

Article

Flavonoid–Antibiotic Synergy: A Review of in Vitro Evidence and Molecular Docking Studies

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Abstract: Due to the worldwide emergence of antimicrobial resistance, plant-based natural compounds, especially flavonoids, are becoming increasingly attractive for the development of new antimicrobial drugs. In this narrative review, the potential of combining in vitro assays of plant flavonoids antibacterial and synergistic activity with in silico molecular docking is examined to gain a better insight into their possible mechanisms of action. This review focuses on publications from 2021 to 2026 in the scientific literature that have been indexed in major databases, especially Scopus and PubMed, which also include computational modeling studies based on AutoDock Vina, along with the assessment of biological activity. Based on the literature reviewed, the use of computational methods in pharmacognosy drug discovery can be beneficial to identify and prioritize bioactive compounds, thereby saving time and resources for preliminary screening. The molecular docking analysis offers useful predictions of ligand–target interactions, binding orientations and binding affinities that can shed light on the observed antibacterial activity in experiments. Predicted binding energies however should be used in conjunction with experimental evidence and not as a clear indicator of biological activity or synergism. In conclusion, it can be said that this study has highlighted the importance of the scientific value of using both the experimental study and the structure-based computational study to enhance the evaluation of plant flavonoids as antibacterial agents. It also emphasizes the need for uniform extraction methods, antimicrobial evaluation and molecular docking studies to ensure reproducibility and comparability of results. Therefore, molecular docking is suggested as a complementary screening tool for prioritizing some of the promising phytochemicals of the identified compounds for further detailed experimental validation.

Keywords: Plant-Derived Flavonoids, Molecular Docking, Antimicrobial Resistance, Pharmacognosy, Computer-Aided Drug Discovery, Autodock Vina

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1. Introduction

Historically medicinal plants have been a rich source of bioactive compounds to be used as effective antimicrobial medicines from which excellent treatments have been derived [1]. Multidrug resistant (MDR) bacterial strains are however, a serious problem and are emerging as a "silent pandemic" in the world today [2], [3]. Epidemiological studies and surveillance reports from around the world from 2022-2025 show a dramatic and unprecedented rate of treatment failure due to antimicrobial resistance [4]. But such authoritative reports are worried that the crisis will push AMR-caused deaths beyond the terrifying level of 10 million per year by 2050 with devastating global economic losses following suit. [5], [6]. On the other hand, scientists are looking to traditional pharmacopeias to study their plant secondary metabolites, particularly flavonoids, for potential resistance-related mechanisms such as efflux or biofilm activity that have become resistant to antibiotics. This is worsened by a clear lack of innovation in the

pharmaceutical sector in terms of new antibiotic development in the pipeline [8]. The threat is the highest in ESKAPE group of pathogens. In particular, very sophisticated survival strategies have been developed by recently isolated strains of both methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*, that involve over-expression of efflux pumps, leading to a significant decrease in the effectivity of the drugs employed for controlling these bacteria, especially the beta-lactams, and it is imperative that alternative therapeutics with novel mechanisms of action be developed to prevent the rapid emergence of bacterial resistance to these currently used drugs. [9], [10], [11].

In this crucial time, research on flavonoids in pharmaceutical and pharmacognosy between 2021 and 2026 clearly points to flavonoids as one of the most promising therapeutic routes [4], [12]. Flavonoids are polyphenolic secondary metabolites found broadly in the plant kingdom and are easily extracted from the leaves and stems of medicinal and aromatic plants like basil, thyme and coriander. Specific flavonoids like quercetin, luteolin and rutin, when given in combination with the standard antibiotic, are very effective synergistic agents, due to their immense structural diversity, which explains the presence of more than 8,000 different compounds [14], [15]. In fact, these combinations have been shown recently in vitro to cause a dramatic drop in the minimum inhibitory concentration (MIC), and thus increase the efficacy of the standard drugs, delay the appearance of new resistance patterns and decrease the cellular toxicity of high doses of antibiotics. The unique property of these compounds is that they are able to interfere with several pathways within bacterial cells, such as disrupting the cytoplasmic membrane and destabilizing the cell wall, preventing the formation of the complex biofilms that physically block drug penetration, and being modern inhibitors of bacterial quorum sensing, the process by which bacteria coordinate their behavior [16-18].

Although it has been well documented that it has efficacy in in vitro systems, contemporary drug discovery requires screening tools that are highly accurate and efficient, and can rapidly identify promising lead compounds for further development to expensive clinical trials. This explains why the last five years' research literature displays an appropriate and vigorous trend toward the incorporation of computational molecular docking methodology into the standard "wet lab" evaluations. The advanced simulation algorithms, especially software like AutoDock Vina and AutoDock 4 have a very predictive environment. They not only calculate the free binding energy but they carefully describe certain chemical and spatial interactions (e.g. hydrogen bonding, hydrophobic interactions) between the active binding pockets of target enzymes. The high throughput virtual screening allows researchers to filter out compounds that may lack biological activity, thus saving significant time and expense in the lab. Theoretical binding estimations are derived from updated protein crystal structure, which can provide insight into the possible mechanism of bacterial efflux pump inhibition, by using computational modelling as an integrative approach to decipher the very specific interactions between flavonoids and important bacterial enzymes, such as DNA gyrase. [24], [25].

This review article then seeks to emphasize the structural properties of plant flavonoids and their synergistic anti-bacterial activity as a foundation, with the goal of focusing attention on the plant flavonoids. The study specifically centers on critically analyzing literature published during the timeframe of 2021-2026, which can successfully describe the dual methodology, from in vitro laboratory experiments to in silico computational simulations, of those works. Finally, a long-term objective will be to develop an overall framework, based on scientific evidence, that could improve the economic and scientific productivity of finding the next generation of antibacterial agents.

2. Materials and Methods

A structured narrative review methodology was used to enable a comprehensive synthesis of the existing literature and to ensure transparency of the synthesis. A thorough literature search was performed in the top academic databases, namely Scopus and PubMed, and included articles published from January 2021 until early 2026 that were published in peer-reviewed journals. The search strategy included the use of logical

operators, functional keywords such as plant flavonoids, antibacterial synergy, molecular docking, AutoDock Vina, and pharmacognosy.

The selection of the articles was limited to original research papers, reviews that focus on the main, and exclusively English-language articles that explicitly studied the antibacterial activity of plant flavonoids against resistant strains. To comply with the central theme of this review, and specifically with the goal of this review, only studies that used a dual methodology were selected. This requires that the papers included in the package be *in vitro* microbiological assessments (like MIM) and *in silico* computational assessment (molecular docking) to confirm their results. Research based on a single computational prediction without experimental testing was systematically omitted, as was research on the use of entirely synthetic flavonoids, not derived from natural botanical sources. The selection was highly rigorous so as to ensure that the synthesized data reflected the most reliable and latest research at the crossroads of traditional pharmacognosy and computational drug design.

3. Results

3.1. Extraction Techniques: Balancing Structural Integrity and Production Efficiency

The extraction of flavonoids from plant tissue is a crucial operation that can influence the chemical profile of an extract, the amount of the target compounds extracted and subsequent biological evaluations[26]. However, one should make a distinction between extraction and isolation and purification. Extraction is the process of transferring a mixture of compounds to a solvent, whereas isolation and purification is the process of obtaining individual ones with a given degree of purity[27]. Chew et al. (2022) studied the recovery of compounds in male papaya leaves (*Carica papaya* L.) and found that process efficiency and compound recovery are in conflict[27]. The optimized extraction yield of rutin by UAE was obtained with 51.5% ethanol, 70.5 minutes of extraction time, 355 μm of particle size and 1:108.6 w/w solid to solvent ratio. Whereas microwave-assisted extraction (MAE) was more efficient in terms of solvent and energy consumption, UAE yielded a higher yield in the conditions studied. The results reported in [28–30] indicate that the extraction processes should be evaluated through multiple performance indicators and not just on the basis of compound yield.

Additionally, recent experimental studies have confirmed the use of combined or sequential methods for better recovery of flavonoids. They are however only effective in the presence of the plant material, the desired compound and the operating conditions [31]. Chew et al (2023) compared MAE and UAE with two sequential extraction methods: microwave–ultrasound-assisted extraction (MUAE) and ultrasound–microwave-assisted extraction (UMAE) in a study on female papaya leaves. Maximum rutin content of 18.46 ± 0.64 mg/g was obtained from MUAE and 18.43 ± 0.81 mg/g was obtained from UMAE under optimum experimental conditions. The solid to liquid ratio was found to be most significant extraction variable [32], [33]. The results indicate that the extraction processes can be effective in combination, but they do not prove to be the best process for all plant materials. However, due to the differences of raw material and experimental conditions, direct comparison with the papaya study needs to be carefully considered [34].

Increased yield doesn't mean that the identity of the original compound is preserved. Ekaette et al., in 2021, found that sonication could lead to hydrolysis of rutin to derivatives such as quercetin depending on the energy, temperature and medium's acidity [35]. In a citric acid medium, quercetin yield increased from $0.34 \pm 0.09\%$ at an energy density of 0.1 kJ/cm³ and 68°C to $2.23 \pm 0.04\%$ at 7.0 kJ/cm³ and 86°C [36]. The observed increase in total phenolics or total flavonoids in water for this study was found at 0.45 ± 0.02 mg catechin equivalents/mg rutin hydrate at 47°C with an energy density of 27 kJ/cm³, indicating that the total flavonoids or total phenolics can increase as the starting compound transforms. These types of measurements are therefore not sufficient to prove structural preservation.

Another alternative method to changing the selectivity of an extraction is to use supercritical fluid extraction, SFE, to control pressure, temperature and solvent composition. As published in 2022, the recovery of lipids and phenolic compounds from sorghum bran by the use of supercritical carbon dioxide (SCCO₂) with co-solvents was

studied [37]. This study suggests that the composition of the co-solvent is important to yield phenolic compounds by SFE and also that the yield of phenolics was 150 ± 3 mg gallic acid equivalents/100 g dry bran and yield of flavonoids was 99.6 ± 4 mg catechin equivalents/100 g dry bran in the best conditions. Values are quoted as gallic acid or catechin equivalents, however, not as the quantity of any particular purified compound, such as rutin or quercetin [38].

Proper chemical characterization and control of solvent effects are also necessary for a reliable assessment of antibacterial activity. Ardalani et al. (2021) introduced a microplate growth inhibition assay, that was combined with liquid chromatography, to identify antibacterial activity in crude extracts against *Staphylococcus aureus* in 2021. Validation of the assays involved testing interferences due to coloured extracts and the effects of dimethyl sulfoxide (DMSO) and repeatability in assays performed on different days [39]. They provided support for inclusion of solvent controls and extract background controls when interpreting bacterial growth inhibition. It is only with proper chemical characterization and separation or purification that activity can be attributed to a specific flavonoid. Testing of a multicomponent extract can prove the activity of an extract but it will not prove the activity of a specific compound in the extract. Likewise, it is a common mistake to assume that degradation products give false positive synergy without first testing the experimental system of interest.

In computational analysis, selection of the ligand should be based on those that have already been identified in the tested sample. An activity of an extract may be very similar to the one of a docking constituent despite the very different composition of the actual extract. Eberhardt et al. described in 2021 a new version of AutoDock Vina (1.2.0) that added more scoring functions and the capability for multiple ligands docking. However, molecular docking is still a semi-empirical technique. Predicted binding conformations and scores can be used to make hypotheses about binding to a bacterial target, but alone are not evidence of enzyme inhibition or of antibacterial synergy [40]. Optimized extraction conditions, compound identity and content, control of solvent interference and experimental testing of the computational hypotheses are essential in order to achieve an optimized connection between extraction and biological testing and molecular docking. The agreement between observation and prediction from the docking is a result to be discussed, not a foregone conclusion when a modern extraction technique is used. The results indicate that the type of extraction method should depend on the utilization of the extract. In studies where flavonoids have been shown to have a beneficial effect against bacteria, it is not enough to recover more of these substances, it is also important to identify the flavonoids that are present and to determine if they are chemically intact. Thus, emphasis in this review will be placed on the recovery of identified compounds as well as total flavonoid measures. This gives a better correlation between the extraction and biological testing and compound selection for molecular docking.

3.2. Antibacterial Synergy: Flavonoids as Antibiotic Adjuvants

Flavonoids are being studied for their direct antibacterial activity in addition to their role in enhancing the efficacy of other antibiotic drugs. This second function could make them useful antibiotic adjuvants, if an antibiotic without them is ineffective against a resistant strain. An improvement in the activity of a combination, however, should not be considered synergy only. The fractional inhibitory concentration index is the sum of the ratios of the MICs of the agents combined and the MICs of the agents in single agent assays in checkerboard assays. An index of 0.5 or lower typically indicates synergy, the thresholds for other types of interactions vary across studies [44]. Time-kill studies are used to complement this measurement to determine the viable bacterial counts over the course of treatment [45]. Common parameters for synergy are when the log reduction is at least 2 log 10 units per ml compared to the most active individual agent. Bactericidal activity, on the other hand, is determined by the decrease from the starting bacterial population [46]. Separate endpoints will help to distinguish between enhanced growth inhibition, synergistic interaction, and bacterial killing.

The distinction is useful as demonstrated by experimental studies of Quercetin. In 2023, Odabaş Köse and colleagues investigated the efficacy of quercetin in combination

with colistin, amikacin and meropenem against colistin-resistant *Acinetobacter baumannii* [47]. The minimum inhibitory concentration of colistin was reduced 4- to 16-fold with the combination of quercetin with colistin, and a 16- to 64-fold reduction in the minimum inhibitory concentration occurred in conjunction with combination with amikacin. Time-kill studies confirmed these results with both combinations demonstrating bactericidal activity after 24 hours [48], [49]. The effect of the combination of quercetin with antibiotics was not the same for all strains: the combination of quercetin with meropenem was considered indifferent, according to the criteria of the study [50].

The outcome may also vary depending on the bacterial isolate, even if the compounds in the combination are identical. In this 2021 study, Lin et al. showed this with their study of quercetin and colistin on 12 colistin-resistant clinical isolates, including 6 *Escherichia coli* and 6 *Klebsiella pneumoniae*. Synergistic interactions were revealed in 8 isolates and additive interactions in 4 isolates by checkerboard testing, and colistin MIC were reduced 2-32-fold throughout the isolates [51]. These findings indicate that a combination could be more effective than either agent alone, but not necessarily synergistic. They also emphasize the need to study multiple isolates and report on each individual isolate. One positive outcome does not imply that the same interaction will be found in a bacterial species or that the same interaction will be found with isolates that have different resistance mechanisms.

Luteolin and apigenin also have been reported to cause antibiotic enhancement, but the significance of this varies depending on the experimental model. Kincses and colleagues assessed the activity of phenylpropanoids, flavonoids and various antibiotics at subinhibitory concentrations in 2024. Luteolin inhibited *Staphylococcus aureus* ATCC 29213 with a minimum inhibitory concentration of 100 micromoles per litre of ciprofloxacin, which was reduced to 0.3125 micromoles per litre. A higher concentration of luteolin was lowered to 0.078 micromoles per litre, which represents an approximately eightfold decrease, when the concentration of luteolin was increased to 200 micromoles per litre [52], [53]. In the strain of methicillin-resistant *Staphylococcus aureus* (MRSA) tested, apigenin, when paired with ciprofloxacin, reduced the minimum inhibitory concentration of the antibiotic from 1.25 to 0.3125 micromoles per litre, a 4,000-fold increase. When combined with ciprofloxacin, apigenin reduced the minimum inhibitory concentration of the antibiotic from 1.25 to 0.3125 micromoles per litre, a 4,000-fold increase, in the tested strain of methicillin-resistant *Staphylococcus aureus* (MRSA). These observations help to confirm antibiotic potentiation and prove that the luteolin combination is concentration-dependent. However, the study determined the antibiotic susceptibility in the presence of fixed sub-inhibitory concentrations of natural compounds. The results must be differentiated from a complete checkerboard classification based on the fractional inhibitory concentration index (FICI) [54].

In 2024, Tang et al. provided a wider range of computational and experimental arguments. They studied apigenin as an adjunct drug to the colistin resistance of *mcr-1*. This process of virtual screening narrowed the number of compounds to be tested in the laboratory to 19,000. After virtual screening, the researchers chose apigenin for testing in the laboratory. The fractional inhibitory concentration indices for three strains containing *mcr-1* from checkerboard assays were 0.188, 0.125 and 0.188, respectively. The combination of apigenin and colistin was effective against the bacteria within 6-12 hours of the time-kill experiment, with 32 micrograms per milliliter of apigenin, and 4 micrograms per milliliter of colistin producing effective killing [55]. The researchers also tested the proposed interaction using molecular simulations, protein thermal stability experiments and bacterial membrane assays [55]. This approach of combining methods gave support in addition to the predictions of docking. The activity against the strains without *mcr-1* also indicated that other mechanisms might play a role in the antibacterial effect besides the one that is encoded by *mcr-1* [56].

These findings together give a basis for investigating flavonoids as antibiotic adjuvants, and demonstrate that they have an antibiotic dependence, concentration dependence and strain dependence. Some studies provide evidence of damage to the membrane, which may account for the increased activity, but does not provide an

explanation for all of the flavonoid–antibiotic interactions [57]. The most informative investigations relate quantitative interaction measurements to time-kill results and experimental examination of the proposed mechanism. The process can be aided by molecular docking, which can predict plausible interaction of the compounds and help in the selection of compounds for biological testing, synergy, however, must be demonstrated through biological studies [58]. The studies mentioned above were not done to test the efficacy and safety of antibiotic dose reduction in patients and so cannot assess the efficacy and safety of similar dose reduction in clinical treatments. Their role is to look for interesting combinations and mechanisms to further explore in the preclinical stage.

The results indicate that the effect of each individual combination of flavonoids and antibiotics must be determined based on their individual evidence, as effects seen with one antibiotic or isolate may not be seen with another. Hence, the results obtained from the checkerboard and time-kill assays are given greater weight than those obtained from a decrease in the antibiotic's minimum inhibitory concentration, when both assays are used. This is encouraging, but doesn't prove synergy, just potentiation. This is an important difference to recognise to find combinations to explore and to limit the conclusions to what is actually found in evidence.

3.3. Molecular Docking: Linking Antibacterial Activity to Plausible Molecular Interactions

A molecular docking study can be used to explore the possible interaction between the relevant molecular target and a flavonoid which has already been found to have an antibacterial effect in the laboratory. This method could be applied in the study of interactions between antibiotic adjuvants and resistance enzymes and transport proteins [59]. Docking can suggest potential binding modes and priorities compounds or interactions for further investigation, although the accuracy and reliability of these predictions rely on the specific target and the docking algorithm used [60]. In their 2021 study on the evaluation of AutoDock Vina Version 1.2.0, Eberhardt and colleagues showed this limitation. They used 102 targets and the Vina scoring function was able to reproduce the position of the experimental ligand within a root mean square deviation of 2 ångströms, in 68% of cases when only the best score was taken into account [61]. This rose to 84% when the three best-ranked poses were looked at. These percentages reflect the pose-prediction success for that benchmark, and not how likely a compound is to display antibacterial activity. The docking outcome, therefore, should be used to inform experimental investigation and not as evidence of a biological effect.

For flavonoids as inhibitors of the OXA-48 beta-lactamase, a helpful case study was offered by Zhang and collaborators in 2022, who reported docking scores of –6.28, –6.21, and –7.14 kilocalories per mole, for quercetin, luteolin, and fisetin, respectively. Experimental enzyme assays gave corresponding inhibition constants of 1.31 ± 0.21 , 0.82 ± 0.05 and 0.62 ± 0.06 micromoles per litre [62]. Enzyme kinetics revealed noncompetitive inhibition, and other binding studies confirmed that there was an interaction between the enzyme and quercetin. Antibiotic enhancement was seen with modified *Escherichia coli* strains in contrast to the four compounds tested, which did not affect antibiotic M.I.C. in the parental OXA-48-producing strain [63]. This contrast is significant because if a compound is found to be active in vitro against a single enzyme it does not prove that it can be taken up by the bacterial cell to reach the enzyme in an effective way.

Some studies have tried to look at interactions with genetic material, not resistance enzymes. In 2023, Tagrida et al. compared the effects of quercetin and hyperoside with *Pseudomonas aeruginosa* and *Shewanella putrefaciens* [64]. They were analysed for docking with a DNA double helix and proposed binding to the minor groove, specifically in adenine–thymine rich regions. Quercetin was reported to have a docking score of –10.7 kilocalories per mole [65]. However, hyperoside proved to have lower minimum inhibitory and minimum bactericidal concentrations in the antibacterial assays than quercetin against both organisms. The docking results thus provided a possible mode of interaction in addition to the experimental results. They failed to make an independent finding that the growth inhibition was primarily due to DNA binding.

In a 2024 *mcr-1* study by Tang et al., a more direct link to antibiotic synergy was observed in the apigenin and colistin resistance. The researchers performed a computational screening of over 19,000 compounds, and apigenin was among the top 10 best candidates [55], [66]. The fractional inhibitory concentration indices were subsequently obtained for three *mcr-1*-positive strains, which were 0.188, 0.125 and 0.188 in the subsequent checker board testing. Molecular simulations, protein thermal stability experiments and membrane related assays were also used to explore the proposed mechanism, which was found to be effective for the killing of the bacteria in combination with colistin at the concentrations of 32 micrograms per millilitre and 4 micrograms per millilitre, respectively [67]. Computational screening was used to select compounds in this study and biological assays were used to confirm the interaction between the two agents. The different roles make it easier to understand the effect of docking [68].

Another possible source for flavonoid antibiotic adjuvants is the efflux transporters. Lu et al. studied apigenin, chrysin, glycitein, and hesperetin as efflux pump inhibitors in drug-resistant *Escherichia coli* (*E. coli*) in 2025 [69]. Binding of apigenin and chrysin was reportedly favourable in the AcrB docking test, with the scores of -8.8 to -8.9 kilocalories per mole in the distal binding pocket. As contacts were predicted, hydrophobic, as well as aromatic contacts, were made between the residues of phenylalanine 610 and phenylalanine 628 [70]. The observations support a hypothesis for the proposed transport-related effect, namely that it is a structural effect. However, the docking scores are also dependent on the scoring function, and the computational setup, so that scores generated with different protocols cannot be considered as directly interchangeable measures of affinity [71].

A 2026 work further demonstrates the need for different targeting of the bacterial target from the host target. Luteolin was found to have a minimum inhibitory concentration of 1 milligram per millilitre and a minimum bactericidal concentration of 2 milligrams per millilitre against multidrug-resistant *Escherichia coli* (MDREC) by Yang and colleagues [72]. Their docking analysis focused on proteins that are present in the host and work with inflammation and cellular regulation processes such as tumour necrosis factor alpha, interleukins, nuclear factor kappa B, and tumour protein p53 [73]. Additionally, it was conducted a 100-ns MD simulation of luteolin with tumour necrosis factor alpha (TNF- α) that investigated potential host-related effects during an infection. These should thus not be considered to represent direct inhibition of a bacterial resistance enzyme by luteolin and should be viewed in isolation of the antibacterial assays [74].

These studies, when combined, demonstrate the importance of the value of docking based on the level of association to the biological question. In this review, the investigations that are based on computational predictions and then tested with binding experiments, enzyme assays and/or well-defined antibacterial interaction tests are emphasized. It's helpful to have a favourable score to begin with, but a good guess is strengthened when the proposed target and mechanism are looked at in the laboratory. This way, the docking can play a role in explaining an antibacterial activity without having to establish effects which can only be demonstrated in a biological experiment.

3.4. Evidence Synthesis

The information on extraction, the synergistic effect against bacteria and the molecular docking provides an issue that goes beyond the fact that flavonoids have antibacterial activity, it is also a question of how convincing is their activity? The initial step is to determine what compounds are present in the sample that is tested. An increase in the extraction yield can be a sign of either increased recovery of the desired compound or chemical modification of the compound or the formation of a derivative [75], [76]. The strength of the evidence will thus rely on the relationship between the recovered compound, the tested sample, and the compound that is determined to interact with the intended target [77], [78]. If the testing for antibacterial activity is performed on a multi-component extract and docking on an individual compound without a known contribution in the mechanism, the relationship between activity and proposed mechanism of action is not fully complete.

Comparing the studies also shows that a distinction must be made between the interactions with the target, whole-cell activity, and therapeutic benefit. An isolated enzyme could be hindered by a flavonoid, which would not enhance antibiotic activity against intact bacteria, as in the OXA-48 study [63], [79]. Similarly, combining two compounds may have a synergistic effect against one isolate and an additive effect against another, even if the same two compounds are combined. [80] Interacting with a target is one thing, actually achieving the target within the bacterial cell and creating an effect under tested conditions is another. The results of laboratory or animal studies, likewise, cannot prove efficacy or safety in humans.

In this evidence base, synergy comes down to a result, not an inherent nature, of a specific combination under specific circumstances, not a specific flavonoid. The interaction may change if there is a change in antibiotic or bacterial isolate, and the method of assessing the interaction may influence how it is classified [81]. Therefore, a decrease in the MIC should not be interpreted as synergy and greater killing of the bacteria. They are observations that are related, but they are answering different questions. When there is an agreement between independent assays, it provides support to the interpretation, when there is disagreement, it must be examined instead of ignored. For the purpose of this review, combinations which do not demonstrate synergy are also useful for shedding light on conditions of enhancement and boundaries thereof.

Molecular docking is most useful when it results in questions that can be answered experimentally: Will the compound bind to the target protein? Will the compound change the protein's function? Can the change in function explain the antibacterial effect? Their proposed mechanisms receive greater support from the studies that have included both computational prediction and direct binding measurements and enzyme assays [82]. Who is the target also plays a role. An interaction with an inflammatory protein in the host is a suitable explanation for antibacterial activity or resistance modification while an interaction with a bacterial inflammatory protein is suitable for the response to infection [83]. Separate interpretations of these assays ensure that each effect of the flavonoids is evaluated and not mistaken for an antibacterial synergy.

In our view, the evidence moves the debate focus from efficacy in general to which compound is effective against which antibiotic against which isolate and in which conditions. This more specific approach allows the findings to be easily interpreted and compared. It also implies that finding more active compounds is not the only research need, but highlighting the definite link between compound structure (chemical identity), antibacterial interaction, molecular mechanism and feasibility as a therapeutic agent. It is combinations that still have a convincing effect after taking their limitations and other possible explanations into account that are worth pursuing and not those that have the highest extraction yield or the lowest docking score.

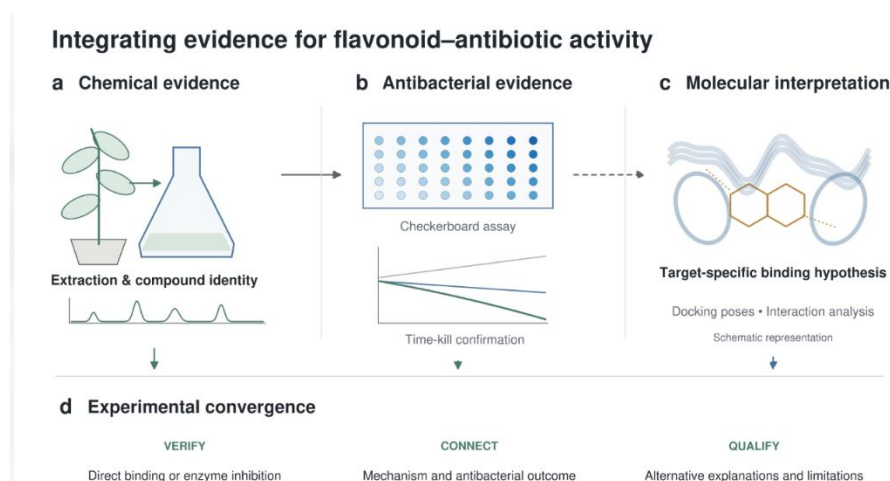


Figure 1. The figure highlights the possibilities of combining the results of chemical characterization, evaluation of the interaction between flavonoids and bacteria, and molecular investigation to evaluate the evidence for flavonoids as antibiotic adjuvants.

Table 1. Selected extraction and molecular docking studies discussed in this review (2021-2026).

Plant source / compound origin	Flavonoids	Bacteria evaluated	Docking target	Docking software	Reported docking score / energy, kcal/mol	Citation
<i>Carica papaya</i> L., male leaves	Rutin	Not evaluated	Not applicable	Not applicable	Not applicable, extraction study	Chew et al., 2022
<i>Carica papaya</i> L., female leaves	Rutin	Not evaluated	Not applicable	Not applicable	Not applicable, extraction study	Chew et al., 2023
Commercial compounds, no plant extraction performed	Quercetin, luteolin, fisetin	OXA-48-producing <i>Escherichia coli</i> BW25113 and deletion mutants	OXA-48 beta-lactamase, PDB: 4S2K	AutoDock 4.0	Quercetin: -6.28 , luteolin: -6.21 , fisetin: -7.14	Zhang et al., 2022 (frontiersin.org)
Commercial compounds, no plant extraction performed	Quercetin, hyperoside	<i>Pseudomonas aeruginosa</i> PSU.SCB.16S.12, <i>Shewanella putrefaciens</i> TBRC 5775	Model DNA double helix, PDB: 453D	AutoDock Vina 1.1.2, AutoDockTools 1.5.7 for preparation	Quercetin: -10.7 , hyperoside: -10.0	Tagrida et al., 2023 (MDPI)
Commercial apigenin from TargetMol, no plant extraction performed	Apigenin	<i>E. coli</i> and <i>Klebsiella pneumoniae</i> , including mcr-1-positive strains	MCR-1 protein, PDB: 5GRR	Smina, version 2020	Numerical docking score not verified in the material examined, experimental dissociation constant: 4.35 μM , which is not a docking energy	Tang et al., 2024 (Microbiology Spectrum)
Commercial compounds from Sigma-Aldrich, no plant extraction performed	Apigenin, chrysin, glycitein, hesperetin	Drug-resistant <i>E. coli</i> , experiments included the Kam3-AcrB model	AcrB efflux transporter, PDB: 4DX5	AutoDock Vina 1.2.0	-8.8 to -8.9 for apigenin and chrysin in the distal binding pocket	Lu et al., 2025 (pdfs.semanticscholar.org)
Commercial luteolin from MACKLIN, purity >98%, no plant extraction performed	Luteolin	Multidrug-resistant <i>E. coli</i> BA1	Host targets: TNF- α , IL-6, IL-1 β , NF- κ B and TP53	AutoDock 4.2, GROMACS 2022 for subsequent molecular dynamics	TNF- α : -8.1 , IL-6: -7.2 , IL-1 β : -7.2 , NF- κ B: -7.6 , TP53: -7.8	Yang et al., 2026 (PMC)

4. Discussion

The reviewed evidence indicates that flavonoid–antibiotic activity is best interpreted as a chain of linked observations rather than as a property inferred from a single assay. Extraction and chemical characterization establish which compounds are actually present, checkerboard and time-kill experiments determine whether a combination produces potentiation, synergy, or bactericidal enhancement, and molecular docking provides a plausible structural explanation that must remain subordinate to biological validation. Across the studies synthesized here, quercetin, luteolin, apigenin, and related flavonoids frequently enhanced antibiotic activity, but the magnitude and classification of the interaction varied with the antibiotic, bacterial isolate, concentration, and resistance mechanism. The strongest evidence therefore comes from studies in which quantitative interaction measurements converge with time-kill data and direct target or mechanism assays. Docking scores alone cannot establish antibacterial efficacy, because predicted affinity does not demonstrate target engagement in intact bacterial cells. These findings support an integrated experimental–computational workflow in which docking is used to prioritize and explain candidates, while microbiological assays remain the principal basis for claims of synergy.

4.1. Research Gaps and Future Directions

Based on the evidence discussed in the previous sections, we regard the major gap in the research as the incomplete link between the observed antibacterial activity and the proposed molecular mechanism to explain this activity. The continuous chemical characterization, biological testing, and target validation conducted on the same compound should form a series of investigations in the future where each stage builds upon the other. If using a multicomponent extract, it is recommended that the constituents chosen for computational analysis are identified and quantified, and their contribution to the measured activity is examined. Individual compounds, selected fractions, and reconstructed combinations (where appropriate) should be tested to help determine whether the proposed explanation explains the activity of the original extract.

In an effort to facilitate the comparability of results across studies and enhance reproducibility, we recommend a common reporting format for compound identity, purity, characteristics and resistance of bacterial strains, culture conditions, exposure periods, solvent controls, and biological replication. Criteria for interaction should be established prior to analysis, and the activity of each agent should be reported in addition to their activity in combination. These combinations that are promising should then be tested for time-kill to see if the interaction persists through time and if the interaction enhances the killing of the bacteria. In the case of results that do not show synergy, this should also be reported, as it helps to define the boundaries of a combination and direct further experiments.

The same attention should be paid to the computational part and be documented. The biological consideration for choosing a target, preparation of protein and ligand, appropriate protonation states, search region, version of software, scoring function, and search parameters are recommended to be explained and reported. The selected protocol must be tested against an appropriate reference system, for example redocking a crystallographic ligand if they are available, or if such are not available, examining compounds of known activity against the target. Docking results can then be used to identify specific interactions to test in experiments, and one can analyse the result according to whether those interactions provide a reasonable explanation for the biological results.

This should lead to direct assessment of binding/target function and exploration of other explanations for molecular and antibacterial results that do not agree. Follow up experiments might involve testing of membrane permeability, membrane efflux or testing differences between strains with different target or resistance mechanism to investigate the research question. Effects on host proteins should be evaluated independently from mechanisms suggested to have direct effect on bacteria, and the potential contribution of host proteins to the overall response to infection should be investigated.

The focus here is to create studies with a compound, interaction and mechanism that can be followed and tested in our opinion. The goal of the standardization should be to help achieve this by ensuring that the key measurements and reporting procedures are comparable so as to allow for adjustments needed by the various experimental systems. The evidence generated would give a better grounding to help clarify which combinations to pursue further and help identify the questions that need to be answered prior to therapeutic development.

5. Conclusion

Overall, the literature reviewed from 2021–2026 supports plant-derived flavonoids as promising antibiotic adjuvant candidates, but the evidence is highly dependent on compound identity, bacterial strain, antibiotic partner, and experimental design. Quercetin, luteolin, apigenin, and related flavonoids have shown meaningful reductions in antibiotic MICs and, in selected studies, synergistic or bactericidal effects confirmed by checkerboard and time-kill assays. Molecular docking contributes mechanistic hypotheses by identifying plausible interactions with targets such as OXA-48 β -lactamase, MCR-1, DNA, and efflux transporters, but predicted binding energies should not be interpreted as independent proof of antibacterial activity or synergy.

The most reliable future studies should connect chemical characterization, standardized antimicrobial interaction testing, target-specific docking, and direct experimental validation within the same study. Particular attention is needed to compound purity, solvent controls, strain-specific resistance mechanisms, reproducible docking protocols, and confirmation of target engagement. Such integration will improve comparability across studies and help distinguish compounds that merely produce favourable computational scores from flavonoid–antibiotic combinations with reproducible biological effects and realistic potential for further preclinical development.

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