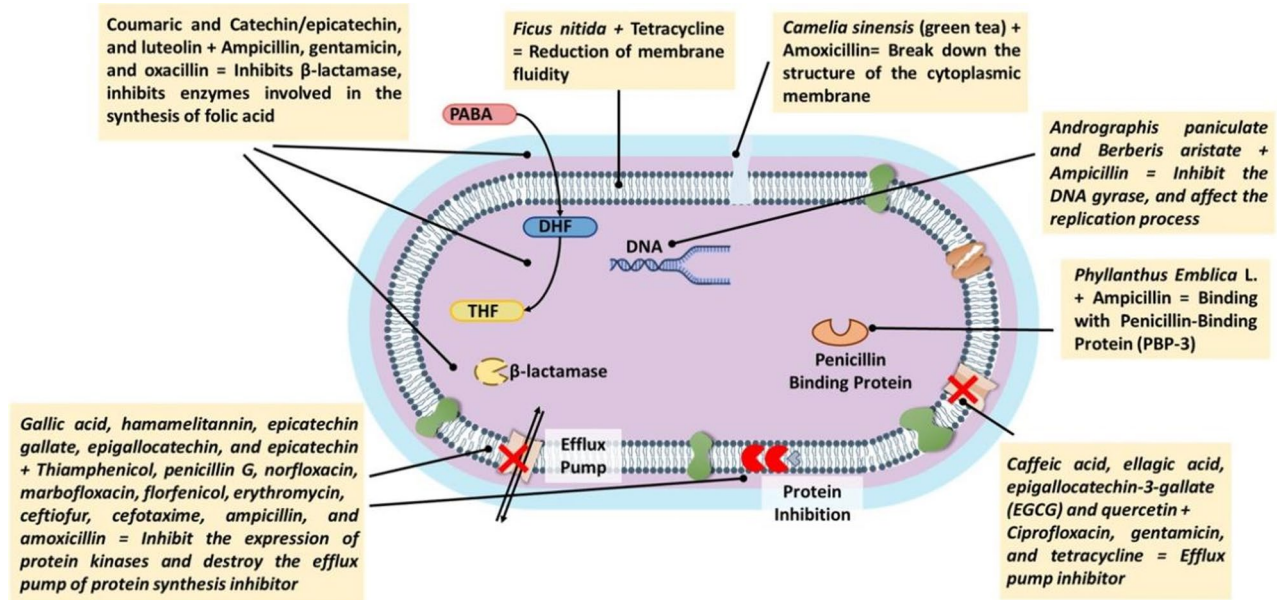


Fig. 6 Overview mechanism of action of synergistic combination of plant extracts, phytochemicals and antibiotics against drug-resistant bacteria

7 Mechanisms underlying the synergistic combination of plant extracts, phytocompounds, and antibiotics against bacterial pathogens

This review section aims to comprehensively analyze potential mechanisms underlying the synergistic effects of plant extracts and phytocompounds, as well

as antibiotic combinations, against pathogenic bacteria. These mechanisms are further elucidated and summarized in Tables 1 and 2 and are visually illustrated in Fig. 6.

1. Inhibiting active site modification: Numerous phenolic compounds, namely magnatriol B, momorcharaside B, pinosresinol, and tiliroside, derived from traditional Chinese medicine, have been recognized as potential inhibitors of enzymes such as PBP2a and PBP4. These enzymes are known to modify active sites and promote antibiotic resistance, particularly in MRSA. Consequently, the combination of β -lactam antibiotics with phenolic compounds has been accorded to exhibit synergistic anti-bacterial effects against MRSA [98].
2. Inhibition of enzymes that modify antibiotics: The investigation of plant extracts and phytochemicals as potential β -lactamases inhibitors is an ongoing area of research. Enzymes in this class are responsible for degrading or modifying antibiotics, thereby playing a vital role in the development of antibiotic resistance [99]. The study by Yam et al. [100] revealed that extracts and chemicals derived from *Camellia sinensis* (green tea) can inhibit β -lactamase enzymes. This inhibition has been shown to reverse resistance in *S. aureus* strains. Baicalin, a flavonoid derived from *Scutellaria amoena*, has been reported to possess inhibitory activity against β -lactamases, making it a potentially valuable compound for combating against antibiotic resistance.
3. Inhibition of permeability enhancers/efflux pumps: Compounds like conessine (a steroidal alkaloid) have been reported to effectively inhibit efflux pumps such as MexAB-OprM in the pathogen *P. aeruginosa*. This suppression of efflux pumps aids in restoring antibiotic action [101]. Nevertheless, the exact mechanism of efflux pump inhibition remains incompletely elucidated; it is postulated that these phytochemicals interact directly with the pumps, thereby inducing their inactivation. Additional proposed methods may involve the exhaustion of energy or the formation of complexes with antibiotics, consequently boosting their penetration into bacterial cells. According to a study by Zloh and his team [102], the increase in antibiotic-inhibitor complex size can confine the antibiotic within cells, resulting in higher intracellular concentrations. This phenomenon contributes to the improved effectiveness of anti-bacterial treatment. Furthermore, it has been observed that a few phytochemicals, like caffeic acid and epicatechin 3-gallate, can interfere with the exterior membrane of bacteria (Gram-negative), leading to an augmentation in membrane permeability. When co-administered with hydrophobic antibiotics

such as rifampicin and erythromycin, these phytochemicals and antibiotics can augment the penetration of antibiotics into bacterial cells. This phenomenon increases intracellular antibiotic concentrations, enhancing anti-bacterial effects [103].

8 Synergy potential of EOs with antibiotics against pathogenic bacteria

Multiple studies have highlighted the synergistic augmentation of quantifiable anti-bacterial antibiotics when co-administered with EOs derived from diverse medicinal plants. These studies involve bacterial species like *Acinetobacter*, *Escherichia*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, and *Salmonella*. The comprehensive information of these findings has been concisely presented in Table 3. The effectiveness of EOs in killing bacteria hinges on their main constituents and the synergistic interactions among them [104]. The fundamental constituents of EOs are illustrated in Fig. 7. It is worth noting that the anti-bacterial mechanisms of different anti-bacterial antibiotics may vary. As a result, the anti-bacterial activity of EOs generally involves multiple concurrent mechanisms of action rather than a single mechanism. From this, it can be inferred that the anti-bacterial action of EOs primarily targets different sites within microorganisms [105].

Fadil and his co-workers [120] evaluated the synergistic interactions between antibiotics such as cefixime, ciprofloxacin, gentamicin, and pristnamycin and EOs derived from Moroccan thyme spp., specifically *Thymus broussonetii* and *Thymus maroccanus*. A total of 80 combinations of EOs and antibiotics were tested. The study results revealed that 71% of the combinations showed complete synergism, 20% exhibited partial synergism, and the remaining 9% showed no effect. Notably, a synergistic effect was observed with carvacrol, the primary constituent of *T. broussonetii* and *T. maroccanus*, when combined with ciprofloxacin. Fadil et al. [121] conducted a subsequent study to investigate the anti-bacterial potential of *Thymus riatarum* EOs in an in-vitro condition. The current study used different assays that specifically target human pathogenic isolates. The phytochemical analysis of EO obtained from *T. riatarum* showed that approximately 20 diverse compounds were present, with borneol (41.67%), terpinen-4-ol (8.65%), and trans-caryophyllene (7.59%) as the primary constituents.

Furthermore, the *T. riatarum* EO was investigated for its ability to inhibit efflux pump mechanisms in Gram-negative bacteria resistant strains. The obtained results also showed a significant enhancement when

Table 3 From 2017–2025, various studies of the synergetic combination of EOs along with antibiotics against pathogenic bacteria and their plausible mechanism

Study country	Bacterial pathogen	Synergistic combination (EOs + Antibiotics)	Synergy assay type	Possible major compounds	Possible mechanism of synergism	Outcomes	References
Burkina Faso	<i>Staphylococcus aureus</i> ATCC 2523, <i>Escherichia coli</i> ATCC 25,922, <i>Pseudomonas aeruginosa</i> ATCC 9027, <i>Enterococcus faecalis</i> 0366 V, <i>Salmonella typhi</i> SKN 1152, <i>Bacillus cereus</i> 0998 V, <i>Bacillus subtilis</i> 0486 V, and <i>Yersinia enterocolitica</i> 0938 V	<i>Hyptis suaveolens</i> and <i>Lagerflora aurita</i> + Ciprofloxacin, amoxicillin + clavulanic acid, and colistin	Checkerboard	β -caryophyllene, sabinene, terpinolene and limonene	Peptidoglycan and cytoplasmic membrane damage	Checkerboard assays revealed synergistic effects resulting in reductions of 93.69% and 87.73%, respectively, in amoxicillin+clavulanic acid and colistin MICs	[106]
Brazil	Multidrug-resistant <i>Acinetobacter baumannii</i> (clinical)	<i>Lippia origanoides</i> + Meropenem, ciprofloxacin, and gentamicin	Checkerboard	Carvacrol, <i>p</i> -cymene, and thymol	Cell membrane disruption	The combinations with antibiotics revealed significant synergistic effects, lowering the minimum inhibitory concentration of the tested antibiotics	[107]
Romania	<i>Staphylococcus aureus</i> (clinical), <i>Acinetobacter baumannii</i> (clinical), <i>Pseudomonas aeruginosa</i> (clinical), and <i>Klebsiella pneumoniae</i> (clinical)	<i>Tanacetum vulgare</i> + Cefepime, amoxicillin, clavulanic acid and tobramycin	ND	Sabinene, eucalyptol, trans- β -ocimene, linalool, filifolone, thujone, camphor, cis-carveol, sabinol, isovalerate, chrysanthenone, terpinen-4-ol, germacrene D, cedrol, cubenol, ledol, globulol, ketone, and spirojatanol	Cell lysis by disintegration of cell membrane	Synergistic activity with different classes of antibiotics have also been noted	[108]

Table 3 (continued)

Study country	Bacterial pathogen	Synergistic combination (EOs + Antibiotics)	Synergy assay type	Possible major compounds	Possible mechanism of synergism	Outcomes	References
India	<i>Escherichia coli</i> MTCC 2127, <i>Klebsiella pneumoniae</i> MTCC 7172, <i>Micrococcus leuteus</i> MTCC 4821, <i>Staphylococcus aureus</i> MTCC 7443, and <i>Bacillus subtilis</i> MTCC 2389	<i>Nardostachys jatamansi</i> + Amoxicillin, erythromycin, chloramphenicol, and ampicillin	ND	Guaia-6,9-diene, calarene, jatamansone, α -gurjunene, valencene, α -maaliene, sprojatamol, and caratol	Efflux pump inhibition, β -lactamase inhibition, inhibition of shared metabolic pathways, and membrane permeabilization	The results of <i>Nardostachys jatamansi</i> EO interactions with conventional antibiotics revealed that amoxicillin, erythromycin, chloramphenicol, and ampicillin MICs were reduced by 5 to 10-fold, 4 to 9.09-fold, 4 to 10.5-fold, and 4 to 8.0-fold, respectively	[109]
Brazil	<i>Staphylococcus aureus</i> ATCC 29,213, antibiotic-resistant <i>Staphylococcus aureus</i> UF-PEDA-659, UF-PEDA-671, UF-PEDA-691, UF-PEDA-705, UF-PEDA-731, and UFPEDA-802 (clinical)	<i>Eugenia stipitata</i> McVaugh + Ciprofloxacin, and gentamicin	ND	Sesquiterpenes	Destabilization in the membrane, promoting the extravasation of proteins, genetic material, and potassium ions	Showed a synergistic effect with all investigated antibiotics	[110]
Portugal	<i>Staphylococcus aureus</i> ATCC 25,923, <i>Staphylococcus aureus</i> 03/10 (clinical), Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) 12/08 (clinical)	<i>Thymus zygis</i> + Ampicillin, ciprofloxacin, and vancomycin	Checkerboard and Time-kill	Thymol, carvacrol, and p-cymene	Disrupt cell membrane integrity	EO demonstrated synergistic interactions in combination with ampicillin, ciprofloxacin or vancomycin against <i>S. aureus</i> strains	[111]

Table 3 (continued)

Study country	Bacterial pathogen	Synergistic combination (EOs + Antibiotics)	Synergy assay type	Possible major compounds	Possible mechanism of synergism	Outcomes	References
Algeria	<i>Acinetobacter baumannii</i> IRAB S3310), and methicillin-resistant <i>Staphylococcus aureus</i> MRSA S19 (clinical)	<i>Artemisia herba alba</i> Asso and <i>Mentha pulegium</i> L. + Oxacillin, cefoxitin vancomycin, ticarcillin, piperacillin, ticarcillin-clavulanic acid, cefotaxime, ceftazidime, cefepime, imipenem amikacin, tobramycin, ciprofloxacin, nalidixic acid, aztreonam	Time-kill	Pulegone, neoisomenthol, camphor, α -thujone, 1,8-cineole, β -thujone, bornéol, camphene, and p-cymene	ND	For IRAB S3310, the strongest synergistic effect was observed with <i>M. pulegium</i> in association with amikacin	[112]
Morocco	<i>Bacillus subtilis</i> ATCC 9524, <i>Escherichia coli</i> ATCC 8739, <i>Klebsiella pneumoniae</i> (clinical), <i>Staphylococcus aureus</i> CCMM B3, <i>Micrococcus luteus</i> ATCC 10,240, and <i>Pseudomonas aeruginosa</i> DSM 50,090	<i>Thymus atlanticus</i> (Ball) Roussine + Ciprofloxacin	Checkerboard	γ -Terpinene, thymol, o-Cymene, carvacrol, monoterpenes, and sesquiterpenes	Alter phospholipid bilayer	The maximum synergistic effect was observed for <i>K. pneumoniae</i> with a 256-fold gain of antibiotic MIC	[113]
Brazil	<i>Staphylococcus aureus</i> ATCC 6538, 700,698, <i>Staphylococcus aureus</i> (clinical)	<i>Croton tetradenius</i> Baill, and <i>Croton pulegioidorus</i> Baill. + Ampicillin, oxacillin, and benzylpenicillin	Checkerboard	p-cymene, camphor, α -phellandrene, trans-chrysanthenyl acetate, and α -terpinene	Beta-lactamase inhibition, disruption of both cell membrane and cell wall, accompanied by diminished action of the bacterial efflux pumps	In combination with antibiotics, both essential oils showed synergisms effect with oxacillin and ampicillin	[114]
Brazil	<i>Klebsiella pneumoniae</i> (clinical), and <i>Serratia marcescens</i> (clinical)	<i>Cinnamomum cassia</i> L. + Polymyxin B	Checkerboard and Time-kill	Trans-cinnamaldehyde	Cell membrane leakage	The association between <i>Cinnamomum cassia</i> L. essential oil (CCeo) and polymyxin B showed a decrease in terms of viable cell counts on survival curves over time after a 4 h-treatment with a FIC index value of 0.006	[115]

Table 3 (continued)

Study country	Bacterial pathogen	Synergistic combination (EOs + Antibiotics)	Synergy assay type	Possible major compounds	Possible mechanism of synergism	Outcomes	References
Brazil	<i>Acinetobacter baumannii</i> (clinical)	<i>Origanum vulgare</i> + Polymyxin B	Checkerboard	Carvacrol, β -caryophyllene, γ -terpinene, p-cymene, and thymol	Destabilization in the membrane	Oregano essential oil (OEO) presented 16-fold reduction of the polymyxin B MIC	[116]
Morocco	<i>Bacillus subtilis</i> ATCC 9524, <i>Escherichia coli</i> ATCC8739, <i>Klebsiella pneumoniae</i> (clinical), <i>Micrococcus luteus</i> ATCC 10240, <i>Pseudomonas aeruginosa</i> (DSM 50090 (clinical)), and <i>Staphylococcus aureus</i> CCMMB3	<i>Senecio anteuphorbium</i> + Ciprofloxacin	ND	γ -selinene, cyperene, γ -cadinene, and α -cyperone	Increase of bacterial membrane permeability	The combination of the studied EO with ciprofloxacin showed a significant decrease in their individual MICs	[117]

Table 3 (continued)

Study country	Bacterial pathogen	Synergistic combination (EOs + Antibiotics)	Synergy assay type	Possible major compounds	Possible mechanism of synergism	Outcomes	References
Italy	<i>Acinetobacter baumannii</i> ATCC19606, <i>Bacillus cereus</i> ATCC10876, <i>Bacillus subtilis</i> ATCC6633, <i>Enterococcus faecalis</i> ATCC29212, <i>Klebsiella pneumoniae</i> ATCC 19,883, <i>Escherichia coli</i> ATCC25922, <i>Pseudomonas aeruginosa</i> ATCC27853, <i>Staphylococcus aureus</i> ATCC29213, <i>Staphylococcus aureus</i> ATCC6538p, and <i>Staphylococcus aureus</i> ATCC 43,300 (MRSA),	<i>Mentha piperita</i> L. + Gentamicin, and ampicillin	Checkerboard	α and β -Pinene, eucalyptol, linalool, α -Terpineol, limonene, and menthol	Damage to cell membrane	The synergistic effect of <i>M. piperita</i> EO with gentamicin resulted in a strong growth inhibition for all the bacterial species	[118]
Thailand	<i>Acinetobacter baumannii</i> (clinical)	<i>Zingiber cassumunar</i> Roxb + Amoxicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, ceftazidime, ceftiofur, ceftriaxone, cefotaxime, imipenem, ertapenem, meropenem, amikacin, gentamicin, tetracycline, doxycycline, levofloxacin, ciprofloxacin, and trimethoprim-sulfamethoxazole	Time-kill	Sabinene, terpinene-4-ol, γ -terpinene, and DMPBD	Inhibit folate pathway	The essential oil had a synergistic effect when combined with antibiotics, e.g., aminoglycosides, fluoroquinolones, tetracyclines, and folate pathway inhibitors	[119]

ND = not defined, MIC= minimal inhibitory concentration, FIC= fractional inhibitory concentration

phenylalanine arginyl β -naphthylamide (PA β N) was present as an efflux pump inhibitor on the EO. Furthermore, the tested EO, when administered at a concentration of MIC/4, showed a substantial rise in the susceptibility of numerous resistant isolates to chloramphenicol. Yap and his research group [122] investigated synergistic effects of essential oils derived from diverse sources, namely tea tree, cinnamon bark, marjoram, lavender, and

peppermint, in combination with antibiotics like ampicillin, cefazolin, ceftazidime, cefuroxime, carbenicillin, meropenem and piperacillin using checkerboard assay against *E. coli* capable of producing beta-lactamase. The interactions between these combinations were characterized by calculating the FIC values. The initial findings from the study highlighted a significant synergistic association between cinnamon bark and piperacillin oil,

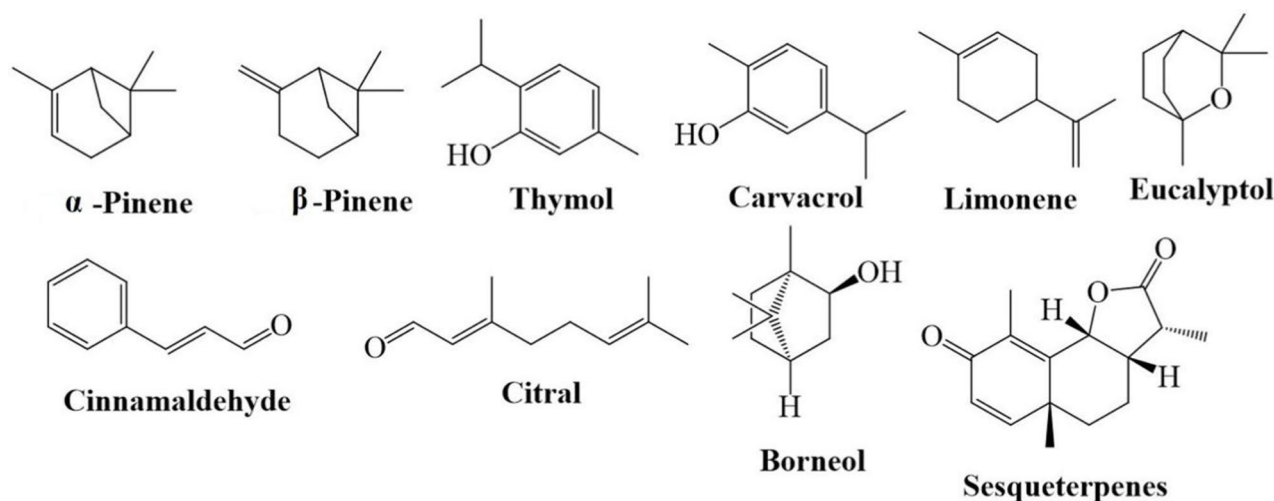


Fig. 7 Diagrammatic illustration of different phytocompounds present in EOs showed synergistic action

peppermint and piperacillin oil and lavender and piperacillin oil when tested against two of the three bacteria under investigation, with FIC indices ranging from 0.26 to 0.5. Aleksic et al. [123] conducted research to analyze the impact of subinhibitory myrtle EO concentrations in combination with regular antibiotics, namely ciprofloxacin and polymyxin B, using the checkerboard method. The study revealed a synergistic effect between the EOs isolated from *Myrtus communis* L. (myrtle) and both anti-bacterial agents against wound isolates of MDR *Acinetobacter baumannii* strains, with FIC indices either less than or equal to 0.50. The concurrent application of subinhibitory doses of EOs and ciprofloxacin resulted in synergistic effects, as evidenced by reduced bacterial growth in most cases. Moreover, a similar outcome was observed with polymyxin B, and the use of myrtle EO restored susceptibility in wound isolates resistant to multiple antibiotics. Utchariyakiat and his research team [124] conducted the checkerboard test to examine synergistic interactions among MDR *P. aeruginosa* strains. Significantly, combining cinnamaldehyde and cinnamon bark oil with colistin resulted in synergistic rates of 10% and 16.7%, respectively. These studies collectively offer valuable insights into the potential synergistic interactions between EOs and antibiotics against MDR bacterial strains. The findings highlight the potential of synergistically using these natural chemicals as a viable strategy to address antibiotic resistance successfully.

9 Mechanism underlying the synergetic combination of EOs and antibiotics against bacterial pathogens

The potential mechanisms explaining the synergistic effects of EOs in combination with antibiotics against bacterial pathogens are summarized in Table 3. The

processes that contribute to pharmacological synergy encompass:

1. Multi-target effect: This mechanism refers to the simultaneous targeting of different sites within the bacterial cell by compounds present in EO and antibiotics [125].
2. Physicochemical or pharmacokinetic effects: The utilization of EOs can potentially have physicochemical or pharmacokinetic effects, leading to improved solubility or bioavailability of antibiotics. This enhancement in solubility and bioavailability can improve the overall efficacy of antibiotics [125].
3. Targeting specific resistance mechanisms: EOs have effectively targeted and inhibited specific bacterial resistance pathways [114].

10 Toxicity concerns of plant extracts and essential oils for potential use as natural anti-bacterial

Plants that act as antibacterials form a notable class of natural preservatives, characterized by secondary metabolites that specifically target microbial cells [126]. Different plant parts, encompassing fruits, seeds, roots, leaves, and peels, exhibit substantial concentrations of plant anti-bacterial agents. These plant anti-bacterial agents comprise a diverse range of phytocompounds, including phenolic compounds (such as anthocyanins, flavonoids, simple phenols, phenolic acids and quinones), tannins, EOs, terpenoids, alkaloids, glucosinolate derivatives and thiols [127, 128]. Pinto and his coworkers [126] have documented that the US FDA (United States Food and Drug Administration) has designated numerous plant extracts as GRAS (Generally Recognized as Safe) and granted the QPS (Qualified Presumption of Safety) approval by EFSA (European Food Safety Authority). Nevertheless, it is

noteworthy that specific approval for their use in diverse food applications beyond these designations may not be explicitly granted. For instance, berberine alkaloids were reported to exhibit low bioactivity when administered orally, and their plasma concentrations showed a non-linear relationship with the administered dose [129]. In another 2010 study carried out on mice, the median lethal dose (LD₅₀) for berberine administered via different routes were determined to be 57.6103 mg/Kg for intraperitoneal, 9.0386 mg/Kg for intravenous, and none for the intragastric (oral) route [130]. In addition, LD₅₀ values for the total extract of *Rhizoma coptidis* in mice were determined to be 2.95 g/Kg, indicating dose- and time-dependent cytotoxicity [131]. As reported in acute toxicity experiments, the LD₅₀ value of *R. coptidis* fibrous roots in kunming mice was > 7000 mg/Kg body weight. However, the administration of a 1.88 g/Kg dose of the body weight of rats (Sprague-Dawley) did not elicit any discernible adverse effects. In contrast, a dosage of 3.76 g/Kg body weight resulted in lung and liver damage [132]. Yi et al. [133] reported that the LD₅₀ values of berberine, coptisine, epiberberine, and palmatine in mice were 713.57, 852.12, 1360, and 1533.68 mg/Kg, respectively. Additionally, it is worth noting that morbidity or mortality was observed in the sub-chronic toxicity studies. The use of EOs in aromatherapy, which encompasses popular variants such as tea tree oil, peppermint, and lavender, has been associated with a range of positive and negative impacts. Notably, specific individuals have reported minor to severe side effects, including the development of dermatitis [134]. However, it is noteworthy that EOs constitute a significant group of phytochemicals that are widely considered non-toxic for human consumption. According to Yu et al. [44], over 300 distinct EOs have been categorized as GRAS. This highlights the potential of these substances for therapeutic applications in humans. Nevertheless, it is imperative to conduct clinical investigations to evaluate the potential toxicity, adverse effects, and risk of poisoning associated with the concurrent use of plant extracts, EOs, and antibacterial antibiotics.

11 Conclusion and future prospects

Synergistic studies using plant extracts and plant-derived EOs have yielded promising results. Still, the exact mechanism of action is based solely on the literature rather than on hard-core experimental findings. Studies on phytochemical synergism were based on commercially purchased phytochemicals. Although, this commercial approach reduces the extraction process from plant materials. But this approach will not serve the purpose of alternative antibiotics, especially when our goal is to explore more plant-based or derived antibacterial materials. Although most studies used clinical

bacterial isolates, they clearly demonstrated the importance of synergism. Significant advancements have been made through in-vitro studies, and the future focus lies on in vivo validation, safety evaluation, pharmacokinetic profiling, and clinical trials. The use of artificial intelligence (AI) significantly transforms the medical sector by optimizing processes and improving outcomes. The incorporation of AI into natural product discovery has significantly enhanced the efficiency of identifying bioactive compounds by enabling activity prediction, mechanistic interpretation, and compound prioritization. Advanced computational methods, including tree ensemble models, graph neural networks, and self-supervised molecular embeddings, facilitate the analysis of complex mixtures, isolated metabolites, peptide analogs and optimization of plant-based antibiotic adjuvants. Furthermore, network pharmacology employs herb–ingredient–target–pathway interaction networks to identify potential synergistic effects and elucidate their underlying mechanisms [135].

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43088-026-00815-1>.

Supplementary Material 1

Acknowledgements

We would like to mention sincere vote of thanks for participating universities in conducting this studies.

Author contributions

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Funding

Open access funding provided by University of Debrecen.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 13 April 2026 / Accepted: 28 July 2026

Published online: 01 September 2026

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