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REVIEW

Plants as sources of new antimicrobials and resistance-modifying agents

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Infections caused by multidrug-resistant bacteria are an increasing problem due to the emergence and propagation of microbial drug resistance and the lack of development of new antimicrobials. Traditional methods of antibiotic discovery have failed to keep pace with the evolution of resistance. Therefore, new strategies to control bacterial infections are highly desirable. Plant secondary metabolites (phytochemicals) have already demonstrated their potential as antibacterials when used alone and as synergists or potentiators of other antibacterial agents. The use of phytochemical products and plant extracts as resistance-modifying agents (RMAs) represents an increasingly active research topic. Phytochemicals frequently act through different mechanisms than conventional antibiotics and could, therefore be of use in the treatment of resistant bacteria. The therapeutic utility of these products, however, remains to be clinically proven. The aim of this article is to review the advances in *in vitro* and *in vivo* studies on the potential chemotherapeutic value of phytochemical products and plant extracts as RMAs to restore the efficacy of antibiotics against resistant pathogenic bacteria. The mode of action of RMAs on the potentiation of antibiotics is also described.

1 Introduction

Antibiotics have proven to be powerful drugs for the control of infectious diseases and remain one of the most significant discoveries in modern medicine. Their extensive and unrestricted use has, however, imposed a selective pressure upon bacteria, leading to the development of antimicrobial resistance.[1–5] The capacity of bacteria to acquire and transmit genetic determinants of resistance is a conserved evolution strategy and has exacerbated the worldwide resistance problem. Antibiotic resistance is recognized by the World Health Organization (WHO) as the greatest threat in the treatment of infectious diseases.[5–9]

In order to control the occurrence and spread of resistant strains, the WHO has promoted a complex action plan, based on the slogan "*No action today*, *no cure tomorrow*" that includes strategic actions for mitigation, prevention and control.[10] The plan is based on the accomplishment of several objectives: the prudent use of antibacterial drugs (with the correct drug at the right dosage and for the appropriate duration) across all relevant sectors; enhanced infection control and environmental hygienic practices to reduce the transmission of resistant strains; and the strengthening of surveillance systems to monitor antibiotic use and resistant bacteria in human and animal health, including the food chain; and the encouragement of the discovery of new active agents.[9–12]

The quest for new antimicrobials to overcome resistance problems has long been a top research priority for the pharmaceutical industry.[3,5,13] However, in the past thirty years only two

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new classes of antibiotics have entered the market, the oxazolidinones and the cyclic lipopeptides, both of which are used against Gram-positive bacterial infections (Fig. 1A).[10] No new anti-Gram-negative drugs have been developed. The low rate of emergence of new drug classes since the 1960s is evident when one examines the history of the Food and Drug Administration (FDA) approval for antibacterial agents (Fig. 1B). In fact, only six new antibiotics have been approved over the last nine years, and the success of these has been compromised due to the emergence of resistance.[13–15]

An important potential strategy to help combat the resistance problem involves the discovery and development of new active agents capable of partly or completely suppressing bacterial resistance mechanisms.[16] The development of what have been termed resistance-modifying agents (RMAs) represents an attractive strategy to mitigate the spread of bacterial drug resistance since it could facilitate the recycling of well-established antibiotics that are often cheaper and less toxic than new candidate antimicrobials[3,17]

Plants have traditionally provided a source of new chemical entities and numerous clinical studies have proved the therapeutic value of molecules of plant origin on the human organism.[3,18,19] Indeed, higher plant-derived products represent approximately 25% of drugs in current clinical use.[20] Of the more than 350 000 species of higher plants currently recognized, only 5–10% have been investigated and considering that each plant species may contain 500–800 different secondary metabolites, the

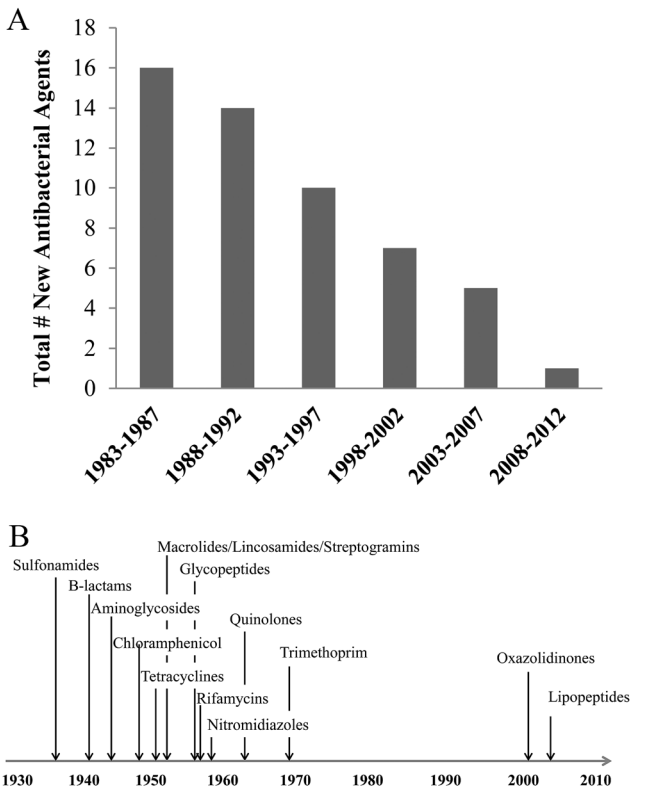

Fig. 1 Antibiotic approvals from 1983 to present (data obtained from IDSA's report[158]) in which most of these drugs (75%) are from two classes, β-lactams and quinolones (A); History of antibacterial drug introductions and approval (B).[159]

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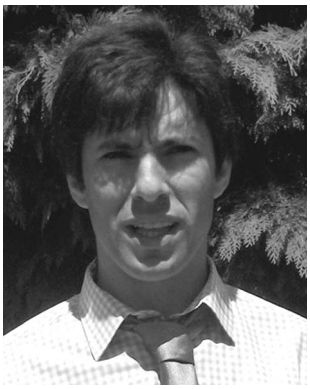

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potential for the discovery of new therapeutic products in this largely untapped resource is considerable.[3,21–23]

With respect to RMAs, several studies have provided clear evidence that plant-derived products can be used to improve the therapeutic efficacy of antibiotics.[5,17,24–27] This article therefore reviews data on the combinatorial interaction of plant-derived products as RMAs, with antibiotics for the treatment of infectious diseases. The mode of action of RMAs for the potentiation of antibiotic activity is also described. Additionally, *in vivo* and toxicity data, where available, are reviewed.

2 Bacterial resistance to antimicrobials

The overuse, underuse and general misuse of antibiotics are major factors in the emergence and dissemination of resistance.[10,28] Indeed, infectious diseases are still one of the most important causes of human mortality.[10] The concept of drug resistance is more complex than it seems. Microbial susceptibility is a continuum that reflects phenotypic and genotypic variations in natural microbial populations.[29] The selection of resistant variants is determined by the level of exposure of the pathogen to an antibiotic, as well as by the pharmacokinetic and pharmacodynamic properties of the antibiotic.[30] Microbial resistance to antimicrobials may occur through the emergence of pre-existing but previously unexpressed resistance phenotypes, or through inherent insusceptibility to antibiotics as a consequence of general adaptive processes. However, the most commonly described form of bacterial resistance occurs either by genomic mutation or through the acquisition of new genetic information encoding for resistance elements.[1,3,8,28,31,32] The major mechanisms of bacterial resistance to antimicrobials are demonstrated in Fig. 2 and include drug inactivation, target modification, alteration in the accessibility to the target through drug efflux and decreased uptake.[1,8,33–35] The ecological impact and roles of the resistance genes and mutations are not completely understood and with some exceptions, neither are the biochemical mechanisms of acquired resistance to most classes of antibiotics.[29,36]

The resistance of pathogenic microorganisms to individual antibiotics is by itself a great problem. However, the emergence of multidrug resistant (MDR) strains represents an increasing complication in the treatment of bacterial infections.[3,12,37] Approximately 50% of hospital-acquired infections worldwide are caused by MDR microorganisms.[38] MDR bacteria may persist for prolonged periods and cause epidemics.[39] Among the most problematic MDR bacteria are vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant MRSA and bacteria producing extended-spectrum β-lactamases (ESβL), such as *Pseudomonas aeruginosa*, *Helicobacter pylori*, *Acinetobacter baumannii*, *Escherichia coli* and *Klebsiella pneumoniae*.[13,25,40] Other important MDR pathogens include *Mycobacterium tuberculosis*, *Legionella pneumophila*, penicillin-resistant *Streptococcus pneumonia*, and *Shigella* and *Salmonella* species.[9,39] MRSA merits special attention since it is responsible for a high-level of hospital-acquired infections.[5,15,26,41–43] In fact, few agents can treat infections caused by this bacterium since many MRSA strains are resistant not only to almost all kinds of β-lactams but also to macrolides, quinolones and even to aminoglycosides.[24,42]

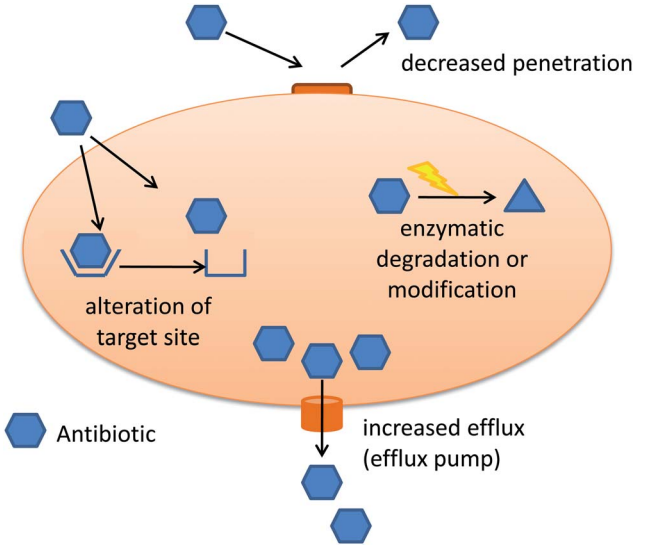

Fig. 2 Mechanisms of resistance to antimicrobials: active drug efflux systems from the cell *via* a collection of membrane-associated pumping proteins that effectively remove toxic compounds from cells; mutations resulting in altered cell permeability; enzymatic degradation of antimicrobials by the synthesis of modifying- or inactivating-enzymes that selectively target and destroy these compounds; alteration/modification of the target site, *e.g.*, through mutation of key binding elements, such as ribosomal RNA.

Additionally, tuberculosis continues to be a major problem, with an estimated 8.8 million cases (1.1 million deaths) worldwide in 2010. The reemergence of tuberculosis is partially associated with the development of mycobacterial resistance against two of the most important tuberculosis drugs, rifampicin and isoniazide, without any new drug being commercialized since 1964.[39,44]

3 RMAs for co-therapeutic use

Several approaches, such as the use of vaccines, monoclonal antibodies, immuno-regulatory cytokines and hematopoiesis-stimulating factors may have utility in the control of antibiotic resistant infectious diseases.[36] The discovery of new effective antimicrobials however remains an urgent requirement, given the continued emergence of resistance to the newer generations of drugs.[45] In some cases, combining established antimicrobials has extended the useful life of an antibiotic. Cases of synergy (where the combined effectiveness of two drugs is greater than the sum of individual activities) between a broad range of antibiotics have been reported. These combinatorial activities may be due to multi-target effects, based on improved solubility, resorption rate, enhanced bioavailability and also to the interaction of products able to modify or inhibit bacterial resistance mechanisms, caused by the combination of selected products and antibiotics.[46,47] The reversal of multi-drug resistance represents a promising approach to mitigate the spread of resistance. Since the understanding of the molecular mechanisms of synergy is the basis for a new strategy for the eradication of resistant pathogens, it is important to understand in detail the mode of action of RMAs. Several mechanisms have been proposed and are discussed in the following section.

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3.1 RMAs acting on the modified target sites of antimicrobial action

Modification of target sites is a common mechanism of resistance and may occur for diverse classes of antibiotics, which include tetracyclines, β-lactams and glycopeptides.[36] In the case of β-lactam antibiotics, their targets are the penicillin-binding proteins (PBPs), which are important cell-wall structural enzymes present in almost all bacteria.[38] MRSA achieves high-level resistance to β-lactam antibiotics by the acquisition and expression of the *mecA* gene, which codes for an altered transpeptidase additional to PBP (PBP2a).[36,48–50] PBP2a confers resistance to β-lactam antibiotics due to its low affinity for penicillins.[43] It is therefore of great interest to find co-therapeutic agents that inhibit PBPs. Some agents have already demonstrated the potential to either inhibit the modified targets or exhibit a synergy by blocking one or more of the other targets in the metabolic pathway, thus causing cell death.[51] A number of β-lactam antibiotics, including modified cephalosporins, carbapenems and trinem have been designed with enhanced activity against PBP2a.[51] Another example is the new glycopeptide antibiotics with activity against vancomycin- and teicoplanin-resistant Gram-positive bacteria, which express modified peptidoglycan structures resistant to the binding of the older glycopeptides.[36]

3.2 RMAs as inhibitors of bacterial enzymes that inactivate antibiotics

Enzymatic degradation or modification of antimicrobials is also an important mechanism of bacterial resistance.[11,46] The resistance to β-lactams mediated by the overproduction of β-lactamases that partially hydrolyze methicillin and related penicillins is probably the most widespread example of antibiotic inactivation involving enzymes.[50] Bacterial enzymes that degrade or modify antibiotics are themselves important targets for drug action since their inhibition protects the antibiotic from degradation.[36] The co-administration of β-lactamase inhibitors is an important strategy for restoring the activity of β-lactam antibiotics, enabling the β-lactam antibiotic to more effectively interact with the PBPs.[52] Inhibitors of β-lactamases have long been known and their administration with antibiotics has been associated with considerable success, proving to be one of the most effective antibiotic combinations.[51,53] An important example of these inhibitors is clavulanic acid, which binds with high affinity to many bacterial β-lactamases, thus protecting antibiotics from destruction, and is available commercially in combination with amoxicillin as Augmentin® and Ticarcillin (Timentin®).[36,53,54] Other inhibitors of β-lactamases are sulbactam, marketed in combination with ampicillin (as Unasyn®), and tazobactam, marketed in combination with piperacillin (as Tazocin®).[36,52,54] New generations of drugs with enhanced activity have been developed to counter antibiotic resistance to β-lactamases, such as 3rd and 4th generation cephalosporins, carbapenems or quinolones.[10,38,55] Some of them, *e.g.* the isoxazolyl penicillins, imipenem and meropenem, are more resistant to enzymatic inactivation.[36] However, enzymes such as active-site serine carbapenemases, plasmid-encoded class C cephalosporinases and acquired metallo-β-lactamases have also emerged.[10,53] There are already diverse reports demonstrating bacterial resistance to the last line of antibiotic defense, underlining the importance of the quest for novel therapeutic strategies.[14,38,51]

3.3 RMAs as membrane permeabilizer agents

The outer membrane (OM) of Gram-negative bacteria serves as a selective barrier for many external hydrophobic solutes due to its high lipopolysaccharides (LPS), content and it forms specific contacts with integral outer membrane proteins (OMPs), such as porins (*e.g.*, OmpF in *E. coli* and OprD in *P. aeruginosa*), which act as entry and exit points for antibiotics and other organic chemicals.[13,51,56–58] Antimicrobial resistance may occur following the loss or modification of certain OMPs, as a consequence of decreased expression, point mutations or insertion sequences in porin-encoding genes, producing proteins with reduced permeability to antibiotics.[38,52] Reduced OM permeability may result in reduced antibiotic uptake[11] and thus, porin mutations can confer resistance to β-lactams, carbapenems, fluoroquinolones, tetracyclines, sulfonamides and chloramphenicol.[38,52]

Many compounds have been reported to affect membrane permeability with activity against a taxonomically diverse range of microorganisms,[59] mainly due to the perturbation of the lipid fraction of the cell membrane and, owing to their lipophilic character, the increase of membrane permeability.[60] Such permeabilizers, as they have been termed, can non-specifically enhance the permeability of bacterial cells to exogenous products, including antimicrobial agents and may therefore potentiate the antibacterial potential of antibiotics that interact with intracellular targets.[61]

3.4 RMAs as inhibitors of efflux pumps

The expression of efflux pumps, which extrude antibiotics from the bacterial cell, is one of the most important mechanisms of microbial resistance to antimicrobials and can be observed in many clinically relevant pathogens.[62–64] Although some are drug-specific, many efflux systems may transport a range of products with different structures and classes, contributing significantly to multidrug resistance.[34,65] Antibiotic efflux transporters have been classified into five main families based primarily on amino acid sequence homology. These are the major facilitator superfamily (MFS), the resistance-nodulation-division (RND) family, the small multi-drug resistance (SMR) family, the ATP binding cassette (ABC) family and the multiple antibiotic and toxin extrusion (MATE) family.[66,67] The efflux of drugs from Gram-positive bacteria is mainly mediated by a single cytoplasmic membrane-located transporter of the MF, SMR or ABC families.[66,68] Among Gram-negative bacteria, multidrug efflux pumps belonging to RND and SMR families are common.[69] The analysis of antibiotic efflux transporters has already been extensively reviewed.[66,67] It is, however, interesting to highlight some important MDR efflux pumps, such as the MDR protein NorA of *S. aureus* (significant for several fluoroquinolones, monocationic dyes and disinfectants), which is probably the most intensively studied pump in pathogenic Gram-positive bacteria and may be responsible for at least 10%

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of antibacterial resistance in MRSA strains;[22,70] Bmr in *Bacillus subtilis*, which mediates tetracycline efflux; TetK (for tetracyclines) and MsrA (for macrolides) in *S. aureus*; QacA, which is responsible for the export of several antimicrobial compounds and for acriflavine and ethidium bromide (EtBr) resistance.[5,22,37,66,71] The ABC transporter LmrA from *Lactococcus lactis* has also been intensively studied for its role in MDR.[72] The AcrAB-TolC, which is the main efflux transporter in *Enterobacteriaceae* and extrudes diverse products (*e.g.* tetracyclines, fluoroquinolones and chloramphenicol), and the MexAB-OprM in *P. aeruginosa*, responsible for resistance to β-lactams, quinolones, tetracyclines and trimethoprim, are examples of the RND family.[17,66–68,73]

Since most bacteria can acquire MDR efflux pumps, the inhibition of these systems is a promising strategy for potentiating antibiotic effectiveness.[46] An effective efflux pump inhibitor (EPI) would help to restore drug susceptibility in some resistant clinical strains by decreasing the antibiotic dose required for bacterial inhibition.[73–76] Consequently, substantial efforts are needed to develop new, safe and effective bacterial EPIs that could be used in antimicrobial therapy.[73,77] Moreover, a strategy already proposed to mitigate efflux-mediated resistance is the synthesis of analogs of macrolides, quinolones and tetracyclines that are not recognized by efflux pumps.[36]

4 Plants as sources of new co-therapeutics and resistance-modifying agents

Plant extracts have been utilized for centuries in the name of human health, particularly in Asia. The purpose was to accelerate wound healing and to treat common infectious diseases. Such traditional medicines are still utilized in the routine treatment of various diseases. Examples include the use of bear-berry (*Arctostaphylos uvaursi*) and cranberry juice (*Vaccinium macrocarpon*) to treat urinary tract infections, or essential oils of tea tree (*Melaleuca alternifolia*) as active ingredients in many topical formulations to treat cutaneous infections. Essential oils of *Hydrastis canadensis* and *Echinacea* species are also used to "treat" tuberculosis infections.[3,15,69] Due to their curative potential, plant extracts have been investigated for the development of novel drugs to control bacterial infections.[78–80]

Of the many medicines that have higher-plant origins, very few are utilized as antimicrobials. Rather, bacteria and fungi are the leading sources of therapeutic antimicrobials.[81] However, issues of toxicity and resistance have promoted interest in products from other sources, particularly plant secondary metabolites (phytochemicals). Due to the current increase in awareness of antibiotic resistance problems, self-medication with the multitude of plant products from herbal suppliers and natural-food stores is enjoying considerable popularity.[81] The WHO noted that the majority of the world's population depends on traditional medicine for primary healthcare.[82] Indeed, in 2010, the global retail sale of botanical dietary supplements amounted to more than $25 billion in the United States, according to studies published by the University of Illinois at Chicago/National Institutes of Health Center for Botanical Dietary Supplements Research.[83]

Phytochemical products that produce minimum inhibitory concentrations (MIC) in the range 100–1000 μg mL^{-1} in *in vitro* susceptibility tests can be classified as antimicrobials.[61] Many of these products are produced as a mechanism of plant defense in response to tissue disruption or pathogen attack. These are commonly classified as phytoalexins.[61] Other products, known as phytoanticipins, are present constitutively in an inactive form, giving the plant a characteristic odor (such as terpenoids), distinctive pigmentation (*e.g.*, quinones and tannins) or flavor (*e.g.*, the terpenoid capsaicin from chili peppers).[40,61,84] Alkaloids, flavonoids, lactones, polyphenols, glycosteroids, diterpenes, triterpenes and naphthoquinones are other common classes of phytochemicals.[40,51,81,85] Some of these products produced by plants can fight infections successfully.[22] In fact, the relative rarity of infectious diseases in wild plants is an indication of the effectiveness of their innate defense mechanisms.[51,85] Hence, those phytochemicals are believed to work synergistically with other intrinsic products (*e.g.* efflux pump inhibitors), thus playing a role against infection in the plant's defense system.[17,51,86] Besides this knowledge, most of the functions of phytochemical products in plants are still unknown.[61]

Many studies have indicated that a broad range of plant extracts may act against bacterial resistance mechanisms.[3] The majority of these have now been focused on combinations between plant extracts and antibiotics in order to screen for RMAs.[22] The following sections will focus on combinatorial activities of plant extracts and products with antibiotics, mainly due to a resistance-modifying action.

4.1 *In vitro* synergy

When assessing the susceptibility of bacteria to antimicrobial products it is important to promote the uniform application of terminology to the methods used.[61] According to the European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), the dilution methods (agar dilution or broth microdilution) are the designated reference methods for antimicrobial susceptibility testing.[87] There are four possible effects when a combination of antibacterial products is used: indifference (when the combination of antibacterial products promotes equal effects to these of the most active product); an additive effect (when the combination of antibacterial products is equal to that of the sum of the effects of the individual products); synergism (when the combination of antibacterials exceeds the sum of the effects of the individual products); and antagonism (when the combination of antibacterial products promotes a reduced effect compared to the effect of the most efficient individual product).[61] Potentiation is a consequence of the synergism observed when the products are used in antimicrobial chemotherapy. The checkerboard and the time-kill curve methods are the most widely used to analyze synergy. The explanation of these methods can be found elsewhere.[51,88] According to the checkerboard method a fractional inhibitory concentration (FIC) index is obtained. The value of the FIC is a predictor of synergy (synergy occurs at a FIC index ≤ 0.5).[89]

The strategy of resistance-modifying activity implies that an antimicrobial product should be co-administered with an

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Table 1 Synergism between natural products and antibiotics due to resistance-modifying activity

Phytochemical product	Plant source	Antibiotic potentiated	Mechanisms of action
Carnosic acid (**1**); Carnosol (**2**)	*Rosmarinus officinalis*	Tetracycline Erythromycin	MDR efflux pumps inhibition[5,66,71]
Reserpine (**3**)	*Rauwolfia serpentina*	Fluoroquinolones Tetracycline	MDR efflux pumps inhibition[64,66,71,73,94,95]
Totarol (**4**); Diterpene 416 (**5**)	*Chamaecyparis nootkatensis*	Norfloxacin Tetracycline Erythromycin Methicillin	NorA inhibition Interference with PBP2a expression[41,61,96,97]
Berberine (**6**)	*Berberis* spp.	Ampicillin Oxacillin	Intercalation into DNA; Increase membrane permeability[61,63,85,98]
5*-methoxy-hydnocarpin (**7**); pheophorbide *a* (**8**)	*Berberis* spp.	Berberine Others (*e.g.* norfloxacin)	NorA inhibition[37,66,71,86]
Ferruginol (**9**); 5-Epipisiferol (**10**)	*Chamaecyparis lawsoniana*	Norfloxacin Erythromycin Oxacillin Tetracycline	EtBr efflux inhibition[22,41]
Catechin gallate (**11**); Epicatechin gallate (**12**); Epigallocatechin gallate (**13**)	*Camellia sinensis*	β-lactams Norfloxacin Carbapenems Tetracycline	β-lactamases inhibition; PBP2a synthesis inhibition; Reaction with peptidoglycan; EtBr efflux inhibition; TetK inhibition[24,73,99–102]
Methyl-1α-acetoxy-7α-14α-dihydroxy-8,15-isopimaradien-18-oate (**14**); Methyl-1α,14α-diacetoxy-7α-hydroxy-8,15-isopimaradien-18-oate (**15**)	*Lycopus europaeus*	Tetracycline Erythromycin	MDR efflux pumps inhibition[25]
Piperine (**16**)	*Piper nigrum* *Piper longum*	Ciprofloxacin	EtBr efflux inhibition[104,105]
Thymol (**17**); Carvacrol (**18**)	*Thymus vulgaris*	Several	Increase membrane permeability[59,61,107,109,110]
Baicalein (**19**)	*Scutellaria* species	Tetracycline β-lactams Gentamicin Ciprofloxacin	Inhibition of PBP2a; Reaction with the peptidoglycan; NorA inhibition[14,46,111]
2,6-dimethyl-4-phenyl-pyridine-3,5-dicarboxylic acid diethyl ester (**20**)	*Jatropha elliptica*	Ciprofloxacin Norfloxacin	NorA inhibition[22,27]
Ethyl gallate (**21**)	*Caesalpinia spinosa*	β-lactams	Restriction of substrate diffusion for PBPs[24]
Cinnamaldehyde (**22**)	*Cinnamomum zeylanicum*	Clindamycin	CdeA inhibition[90]
Gallic acid (**23**)	Berry extracts	Tetracycline	Increase membrane permeability[78,112,113]
Xanthohumol (**24**); Lupulon (**25**)	*Humulus lupulus*	Polymyxin B sulphate Tobramycin Ciprofloxacin	Increase membrane permeability[46,114]
Tellimagrandin I (**26**); Rugosin B (**27**)	*Rosa canina* L.	β-lactams	Inactivation of PBPs, particularly PBP2a[115,116]
Corilagin (**28**)	*Arctostaphylos uva-ursi*	β-lactams Cefmetazole	Inhibition of PBP2a activity or production[49,116]
Myricetin (**29**)	Widespread among plants including tea, berries, fruits, vegetables and medicinal herbs	Amoxicillin/ clavulanate Ampicillin/ sublactam Cefoxitin	DNA B helicase inhibition[51,117]
Allicin (**30**)	*Allium sativum*	Cefazolin Oxacillin Cefoperazone Vancomycin	RNA synthesis inhibition; Interaction with important thiol-containing enzymes[51,119–121]
Silybin (**31**)	*Silybum marianum*	Ampicillin Oxacillin	MDR efflux pumps inhibition[63,124]
The polyacylated neohesperidoses (**32**) and (**33**)	*Geranium caespitosum*	Berberine Ciprofloxacin Norfloxacin Rhein	MDR efflux pumps inhibition[66,86,125]

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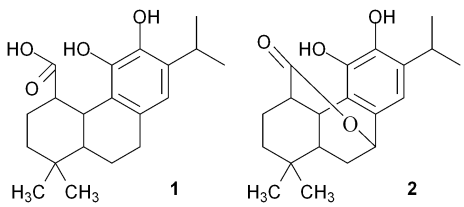
Table 1 (*Contd.*)

Phytochemical product	Plant source	Antibiotic potentiated	Mechanisms of action
Chrysosplenol D (**34**); Chrysoplenetin (**35**)	*Artemisia annua*	Artemisinin Berberine Norfloxacin	MDR efflux pump inhibition[66]
Chalcone (**36**)	*Dalea versicolor*	Berberine Erythromycin Tetracycline	NorA inhibition[66,126,127]
4′,5′-*O*-dicaffeoylquinic acid (**37**)	*Artemisia absinthium*	Berberine Fluroquinolones	MFS family efflux systems inhibition[128]
Genistein (**38**); Orobol (**39**); Biochanin A (**40**)	*Lupinus argenteus*	Berberine Norfloxacin	MDR efflux pump inhibition[129]

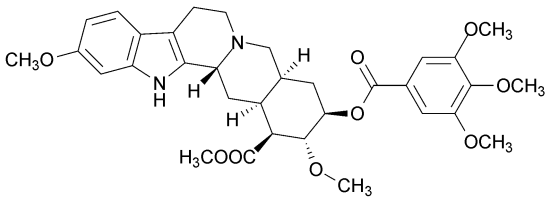
inhibitor that deactivates the bacterial resistance mechanism, thus increasing the effectiveness of the antimicrobial product.[90] These products could be of significant benefit for the treatment of MDR infections. There are diverse phytochemical products which have already demonstrated the potential to act as resistant modifying agents and potentiated the antimicrobial effects of antibiotics (Table 1).

4.2 Plant products with resistance-modifying activity *in vitro*

Rosmarinus officinalis L. (commonly referred to as rosemary) extracts act as a soft analgesic and antimicrobial agent in traditional use.[91,92] Extracts of rosemary provide a major source of antimicrobials, including carnosic acid (**1**) and carnosol (**2**).[5,93] These compounds at 10 μg mL^{-1} were found to potentiate the activity of tetracycline (2- and 4-fold, respectively) against a TetK-possessing *S. aureus* strain and **1** showed an 8-fold reduction in the MIC of erythromycin against *S. aureus* strains expressing MsrA.[22] Additionally, **1** was shown to inhibit EtBr (a substrate for several MDR pumps) efflux in a NorA expressing *S. aureus* strain with an IC_{50} of 50 mM (one quarter of the MIC for the strain).[5,22] However, this activity is likely to be related to the inhibition of efflux pump(s) other than NorA.

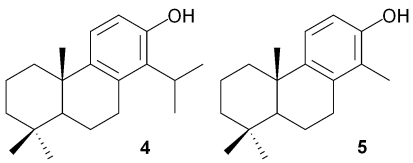


The plant alkaloid reserpine (**3**) produced by*Rauwolfia serpentina*[37]was originally found to inhibit Bmr efflux pumps.[66] Its EPI activity was also demonstrated against the NorA pump in *S. aureus* by reducing the MIC of norfloxacin at least 4-fold.[71,94] Reserpine reduced sparfloxacin, moxifloxacin and ciprofloxacin MICs up to 4-fold in several clinical isolates of *S. aureus.*[95] Gibbons and Udo[64] showed the same reduction for tetracycline in clinical isolates of MRSA with the TetK determinant. Reserpine also inhibited LmrA of *L. lactis*.[73] However, it was found that **3** is toxic for humans at concentrations required for MDR pump inhibition.[80,94] Moreover, bacterial resistance to this natural product was already observed.[51]



3

Totarol (**4**), a diterpene isolated from immature cones of *Chamaecyparis nootkatensis*, reduced the MIC of tetracycline, norfloxacin and erythromycin 4-, 4- and 8-fold, respectively, in MRSA strains,[76] suggesting that this product is a NorA EPI.[76] Totarol also potentiated the activity of methicillin against MRSA by 8 times.[96] Despite the fact that the primary staphylococcal target for **4** is the respiratory chain, it was found that this synergistic activity is due to the interference with PBP2a expression.[96,97] Other studies suggest that this product may affect the production of macromolecules, including DNA and peptidoglycan, causing cell membrane disruption.[61,76,96] Other diterpenes, especially diterpene 416 (14-methylpodocarpa-8,11, 13-trien-13-ol; **5**), also potentiated methicillin, with a reduction in the MIC of more than 256-fold against MRSA, owing to a significant reduction in PBP2a expression.[96]



Berberine (**6**), a hydrophobic alkaloid produced by *Berberis* species,[51] is known to increase membrane permeability and to intercalate into DNA.[85] Yu *et al.*[98] found an additive effect between **6** and ampicillin and a synergistic effect with oxacillin, against MRSA. This product has the potential to restore the effectiveness of β-lactam antibiotics against MRSA.[63,98] However, **6** is rapidly extruded by multidrug efflux pumps.[62] It was observed that *Berberis* species also synthesize 5′-methoxyhydnocarpinD (**7**) and pheophorbide *a* (**8**), which were identified as inhibitors of the NorA MDR efflux pump in *S. aureus.*[22,37,63,73,86] These MDR inhibitors facilitate the penetration of berberine into *S. aureus* by completely inhibiting its efflux from the cell.[46,71,86] This is an important example of synergy

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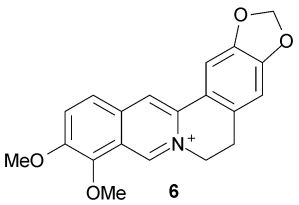
between components from the same plant.[62] 5′-methoxy-hydnocarpin D also had a synergistic effect with several other products exported by NorA, including norfloxacin.[66]



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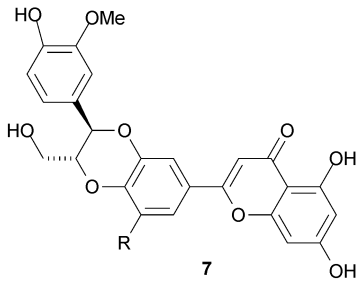

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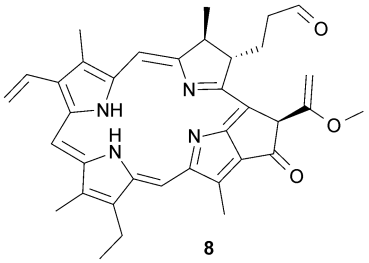
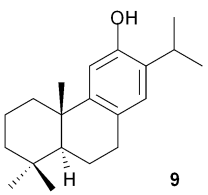


8

Smith *et al.*[41] isolated active compounds from the cones of *Chamaecyparis lawsoniana* and observed that ferruginol (**9**) and 5-epipisiferol (**10**) were effective in increasing the efficacy of tetracycline, norfloxacin, erythromycin and oxacillin against resistant *S. aureus*. It was demonstrated that **9** inhibited the efflux of EtBr by 40%, using a NorA-expressing *S. aureus* strain.[41]



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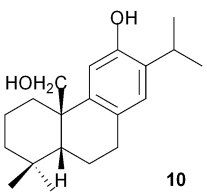
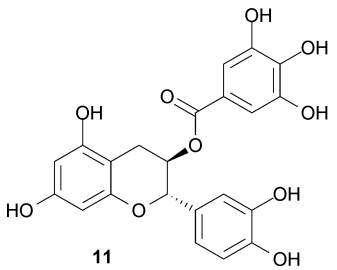


10

Japanese green tea (*Camellia sinensis*) is consumed every day by billions of people worldwide for its antipyretic, antidotal, antidiarrheal and diuretic effects.[99] These benefits of green tea are not only due to the bacteriostatic and bactericidal activities of some constituents but also to the presence of several resistance-inhibitory products.[100] Aqueous extracts of tea, especially catechin gallate (**11**), epicatechin gallate (**12**) and epigallocatechin gallate (**13**), demonstrated the potential to reverse methicillin resistance in MRSA and penicillin resistance in β-lactamase-producing *S. aureus*, apparently by the prevention of PBP2a synthesis and the inhibition of β-lactamase secretion, respectively.[27,100–102] Moreover, in the presence of **11** and **12** the MIC for oxacillin was reduced from 256 and 512 to 1–4 mg L^{-1} against MRSA.[24] Epigallocatechin gallate had much lower activity than **12**, although only differing by an extra hydroxyl group on the B-ring, resulting in a reduction in oxacillin MIC, ranging between 4 and 64-fold.[41] Gibbons *et al.*[103] showed that **12** and **13** caused a 4-fold potentiation of the activity of norfloxacin against a NorA over-expressing *S. aureus* strain. Another explanation for this synergism with β-lactams against MRSA is the possible interference of these products with the integrity of the cell wall through direct binding to peptidoglycan.[99] Additionally, **13** was found to synergistically enhance the activity of carbapenems against MRSA.[51,101] It was also demonstrated that **12** and **13** are able to reverse tetracycline resistance in staphylococcal isolates expressing TetK and TetB efflux pumps, probably due to the inhibition of these pumps.[73,102]



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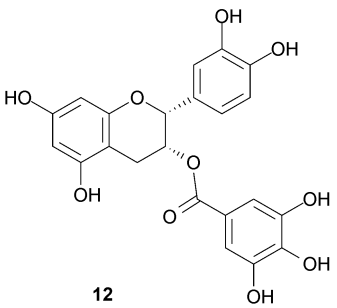

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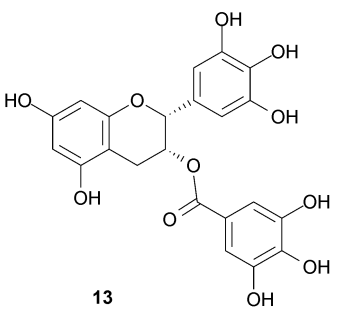
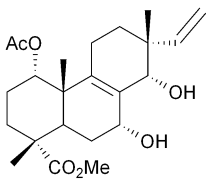


13

Two isopimarane diterpenes, methyl-1α-acetoxy-7α, 14α-dihydroxy-8,15-isopi-maradien-18-oate (**14**) and methyl-1α,14α-diacetoxy-7α-hydroxy-8,15-isopimaradi-en-18-oate (**15**), isolated from an extract of *Lycopus europaeus* by Gibbons *et al.*,[25] demonstrated a 2-fold potentiation of tetracycline and erythromycin against two strains of *S. aureus* possessing the TetK, MsrA and NorA efflux mechanisms.



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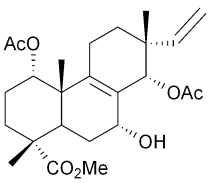

15

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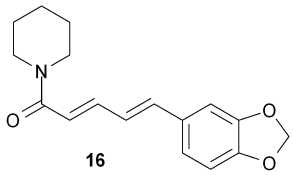
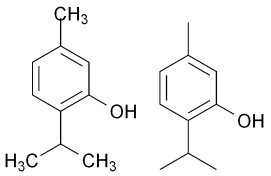
Piperine (**16**), the major plant alkaloid present in black pepper (*Piper nigrum*) and long pepper (*Piper longum*) reduced the MIC of ciprofloxacin against *S. aureus*, including MRSA, and also decreased EtBr efflux.[104,105] Piperine also reduced the MIC of mupirocin against strains of *S. aureus*, including MRSA.[106]



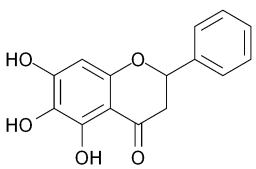
16

Thymol (**17**) and carvacrol (**18**), two main products of the essential oil of *Thymus vulgaris*, reportedly disintegrate the OM and thus increase membrane permeability and fluidity in Gram-negative bacteria, facilitating the penetration of antibiotics.[3,46,59,61,107] The combined application of both products resulted in a stronger antibacterial effect than if applied separately.[108] Synergistic interactions have been reported between **17** and **18**, and 18 antibiotics against *Sphingomonas paucimobilis* and *Klebsiella oxytoca*.[109] These products increased the susceptibility of *Salmonella enterica* Typhimurium SGI 1 to ampicillin, tetracycline, penicillin, bacitracin, erythromycin and novobiocin, and reduced the resistance of *Streptococcus pyogenes* with the macrolide-resistant gene *ermB* to erythromycin.[110]



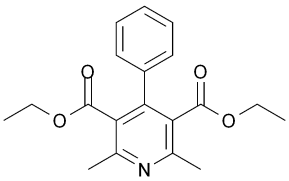
17 **18**

Baicalein (**19**) has been isolated from extracts of the leaves of *Scutellaria baicalensis*, which is one of the most popular herbs in China for the treatment of bacterial and viral infections.[14] Baicalein had a synergistic effect with tetracycline against MRSA with and without TetK-genes and in TetK-overexpressing *E. coli*.[111] This product potentiated the effects of β-lactam antibiotics against MRSA.[46] Chan *et al.*[14] also demonstrated synergy between **19** and ciprofloxacin against MRSA strains and with gentamicin against vancomycin-resistant enterococci (VRE), apparently by the inhibition of the NorA efflux pump. The synergistic actions of **19** on MRSA may therefore involve several mechanisms of action, such as bacterial efflux pump inhibition (different from TetK), PBPs inhibition or cell wall disintegration by interference with the peptidoglycan structure.[14,46]



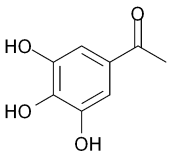
19

2,6-Dimethyl-4-phenylpyridine-3,5-dicarboxylic acid diethyl ester (**20**), a penta-substituted pyridine isolated from an ethanol extract of rhizomes of *Jatropha elliptica*,[27] increased the activity of ciprofloxacin and norfloxacin against NorA-expressing *S. aureus* by 4-fold, suggesting that **20** is an inhibitor of this efflux pump.[22,27]



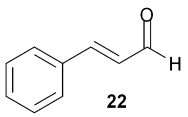
20

Ethyl gallate (**21**) and other alkyl gallates purified from a dried pod of *Caesalpinia spinosa* (tara) reduced the MIC of β-lactams against MRSA and methicillin-sensitive *S. aureus* (MSSA) strains.[24] The mechanism of action of these gallates is still unknown but the possible perturbation of the membrane and the resulting restrictions on its components' fluidity could make the diffusion of substrates for PBPs difficult, especially for PBP2a.[24]



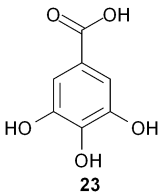
21

Shahverdi and co-workers[90] discovered that cinnamaldehyde (**22**), from *Cinnamomum zeylanicum* bark essential oil, reduced clindamycin resistance in *Clostridium difficile.* CdeA was the first multidrug efflux transporter to be identified in *C. difficile* and **22** may inhibit this efflux pump system.[90] Moreover, **22** reduces the MIC of ampicillin, tetracycline, penicillin, erythromycin and novobiocin.[110]



22

Gallic acid (**23**) from berry extracts has proven to be an efficient permeabilizer for several *Salmonella* strains.[112] Moreover, synergistic interactions were observed with the combination of **23** with tetracycline against *P. aeruginosa* strains[78] and with streptomycin against *E. coli* and *P. aeruginosa.*[113] The OM disintegrating activity of **23** was suggested to be based on the chelation of divalent cations from the OM and, additionally, the partial hydrophobicity of this product also allowed bacterial membrane destabilization.[112]



23

A putatively synergistic effect has been reported for the antibacterial constituents of *Humulus lupulus* (hop), xanthohumol (**24**) and lupulon (**25**), and some antibiotics, such as polymyxin B

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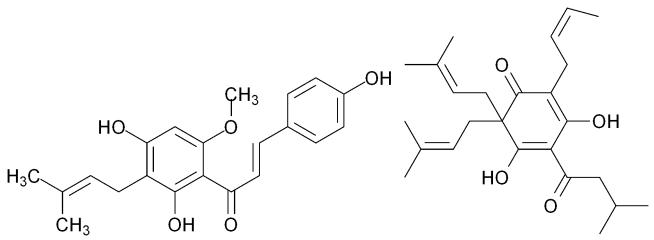
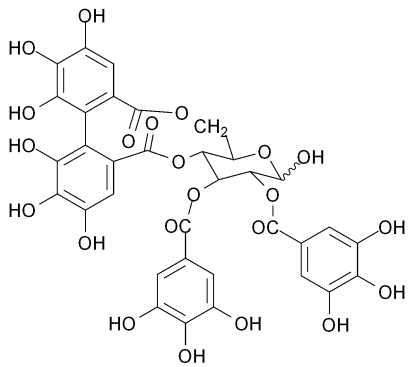
sulfate, tobramycin and ciprofloxacin, against Gram-positive and Gram-negative bacteria.[46] The antimicrobial action of **24** and **25** was due to changes induced in the properties and permeability of the membrane.[114]



24 **25**

Compounds obtained from the extract of petals of *Rosa canina* L. (rose red), tellimagrandin I (**26**) and rugosin B (**27**), markedly reduced the MIC of β-lactams against MRSA.[115] Results indicate that inactivation of PBPs, especially of PBP2a, is the major reason for this reduction.[116]



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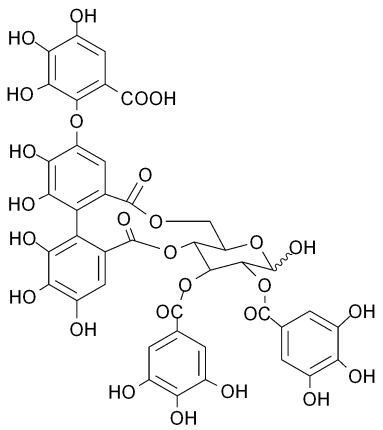
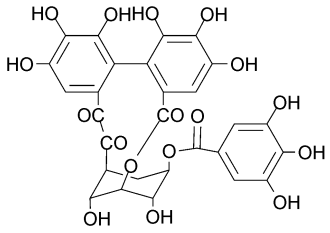


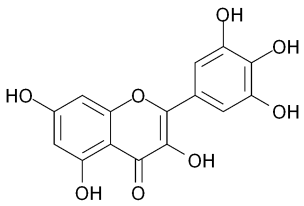
27

Shimizu *et al.*[49] found that an extract of *Arctostaphylos uva-ursi*, the polyphenol corilagin (**28**), markedly reduced the MIC of β-lactams, such as oxacillin and cefmetazole, by 100- to 2000-fold in MRSA. It was suggested that the antimicrobial mechanism of action of **28** involves PBP2a (inhibition of activity or inhibition of synthesis).[49,116]



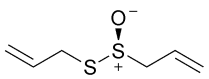
28

Myricetin (**29**), a flavonol found in many vegetables, herbs, berries and fruits, had a significant synergistic activity against ESβL-producing *K. pneumoniae* in combination with amoxicillin/clavulanate, ampicillin/sulbactam and cefoxitin.[117] This product inhibited DNA B helicase in *E. coli*, which plays a central role during DNA replication initiation and elongation.[51,118] Moreover, it is known that **29** inhibits a variety of DNA polymerases, RNA polymerases, reverse transcriptases and telomerases.[118]

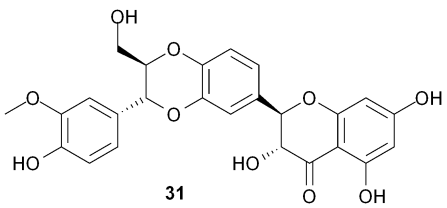


29

Allicin (**30**) is one of the most effective antimicrobial products isolated from garlic (*Allium sativum*) and may potentiate the action of the antibiotics cefazolin and oxacillin against *Staphylococcus* spp. and cefoperazone against *P. aeruginosa.*[119] Allicin also considerably reduced the MIC of vancomycin for VRE strains.[120] Allicin reportedly inhibits bacterial RNA synthesis[51] and interacts with important thiol-containing microbial enzymes, such as cysteine proteinases, acetate kinases, alcohol dehydrogenases, thioredoxin reductases and phosphotransacetyl-CoA synthetases.[121]



Silybin (**31**) is a flavonolignan obtained from silymarin, the standardized extract of the *Silybum marianum*, which is one of the oldest known and most widely used traditional European medicine and has mainly been used for liver disorders.[122] Silybin was shown to be a MDR pump inhibitor.[63,123] This product has been evaluated against 20 clinical isolates of MRSA in combination with ampicillin or oxacillin and the MIC for this combination were reduced by more than 4-fold.[124]



31

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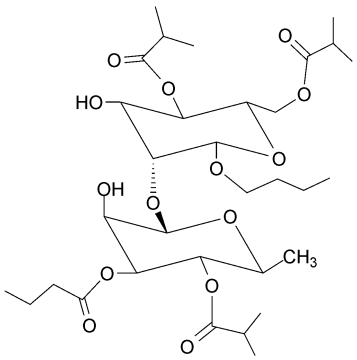
The polyacylated neohesperidosides (**32** and **33**), from *Geranium caespitosum*,[125] increased the activity of ciprofloxacin and norfloxacin, apparently due to MDR pump inhibition.[66,86]



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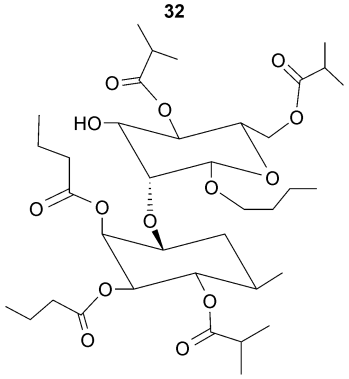
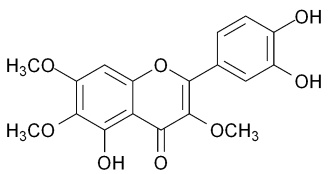


33

The flavones chrysosplenol D (**34**) and chrysoplenetin (**35**), isolated from *Artemisia annua*, potentiated the activity of the antimalarial artemisinin against *Plasmodium falciparum* in the presence of berberine and norfloxacin,[66] due to NorA inhibition.



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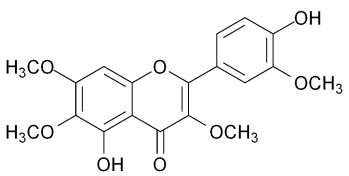
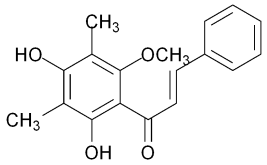


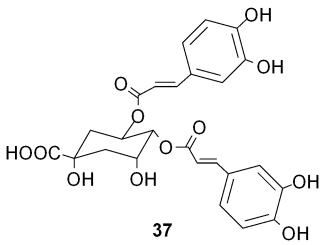
35

An extract of *Dalea versicolor*, chalcone (**36**), reportedly enhanced the activity of berberine, erythromycin and tetracycline against *S. aureus*, apparently by the inhibition of the NorA efflux pump.[66,126,127] Chalcone also increased the activity of berberine against *B. cereus* 30-fold.[126] This is an interesting case where products with strong direct antimicrobial activity and those with MDR pump inhibitory action were found in the same plant.[127]



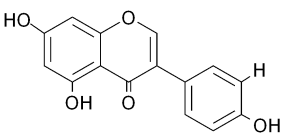
36

4′,5′-*O*-dicaffeoylquinic acid (**37**) from *Artemisia absinthium* has been identified and characterized by Fiamegos *et al.*[128] as an efflux pump inhibitor with the potential to target efflux systems, especially of the MFS family. 4′,5′-*O*-dicaffeoylquinic acid potentiated berberine and fluroquinolones against a wide panel of Gram-positive human pathogenic bacteria.[128]



37

The isoflavones genistein (**38**), orobol (**39**) and biochanin A (**40**), isolated from *Lupinus argenteus*, potentiated the antibacterial activity of α-linolenic acid, also from the same plant, and enhanced the antibacterial activity of berberine and norfloxacin against *S. aureus* cells, indicating that they may inhibit MDR pumps.[129]



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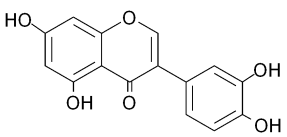

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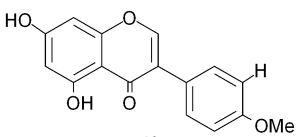

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4.3 Plant extracts with resistance-modifying activity *in vitro*

In addition to the synergistic effects observed for the previously described products, a large number of other *in vitro* studies have reported the capacity of plant extracts to potentiate the activity of antibiotics. Mechanistic studies into such synergistic effects are often complicated by the fact that plant extracts consist of complex mixtures of compounds, several of which can be involved in the final result.[46] The screening of these extracts is, however, expected to provide leads for the isolation of MDR inhibitors.[79] Some examples are described in this section.

Methanolic extracts of *Punica granatum* have shown synergistic activity by the broth dilution method and time-kill assays with chloramphenicol, gentamicin, ampicillin, tetracycline and oxacillin against strains of MRSA and MSSA.[130] The extracts also demonstrated the potential to either inhibit the efflux pump NorA or to enhance the influx of the antibiotics.[130] Aqueous

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crude extracts of *Catha edulis* at sub-MIC potentiatied the action of tetracycline against *Streptococcus sanguis* TH-13 and *Streptococcus oralis* SH-2 (2- and 4-fold, respectively), and penicillin G against *Fusobacterium nucleatum* 9911 (4-fold).[131] Ethanol extracts of *Eugenia uniflora* L. and *Eugenia jambolanum* exhibited synergistic activity with gentamicin against *E. coli*.[132] Synergistic interactions were also observed between extracts of several Brazilian plants and eight antibiotics against *S. aureus*, indicating the presence of a range broad of inhibitors of protein synthesis, cell wall synthesis, nucleic acid synthesis and folic acid synthesis.[18] Methanolic extracts of 19 Jordanian plants had significant synergistic interactions against MRSA and MSSA strains in combination with chloramphenicol, gentamicin, erythromycin and penicillin G.[133] Ethanol extracts of the Chinese plants *Isatis tinctoria*, *Scutellaria baicalensis* and *Rheum palmatum* improved the activity of ciprofloxacin, penicillin, gentamicin and ceftriaxone against antibiotic resistant *S. aureus* strains.[134] *Dalea spinosa* (smoke tree) extracts potentiated antibiotic activity against MRSA, due to MDR efflux pump inhibition.[61,135] Coutinho *et al.*[21] demonstrated that an ethanol extract of *Momordica charantia* L. reduced the MIC for some aminoglycosides against MRSA. Extracts of *Securinega virosa* and *Mezoneuron benthamianum* exerted a potentiation activity against fluoroquinolone-, tetracycline- and erythromycin-resistant *S. aureus* strains.[66] Additionally, 4-fold potentiation of the activity of norfloxacin was observed with ethanol extracts of *M. benthamianum* and, to a lesser extent, with chloroform extracts of *S. virosa.*[80] Coutinho and co-workers[136] reported that the ethanol extract of *Turnera ulmifolia* L. had a synergistic effect with aminoglycosides. Acetone extracts of *Garcinia kola* seeds potentiated the activity of tetracycline and chloramphenicol against *E. coli* and *K. pneumonia*, and amoxicillin and penicillin G against *S. aureus.*[79]

Adwan *et al.*[137] demonstrated a significant reduction in the MIC of several antimicrobial agents against MRSA and MSSA with water extracts of *Psidium guajava*, *Rosmarinus officinalis*, *Salvia fruticosa*, *Majorana syriaca*, *Ocimum basilucum*, *Syzygium aromaticum*, *Laurus nobilis* and *Rosa damascene*. Ethanolic extracts of some Indian medicinal plants, *Acorus calamus*, *Hemidesmus indicus*, *Holarrhena antidysenterica* and *Plumbago zeylanica* demonstrated synergistic activities when combined with tetracycline, chloramphenicol, ciprofloxacin, cefuroxime and ceftidizime against several MRSA strains,[138] and with tetracycline and ciprofloxacin against ESβL-producing MDR enteric bacteria.[139]

Synergistic interactions of crude extracts from *Camellia sinensis*, *Lawsonia inermis*, *Terminalia chebula* and *Terminalia belerica* have been reported with tetracycline against MRSA and MSSA strains and with ampicillin for *Camellia sinensis* extract.[140] Extracts of *Commiphora molmol*, *Centella asiatica*, *Daucus carota*, *Citrus aurantium* and *Glycyrrhiza glabra* had good activity against *S. enterica* serovar Typhimurium overexpressing the AcrAB-TolC efflux protein.[66] Synergy against a MDR *P. aeruginosa* strain was found between ethanolic extracts of *Rhus coriaria* (seed) and some antimicrobial drugs, including oxytetracycline HCl, penicillin G, cephalexin, sulfadimethoxine sodium and enrofloxacins, suggesting the presence of natural inhibitors, including EPIs.[141] The combinations of the methanol extract of the leaves of *Helichrysum pedunculatum* and 8 first-line antibiotics were investigated against several bacterial strains implicated in wound infections and 60% of the interactions were synergistic.[142] The combination of methanol extracts of *Helichrysum longifolium* with penicillin G sodium, amoxicillin, chloramphenicol, oxytetracycline, erythromycin and ciprofloxacin against several bacterial isolates of referenced, clinical and environmental strains resulted in 61.7% synergy for all interactions.[143]

4.4. *In vivo* tests of phytochemicals

Extracts from many plant species have been previously tested against hundreds of bacterial strains *in vitro*, and some of them have demonstrated antimicrobial activity. All these findings have provided good indications for the potential of plants to be applied in combination with antibiotics to treat infectious diseases. Unfortunately, much of the data acquired are only preliminary, having been derived from *in vitro* antimicrobial assays, potentiation assays and efflux studies and the mechanisms of action often remain unexplained.[66] Further research should therefore focus on the use of preclinical animal infection models, followed, where appropriate, by clinical trials, enabling the definition of pharmacokinetic and pharmacodynamic targets and the measurement of many other parameters at the site of infection, such as antimicrobial efficacy, appropriate doses and safety data.[51,144] Thorough investigation is also required to elucidate side effects, and strategies for extraction and preservation of these products.[145] In this respect, according to Harvey,[146] over 100 natural products are currently undergoing clinical trials, with at least the same number of projects in preclinical development. Most of these products are derived from plants and microbial sources and are directed for anti-cancer, antioxidant, anti-diabetic, anti-inflammatory and wound healing activities or for urology, cardiovascular, dermatology, sleep and nervous disorders.[146] Only a few have been tested in animal or human studies for their antimicrobial properties. Fig. 3 shows data about the stages of development of plant products for their anti-infective properties. In this context, studies evaluating RMAs or phytochemical–antibiotic therapies *in vivo* are uncommon. The activity of levofloxacin plus an efflux pump inhibitor was evaluated in an animal model of a *P. aeruginosa* infection caused by a strain overexpressing the MexAB-OprM MDR efflux system and demonstrated efficacy.[147] The combination of piperine with mupirocin in a dermal infection model in mice showed better *in vivo* efficacy when compared with the commercially available formulation of 2% mupirocin.[106] The combination of Japanese green tea extract with levofloxacin against enterohemorrhagic *E. coli* infection in a gnotobiotic mouse model produced an increased survival rate and the prevention of tissue damage.[148] Isbrucker *et al.*[149] developed safety studies of epigallocatechin gallate and demonstrated that this product caused minor dermal irritation in rats and guinea pigs, but not rabbits, and was a moderate dermal sensitizing agent in the guinea pig maximization test. Moreover, the dietary administration of epigallocatechin gallate to rats for 13 weeks was not toxic at doses up to 500 mg kg^{-1} day^{-1},[149] and administration to 40 volunteers for 4 weeks at 800 mg day^{-1} only caused minor adverse effects.[150] Co-administration of an EPI with an antibiotic has already progressed to human clinical trials.[37,75] An aerosolized formulation of the EPI MP-601,205, combined with

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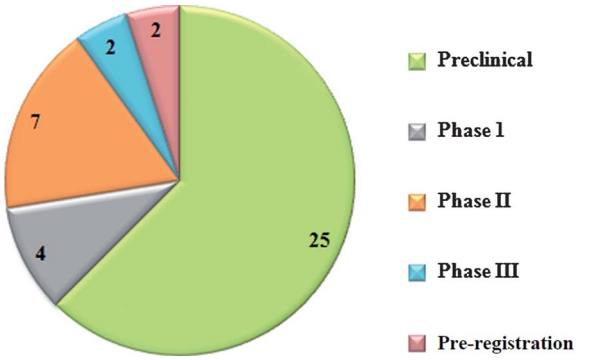

Fig. 3 Number of drugs obtained from plants at different stages of development for their anti-infective properties (data of 2008 obtained from Harvey[146]).

ciprofloxacin, is in phase II of tests for the treatment of pulmonary exacerbations in cystic fibrosis patients.[67]

Other studies have focused on the *in vivo* antimicrobial activity of plant extracts. For example, Chowdhury *et al.*[151] demonstrated that allicin has promising *in vivo* antibacterial activity against *Shigella flexneri* when tested in the rabbit model of experimental shigellosis. Si *et al.*[152] studied the antimicrobial activity of carvacrol, thymol and cinnamaldehyde against *Salmonella* in pig diets and reported that higher concentrations were needed to retain their antimicrobial activity when added to the diets. The effects of oolong tea polyphenols on dental caries in rats were tested and showing that total fissure caries lesions was significantly reduced by the addition of tea polyphenols to the diet or in the drinking water.[153] Tea catechins also inhibited the fluid accumulation induced by cholera toxin in sealed adult mice and reduced fluid accumulation by *Vibrio cholerae* O1 in intestinal loops of rabbits, suggesting that tea catechins may possess protective activity against *V. cholerae* O1.[154] *P. aeruginosa* lung infection of athymic rats was used to test subcutaneous administrations of ginseng, which seems to increase the resistance of the athymic rats to this bacteria.[155]

5 Conclusions and perspectives

Plants represent a renewable and attractive source of antimicrobials with many *in vitro* studies demonstrating the therapeutic potential of phytochemical products as alternatives or potentiators of antibiotics. The rich chemical diversity in plants makes them a potential source of antimicrobials and RMAs. However, the pharmaceutical industry is still acting more on tradition or habit based on past success (antibacterials of microbial origin, of which there are many examples). The current lack of progress suggests that it is time for a paradigm-shift.

The chemical complexity of plant extracts, often undocumented toxicity, poor water solubility and the lack of standardization may be responsible for the apparent lack of industrial interest in phytochemicals.[39,156] Difficulties in access and supply, the inherent slowness of working with natural products and the costs of collection, extraction and isolation are additional limitations.[20,146] Therefore, the discovery of antibiotics of plant origin has been regarded as risky by the industry.[29] The current antibacterial research and development activities are usually based on alterations to existing classes of antibiotics and on screening collections of synthetic products prepared by combinatorial chemistry and computational design.[19,45] These libraries, however, often lack the true chemical diversity that natural products display (extensive functional group chemistry and chirality).[20]

The advent of high-throughput screening methods for assessment of large numbers of plant extracts containing putative biologically active compounds has further encouraged industrial interest in plant research.[20] Recent developments in genomics, proteomics and metabolomics may play a role in the future of antibiotic resistance diagnostics and may allow an accurate characterization of the mechanisms of action of new antimicrobials.[29,51] New investigations should also employ modern methodologies, including the use of generally recognized protocols and standards for microbial testing, as well as standardization of the quality of plants.[157] The application of metabolic engineering to plants will increase the yield of product and boost industrial interest in phytochemical products for antimicrobial therapy.

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