EXPERT REVIEWS

# Plant essential oils and their constituents in coping with multidrug-resistant bacteria

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Antibiotic resistance is documented to be a serious problem that affects the choice of appropriate antibiotic therapy and increases the probability of unfavorable infection outcome. One of the proposed methods to cope with multidrug-resistant (MDR) bacteria is the use of alternative antibacterial treatments, which include natural antimicrobial substances such as plant essential oils (EOs). The aim of the present article is to review published studies on the activity of EOs and their constituents against MDR bacteria and to formulate perspectives for the future. In general, published studies indicate that EOs can be used as effective antiseptics against many species, including MDR bacteria, such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, resistant isolates of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and others; certain EOs may potentiate the effectiveness of antibiotics against MDR bacteria; EOs can be synergistic with bacteriophages; and polymeric nanoparticles can be used for delivery of EOs and enhancement of their activity at the site of infection.

**Keywords:** antibiotics • bacteriophages • combinations • essential oils • MRSA • multidrug-resistant bacteria

## Multidrug-resistant bacteria & methods for coping with resistance

Inappropriate and irrational use of antibiotics provides favorable conditions for selection and spread of antibiotic resistance. Today, the escalation of multidrug-resistant (MDR) bacteria is documented among agents of different infectious processes all over the world [1]. Resistance has emerged towards all classes of antibiotics leading to a continuous need for producing new drugs. However, during the past 40 years, a few new classes of antibiotics have been discovered. One of the reasons for this decline is the challenges in identifying new chemical substances that are simultaneously effective and also nontoxic [2].

Constant antibiotic use leads to bacterial evolution to MDR forms, resulting in human epidemics. Examples of such MDR bacteria are methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE), *Escherichia coli* and *Pseudomonas aeruginosa* resistant to fluoroquinolones, *Klebsiella pneumoniae* resistant to ceftazidime, MDR *Acinetobacter baumannii*, and many other bacteria. The treatment options for these bacteria are increasingly limited, and outcomes of infections are significantly affected [3,4].

Results of several surveillance programs indicate that a high percentage of hospital-acquired infections are caused by MDR strains, such as MRSA and VRE. A third of European countries had an MRSA prevalence among bloodstream-isolated *S. aureus* of over 25% in 2008, according to the study performed by the European Antimicrobial Resistance Surveillance System [5]. Furthermore, it is not only the high prevalence of resistance among bacteria that poses a threat, but also the rapid increase in levels of resistance. Mainous and colleagues analyzed the tendencies of antibiotic resistance in US hospitals during a 10-year period between 1997 and 2006. The results of the study showed a 2.5-fold increase in the number of infection-related hospitalizations with antibiotic resistance [6].

For moderate or severe infections, clinicians commonly initiate antibiotic therapy early and empirically, before the results of cultures and their respective antibiotic susceptibilities are known. The high incidence of MDR bacteria enhances the probability of inappropriate initial empirical antibiotic therapy. Many studies

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have demonstrated the adverse impact of inappropriate initial antibiotic therapy on infection with increased morbidity and mortality [7–9].

The major routes of antibiotic resistance include enzymatic modification or degradation of the antibiotic molecule, alteration of the antibiotic target that prevents binding of the antibiotic and leads to loss of its activity, the efflux of antibiotics from bacterial cells through efflux pumps and changes in bacterial physiology that impact antibiotic susceptibility [2,10].

One of the promising methods in coping with bacterial resistance is the use of alternative classes of antimicrobial agents and the application of synergistic activity between antibiotics, and between antibiotics and non-antibiotics [2]. Promising agents with antimicrobial properties are plant essential oils (EOs) and their constituents [11–15]. Synergistic combinations may be composed of antibiotics and bacteriophages [16], antibiotics and metals with antibacterial activities, especially nano-scaled metals [17,18], antibiotics with quorum-sensing inhibitors [19] and antibiotics with EOs of plants [20–23]. EOs have advantages both in their complex mechanism of action and in their complex healing properties. They usually possess antimicrobial activity not only against bacteria but also against fungi [24], protozoans [25] and viruses [26], which is especially important in mixed infections. Furthermore, EOs also have anti-inflammatory [27], immune modulatory [28], antioxidant [27,29] and regenerative activities [30], which make them promising agents in the treatment of different infections.

The purpose of this review is to summarize the studies on the use of plant EOs and their constituents for coping with MDR bacteria, and to formulate new prospects for future studies on this topic (Figure 1).

## EOs with antibacterial properties: mechanism of action on bacteria

The increasing prevalence of MDR bacteria has led to renewed interest in natural antimicrobial substances, which were used previously but then lost their application since the introduction of antibiotics. The antibacterial properties of plant EOs have been known for many centuries. Even before the discovery of microorganisms, plants were accepted to be effective against infectious diseases [31]. Great success in the treatment of infections in the era of discovery of the first antibiotics diminished the role of natural antimicrobial products in combating bacteria. However, the progressive increase in the spread of antibiotic resistance gave new life to EOs and plant extracts.

Great interest in the antimicrobial properties of EOs nowadays is well illustrated by the number of PubMed publications on this topic. By search criteria 'antibacterial EOs' the authors found 1009 publications up to 2011. Until the 1990s, EOs were not studied widely, mainly because antibiotics were sufficiently effective against bacteria. However, the progressive increase in the spread of resistant bacteria has led to renewed interest in EOs: in the period 1991–1994 and 1995–1999, 19 and 49 articles were published in PubMed, respectively. Since 2000 there has been a rapid increase in the number of publications: reaching 134, 122 and 111 articles in 2009, 2010 and 2011, respectively.

The chemical structure of each EO includes many components, which have been screened for antimicrobial activity; antibacterial components are derived from terpenes, such as, thymol, carvacrol, menthol and geraniol [15].

Among the components of EOs, oxygenated terpenes exhibit high antimicrobial activity compared with their hydrocarbon counterparts. One of the main antimicrobial components is thymol. Its high antibacterial activity is associated with the electron delocalization system and hydroxyl group in its structure. Thymol, citrals, carvacrol and *p*-cymene have been shown to cause membrane permeability by taking part in membrane swelling. *p*-cymene, via its permeabilizing activity, enables the influx of carvacrol into the cell thus achieving a synergistic effect when both components are present [32].

The high antimicrobial activity of EO components with phenolic structures, such as thymol, carvacrol and eugenol, has been demonstrated in many studies [33–36]. Several mechanisms have been proposed as an explanation for this. The hydroxyl group on eugenol might react with proteins and prevent enzyme action. Furthermore, hydrophobic thymol and carvacrol may disturb the outer membrane of Gram-negative bacteria releasing lipopolysaccharides [36].

Cinnamaldehyde is the main component of cinnamon EO, and has demonstrated very high antibacterial activity in many studies [37–39]. Cinnamaldehyde and eugenol demonstrated an ability to inhibit energy metabolism in *Listeria monocytogenes* and *Lactobacillus sakei*. This effect can be explained by interactions between cinnamaldehyde and bacterial cell membranes, leading to membrane disruption. Damage to the cell membrane leads to dispersion of the proton motive force with small ion leakage, while larger cell components, such as ATP, are not leaked. Furthermore, one of the effects of cinnamaldehyde is the inhibition of glucose import or inhibition of glycolysis [40].

The multicomponent chemical composition and complex mechanism of action of EOs give them advantages over traditional that antibiotics possess more specific actions on bacterial structures. The simultaneous

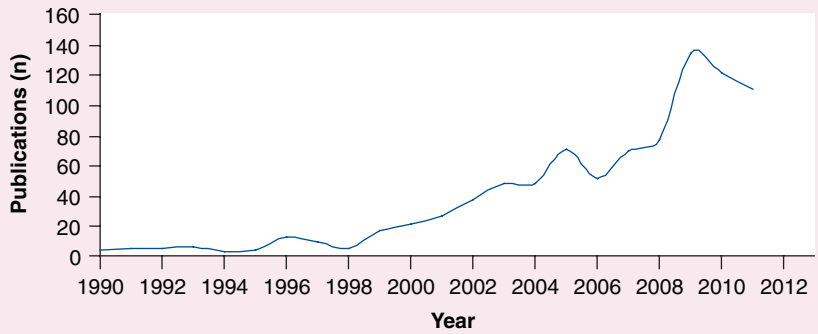


**Figure 1. Dynamics of PubMed publications on antibacterial activity of essential oils.**

effects of EO components on different bacterial sites makes it more difficult for the bacteria to develop resistance.

## Activity of EOs & their constituents against MDR bacteria

### In vitro *activity of EOs against MDR bacteria*

One of the most common examples of an MDR bacterium is MRSA, which was first isolated in 1961; a year after methicillin was introduced into clinical use [5]. Following the emergence of MRSA, a search for coping with this MDR bacterium also began. Alternative antibiotics were proposed, such as glycopeptides (vancomycin). At the same time, the study of natural antimicrobial compounds for the presence of anti-MRSA activity also started. The first EO that demonstrated activity against MRSA was tea tree (*Melaleuca alternifolia*) oil [41]. In the 1990s, this EO was the most widely studied against different microorganisms [42,43]. The potential of tea tree oil in combating MRSA was first hypothesized by Walsh and Longstaff in 1987 [44], and this topic later elicited wide interest among scientists. Carson *et al.* evaluated the susceptibility of 64 MRSA isolates from Australia and the UK to tea tree oil, among them 33 isolates were mupirocin-resistant [41]. The minimal inhibitory concentrations and minimal bactericidal concentrations were 0.25–0.313% and 0.5–0.625%, respectively, which indicates the susceptibility of tested strains to tea tree oil.

After 5 more years, May *et al.* published results of a study on the survival rate of MDR bacteria under exposure to tea tree oil at a final concentration of 5% [45]. Several bacterial strains were used, such as MRSA, VRE, *P. aeruginosa* (including gentamicin-resistant), *Stenotrophomonas maltophilia* and *K. pneumoniae* (including gentamicin-resistant); all these strains belong to MDR nosocomial pathogens. The authors also compared oils with different components: standard oil with 34.8% of terpinen-4-ol and 5.5% of cineole and cloned (modified) oil with 43.1% of terpinen-4-ol and 1% of cineole. All tested strains were killed within 6 h. In addition, modified oil with higher terpinen-4-ol and lower cineole content was more active than standard against all strains.

Since the 2000s, studies of the anti-MRSA activity of EOs have become abundant, and many EOs from different plant families have been shown to possess strong activity against MRSA and other bacterial strains (Table 1).

Extensive research was performed by Chao *et al.* who screened 91 EOs and 64 blends against MRSA [46]. Of the 91 single EOs, 78 exhibited zones of inhibition against MRSA. The highest levels of activity were exhibited by lemongrass, lemon myrtle, mountain savory, cinnamon and Melissa EOs. The authors emphasized that the advantage of EOs and especially of their blends is their multicomponent chemical composition that makes it more difficult for microorganisms to develop resistance simultaneously to all active components present. Moreover, the combined action of EO components may have synergistic effects [46]. An example of this synergy is the interaction between tea tree oil components 1,8 cineole and terpinene. The effect of 1,8-cineole itself is mild, but in combination with terpene 1,8-cineole, it can increase the permeability of the bacterial membrane, thus enhancing uptake of terpene and contributing its activity [47].

EOs have also demonstrated significant activity against other MDR bacteria, such as VRE [42,48–50], *A. baumannii* [48,51], resistant strains of *P. aeruginosa* [52,53], *E. coli* [51,53], *Enterobacter aerogenes* [53], *Enterobacter cloacae* [51] *K. pneumoniae* [51,53] and *Salmonella enterica* serotype *Typhimurium* [53].

Data presented in Table 1 show that many studies on the anti-MDR potential of EOs are performed in Germany, the UK and Turkey; however, global reports demonstrate interest in EOs throughout the world, in spite of the indigenous origin of some plants, such as tea tree. The majority of studied oils were produced from plants of *Lamiaceae* and *Myrtaceae* families. Among EOs from *Lamiaceae*, plants the highest interest from researchers is elicited by lavender, mint and thyme EOs. Among the Myrtaceae family the most widely studied is tea tree oil. Regarding the methods used to assess the activity of EOs against MDR bacteria, published studies mainly examine concentration-dependent activity of EOs using the microdilution method, while time-dependent activity with Time–kill curves has not been investigated well.

Regarding mechanism of action of EOs against isolates with resistance to antibiotics, inhibition of the growth of MDR bacteria is assumed to be caused by the same mechanisms as in sensitive strains regardless of the presence of resistance to antibiotics. These findings support an idea about advantages of EOs owing to their multicomponent composition and complex mechanism of action [54].

Moreover, the great potential of EOs in combating antibiotic resistance is supported by the findings on antiplasmid activity of some EOs. Schelz *et al.* revealed rather high plasmid curing activity of peppermint oil and its major component menthol in *in vitro* experiments on the metabolic plasmid of *E. coli* F'lac K12 LE140 [55]. A concentration of peppermint oil of 0.54 mg/ml caused 37.5% plasmid elimination, while a menthol concentration of 0.325 mg/ml has led to up to a 96% plasmid elimination.

### In vitro *activity of plant components against MDR bacteria*

Only a few studies have investigated the activity of isolated plant components against MDR bacteria. Stermitz *et al.* published very interesting findings about the activity of 5′-methoxyhydnocarpin, isolated from *Berberis fremontii,* as an inhibitor of the NorA efflux pump of *S. aureus* [56]. This strain of *S. aureus* expressed efflux pump, which conferred resistance to quinolones and antiseptics, including the plant product berberine. The authors described complete inhibition of efflux of berberine from *S. aureus*, which is an example of synergy between the molecular components of a medicinal plant.

In some studies, the activity of major components was similar to the activity of the original EO, such as in the study by Hayes and Markovic [57], in which the activity of lemon myrtle (*Backhousia citriodora*) oil against *S. aureus*, *E. coli*, *P. aeruginosa*, *Candida albicans*, MRSA, *Aspergillus niger*, *K. pneumoniae* and *Propionibacterium acnes* was comparable to its major component citral. In other studies, the activity of the components was more pronounced. For example, Loughlin *et al.* investigated the effect of

**Table 1. Studies demonstrating activity of essential oils from different plant families against multidrug-resistant bacteria (methicillin-resistant *Staphylococcus aures* and vancomycin-resistant enterococci).**

| Essential oil | Major components | Method for study of antibacterial activity | Test object including MDR bacteria | Country | Ref. |
|---|---|---|---|---|---|
| *Aristolochioideae* | | | | | |
| *Aristolochia mollissima* | 2,2,7,7-tetramethyltricyclo [6.2.1.0(1,6)]undec-4-en-3-one (15.9 and 13.5% from the rhizome and the aerial part of *A. mollissima*, respectively); (E)-β-santalol acetate (10.3%) and camphene (6.7%) in the rhizome oil; spathulenol (6.8%) in the oil from the aerial part | Disk diffusion, microdilution | 20 bacterial strains, including MRSA, MSSA, *Staphylococcus saprophyticus* | China | [82] |
| *Cupressaceae* | | | | | |
| *Juniperus communis* | Not determined | Microdilution | MRSA, vancomycin-resistant *Enterococcus faecium* | UK | [42] |
| *Geraniaceae* | | | | | |
| *Pelargonium graveolens* | Not determined | Vapour diffusion | MRSA, VRE, *Acinetobacter baumannii* and *Clostridium difficile* | UK | [48] |
| *Lamiaceae* | | | | | |
| *Cinnamomum osmophloeum* | Cinnamaldehyde | Microdilution | *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, MRSA, *Klebsiella pneumoniae*, *Salmonella* spp., *Vibrio parahemolyticus* | Taiwan | [83] |
| *Cinnamomum pubescens* Kochummen | 1,6-octadien-3-ol,3,7-dimethyl (11.55%), cinnamaldehyde (56.15%) and 1-phenyl-propane-2,2-diol diethanoate (11.38%) | Broth microdilution | MRSA, *Bacillus subtilis*, *P. aeruginosa*, *Salmonella choleraesuis* | Malaysia | [52] |
| *Dracocephalum foetidum* | *n*-mentha-1,8-dien-10-al (39.19%), limonene (17%), geranial (4.56%) and neral (3.20%) | Microdilution | *B. subtilis*, *S. aureus*, *Micrococcus luteus*, *Enterococcus hirae*, *Streptococcus mutans*, *E. coli*, MRSA | Republic of Korea | [85] |
| *Lavandula angustifolia* | Not determined | Microdilution | MRSA, vancomycin-resistant *E. faecium* | UK | [42] |
| *Lavandula stoechas* | alpha-fenchone (41.9%), 1,8-cineole (15.6%), camphor (12.1%) and viridiflorol (4.1%) in the leaves; and alpha-fenchone (39.2%), myrtenyl acetate (9.5%), α-pinene (6.1%), camphor (5.9%) and 1,8-cineole (3.8%) in flowers | Broth microdilution | MRSA | Turkey | [86] |
| *Mentha piperita, Mentha spicata, Mentha arvensis* | Not available | Microdilution | *Helicobacter pylori*, *Salmonella enteritidis*, *E. coli*, O157:H7, MRSA, MSSA | Japan | [87] |
| *Perilla frutescens* | Not determined | Broth microdilution | MRSA, MSSA | China | [88] |
| *Salvia rosifolia* | α-pinene (15.7–34.8%), 1,8-cineole (16.6–25.1%), β-pinene (6.7–13.5%), β-caryophyllene (1.4–5.0%) and caryophyllene oxide (1.4–4.4%) | Broth microdilution | MRSA, *E. coli*, *P. aeruginosa*, *Enterobacter aerogenes*, *Salmonella enterica*, serotype *Typhimurium*, *S. epidermidis* | Turkey | [89] |

MDR: Multidrug resistant; MRSA: Methicillin-resistant *Staphylococcus aures*; MSSA: Methicillin-susceptible *S. aureus*; VRE: Vancomycin-resistant enterococci.

**Table 1. Studies demonstrating activity of essential oils from different plant families against multidrug-resistant bacteria (methicillin-resistant *Staphylococcus aures* and vancomycin-resistant enterococci) (cont.).**

| Essential oil | Major components | Method for study of antibacterial activity | Test object including MDR bacteria | Country | Ref. |
|---|---|---|---|---|---|
| *Lamiaceae (cont.)* | | | | | |
| *Satureja cuneifolia* Ten. | Thymol (42.5–45.2%), *p*-cymene (19.4–24.3%) and carvacrol (8.5–13.2%) | Microdilution | MRSA | Turkey | [90] |
| *Satureja montana* | Carvacrol (45.7%), *p*-cymene (12.6%) and γ-terpinene (8.1%) | Microdilution | *E. coli*, MRSA | Croatia | [91] |
| *S. cuneifolia* | β-cubebene (8.7%), limonene (8.3%) and α-pinene (6.9%) | Microdilution | *E. coli*, MRSA | Croatia | [91] |
| *Thymus vulgaris* | Not determined | Microdilution | MRSA, vancomycin-resistant *E. faecium* | UK | [42] |
| *T. vulgaris* | Thymol (48.1%), rho-cymene (15.6%) and γ-terpinene (15.4%) | Disk diffusion, agar dilution | MRSA | Iran | [92] |
| *Mentha piperita* | Not determined | Microdilution | MRSA, vancomycin-resistant *E. faecium* | UK | [42] |
| *Zataria multiflora* | Thymol (38.7%), carvacrol (15.3%) and rho-cymene (10.2%) | Broth microdilution | MRSA, MSSA | Iran | [68] |
| *Myrtaceae* | | | | | |
| *Backhousia citriodora* | Citral (93–98%) | Microdilution | 13 bacterial strains including the clinical strain of MRSA, *P. aeruginosa* and *Clostridium perfringens* | Australia | [93] |
| *B. citriodora* | Citral | Microdilution | MRSA, *S. aureus*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *Propionibacterium acnes* | Australia | [57] |
| *Cleistocalyx operculatus* | γ-terpinene (5.76%), globulol (5.61%), *cis*-linalool oxide (5.21%), acorenol (5.12%) and camphene (4.12%) | Microdilution | MRSA, VRE | Republic of Korea | [84] |
| *Eucalyptus citriodora* Hook | Citronellal (90.07%) and citronellol (4.32%) | Broth microdilution | MRSA | Germany | [94] |
| *Eucalyptus globulus* | Aromadendrene, 1,8-cineole and globulol | Broth microdilution | MRSA, VRE | Germany | [59] |
| *E. globulus* | Aromadendrene (31.17%) in fruit oil and 1,8-cineole (86.51%) in leaf oil | Broth microdilution | MRSA | Germany | [94] |
| *E. globulus* | Eucalyptol (47.2%), (+) spathulenol (18.1%) and α-pinene (9.6%) | Disk diffusion, agar dilution | MRSA | Iran | [92] |
| *E. radiata* Sieber ex DC | 1,8-cineole (82.66%) | Broth microdilution | MRSA | Germany | [94] |
| *Melaleuca alternifolia* | Not determined | Microdilution | MRSA | UK | [44] |
| *M. alternifolia* | Not determined | Disk diffusion, microdilution | Clinical isolates of MRSA and mupirocin-resistant *S. aureus* | The Netherlands | [41] |
| *M. alternifolia* | Not determined | Microdilution | MRSA, vancomycin-resistant *E. faecium* | UK | [42] |
| *M. alternifolia* | Not determined | Microdilution | Clinical strains of MRSA and coagulase-netagive staphylococci, reference strains of *S. aureus* | UK | [43] |

MDR: Multidrug resistant; MRSA: Methicillin-resistant *Staphylococcus aures*; MSSA: Methicillin-susceptible *S. aureus*; VRE: Vancomycin-resistant enterococci.

**Table 1. Studies demonstrating activity of essential oils from different plant families against multidrug-resistant bacteria (methicillin-resistant *Staphylococcus aures* and vancomycin-resistant enterococci) (cont.).**

| Essential oil | Major components | Method for study of antibacterial activity | Test object including MDR bacteria | Country | Ref. |
|---|---|---|---|---|---|
| *Myrtaceae (cont.)* | | | | | |
| *M. alternifolia* | Not determined | Time–kill | MRSA, *P. aeruginosa* | UK | [95] |
| *M. alternifolia* | Not determined | Time–kill | Clinical strains including MRSA, glycopeptide-resistant enterococci, aminoglycoside-resistant klebsiellae, *P. aeruginosa* and *Stenotrophomonas maltophilia* | UK | [45] |
| *M. alternifolia* | Not determined | Microdilution | Clinical strains of MRSA and MSSA | Japan | [96] |
| *M. alternifolia* | Not determined | Agar dilution, microdilution | 64 clinical strains including MRSA, *S. aureus*, *E. faecalis*, β-hemolytic streptococci, coagulase-negative staphylocci, *P. aeruginosa, E. coli* | UK | [97] |
| *M. alternifolia* | Not determined | Microdilution | 13 bacterial strains including the clinical strain of MRSA, *P. aeruginosa* and *Clostridium perfringens* | Australia | [93] |
| *M. alternifolia* | Not determined | Microdilution, time–kill curves | MRSA, MSSA, coagulase-negative staphylococci | UK | [98] |
| *M. alternifolia* | Terpinen-4-ol | Microdilution, time–kill curves | MRSA | USA | [99] |
| *M. alternifolia*, terpinen-4-ol | Terpinen-4-ol | Microdilution, time–kill curves | Clinical strains of MRSA and coagulase-negative staphylococci | UK | [58] |
| *Pinaceae* | | | | | |
| *Abies koreana* | Borneol (27.9%), α-pinene (23.2%), β-pinene (5.8%), terpinene-4-ol (3.8%), bornyl acetate (3.4%) and α-terpineol (3.1%) | Disk diffusion, microdilution | MRSA | South Korea | [100] |
| *Poaceae* | | | | | |
| *Cymbopogon flexuosus* | Not determined | Vapour diffusion | MRSA, VRE, *A. baumannii* and *C. difficile* | UK | [48] |
| *Rutaceae* | | | | | |
| *Citrus bergamia, C. limon* and *C. sinensis* | Not determined | Microdilution, time–kill | Vancomycin-resistant and vancomycin-sensitive *E. faecalis* and *E. faecium* | UK | [101] |
| *Schisandraceae* | | | | | |
| *Kadsura longipedunculata* | δ-cadinene (21.79%), camphene (7.27%), borneol (6.05%), cubenol (5.12%) and δ-cadinol (5.11%) | Broth microdilution | MRSA, VRE, *Streptococcus pyogenes, S. agalactiae* | Germany | [50] |
| *Zingiberaceae* | | | | | |
| *Etlingera elatior* | β-pinene (24.92%) and 1-dodecene (24.31%) | Broth microdilution | MRSA, *B. subtilis, P. aeruginosa, S. enterica* serotype *Choleraesuis* | Malaysia | [52] |

MDR: Multidrug resistant; MRSA: Methicillin-resistant *Staphylococcus aures*; MSSA: Methicillin-susceptible *S. aureus*; VRE: Vancomycin-resistant enterococci.

terpinen-4-ol, the main component of tea tree oil, against MRSA and coagulase-negative staphylococci, and revealed that it had higher effect than tea tree oil itself [58].

Mulyaningsih *et al.* compared the antimicrobial properties of *Eucalyptus globulus* EO and three of its major components, aromadendrene, 1,8-cineole and globulol, against MRSA and VRE [59]. They revealed that aromadendrene was responsible for the activity of EO, while the effects from the other two components was much weaker. Moreover, the authors studied the effect of a combination of 1,8-cineole and aromadendrene. In

the checkerboard method, the effect in most cases was additive, whereas the Time–kill assay indicated synergy, which explains the high activity of EO.

### In vivo *activity of EOs against MDR bacteria*

In spite of the presence of a large number of publications regarding the *in vitro* effectiveness of EOs against MDR microorganisms, there is a significant lack of research into their *in vivo* efficacy, either on experimental animals or in clinical application.

The majority of studies were designed to assess the effect of only one EO, tea tree oil, against only one MDR bacterium, MRSA. While in some studies the beneficial effect from tea tree oil application was evident [60], in other studies the effect was unclear [61]. Sherry *et al.* reported treatment of chronic MRSA osteomyelitis with tea tree oil and eucalyptus-derived commercial formulations named polytoxinol [60]. Dryden *et al.* compared the effect of mupirocin 2% nasal ointment, chlorhexidine gluconate 4% soap, silver sulfadiazine 1% cream versus a tea tree oil regimen, which included 10% tea tree cream and 5% tea tree body wash, on clearance of MRSA carriage [61]. Mupirocin cleared 78% of nasal carriers while tea tree cream only cleared 47% ($p = 0.0001$); however, in clearing superficial skin sites and skin lesions, tea tree oil treatment was more effective than chlorhexidine or silver sulfadiazine.

Edmondson *et al.* published the results of an uncontrolled, open-label, pilot study of effectiveness of 3% tea tree oil solution used for the decolonization of MRSA-positive wounds [62]. Water-miscible tea tree oil solution was applied to 11 patients as a part of the wound cleansing regimen during each dressing change. The effect of tea tree oil application was not as pronounced as expected because none of the patients became MRSA negative. However, tea tree oil demonstrated good wound healing properties, which was established by the reductions in wound size after treatment.

Some studies evaluated the application of EOs for treatment of different infections regardless of antibiotic resistance of etiological agents, and these studies are also of interest because they demonstrate general clinical effectiveness of EOs in infectious diseases. Natural antimicrobial compounds cannot substitute antibiotics in severe systemic infections. However, they can be useful in the treatment of skin and soft tissue, wound, gynecological, respiratory and other infections where local application of an antimicrobial agent is possible.

Orafidiya *et al.* demonstrated the healing properties of *Ocimum gratissimum* EO during topical application onto the wound surface in experiments on rabbits [63]. Tumen *et al.* showed high wound healing and anti-inflammatory potential in EOs of *Juniperus oxycedrus* subsp. *oxycedrus* and *J. phoenicea* in experimental treatment of wounds in mice by topical application of an ointment containing 1% EO [64]. Süntar *et al.* demonstrated remarkable wound healing capacity in an ointment containing olive oil extract of flowering aerial parts of *Hypericum perforatum* L., olive oil, an equivalent mixture of *Origanum majorana* L., *O. minutiflorum* Schwrd. *et Davis* EOs (*O. aetheroleum*) and *Salvia triloba* L. EOs in experimental treatment of wound in rats and mice [65]. A randomized double-blind clinical trial in 60 patients with mild-to-moderate acne vulgaris demonstrated efficacy of topical 5% tea tree oil gel [66]. EOs may reduce the use of traditional antibiotics in such cases and prevent resistance to them. Furthermore, they can be useful in the increase of activity of traditional antibiotics by using combined therapy, as discussed below.

## Combinations of EOs with antibiotics & other antimicrobial agents as a way of coping with bacterial resistance

### *Combinations of EOs with antibiotics*

Potential in coping with antibiotic resistance becomes even more evident during combining EOs or their components with antibiotics. In general, combined use of antimicrobial agents opens a perspective in increasing antimicrobial activity and reducing toxicity of both agents towards human cells [67]. In spite of the presence of a large amount of work dedicated to either the antibacterial activity of EOs, antibiotics or antibiotic–antibiotic combinations, there is a lack of studies on the antibacterial effect of combinations between EOs and antibiotics. Below we characterize some important studies that revealed promising interactions between EOs and antibiotics (Table 2).

Van Vuuren *et al.* studied activity of four EOs (*M. alternifolia*, *Thymus vulgaris*, *Mentha piperita* and *Rosmarinus officinalis*) in combination with ciprofloxacin against *S. aureus* and *K. pneumoniae* in nine different ratios [21]. The study demonstrated the presence of concentration-dependent interactions between EOs and antibiotics: the same EO showed effect from synergistic to antagonistic when combined with ciprofloxacin depending on the ratios of both agents.

Si *et al.* examined combinations between *O. vulgare* EO and different antibiotics against an MDR strain of extended-spectrum β-lactamase-producing *E. coli* [22] . Most combinations were either synergistic or additive with no antagonistic effect.

Mahboubi and Bidgoli studied activity of *Zataria multiflora* EO against clinical isolates of MRSA and methicillin-susceptible *S. aureus* (MSSA) and its interactions with vancomycin [68]. *Z. multiflora* not only demonstrated high activity against both MSSA and MRSA with ranges of minimal inhibitory concentrations and minimal bactericidal concentrations of 0.25–1.00 and 0.5–2.00 µl/ml, respectively, but also enhanced activity of vancomycin. Vancomycin has been commonly used for the treatment of MRSA infections for 30 years; however, since appearance of vancomycin-intermediate *S. aureus* and vancomycin-resistant *S. aureus* there is a need to search for vancomycin alternatives [69]. Combination of EOs with vancomycin may be one of the ways in prolonging its usage against MRSA.

In the study by Rosato *et al.* the effect of combinations between gentamicin and four EOs (*Aniba rosaeodora*, *M. alternifolia*, *O. vulgare* and *Pelargonium graveolens*) was evaluated by the checkerboard method against several reference bacterial strains [70]. A synergistic effect against all tested strains was observed in gentamicin/*A. rosaeodora* and gentamicin/*P. graveolens* combination, while in the other two combinations the effect was from synergistic to indifferent. The highest synergy was observed in combination of gentamicin and *P. graveolens* against *A. baumannii*,

**Table 2. Studies on combinations between essential oils from different plant families and antibiotics.**

| EO | Major components | Antibiotics | Method | Bacterial model | Effect | Ref. |
|---|---|---|---|---|---|---|
| *Asteraceae* | | | | | | |
| *Helichrysum italicum*, geraniol | Neryl acetate, neryl propionate, 4,6,9-trimethyldec-8-en-3,5-dione, 2,4,6,9-tetramethyldec-8-en-3,5-dione, nerol, linalool, guaiol, α-terpineol | Ampicillin, penicillin, norfloxacin | Microdilution | *Enterobacter aerogenes* | Enhancement of activity of all antibiotics | [20] |
| *Geraniaceae* | | | | | | |
| *Pelargonium graveolens* | Citronellol (47.3%), geraniol (9.1%), linalool (8.81%), epizonalene (6%) and caryophyllene (1.13%) | Gentamicin | Checkerboard | Reference strains of *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Serratia marcescens*, *Escherichia coli*, *Yersinia enterocolitica*, *Salmonella typhi*, *Acinetobacter baumannii*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* | Synergistic effect against all tested strains | [70] |
| *Lamiaceae* | | | | | | |
| *Mentha piperita* | Not determined | Ciprofloxacin | Microdilution (checkerboard): combinations of nine ratios of EOs and ciprofloxacin were tested | Reference strains of *S. aureus*, *K. pneumoniae* | Against *S. aureus*: synergy at five ratios and antagonism at the others<br>Against *K. pneumoniae*: synergy at four ratios and antagonism at the others | [21] |
| *Origanum vulgare* | Not determined | 11 antibiotics of different groups | Checkerboard | Extended-spectrum β-lactamase-producing *E. coli* | Sarafloxacin, levofloxacin, maquindox, florfenicol, doxycycline: synergy<br>Polymycin, lincomycin, amoxicillin, ceftiofur, ceftriaxone: additive effect<br>Kanamycin: indifference | [22] |
| *O. vulgare* | Cymenol (58.6%), cymene (25%), α-pinene (5.1%), thymol (3.7%) and eucalyptol (2.8%) | Gentamicin | Checkerboard | Reference strains of *B. cereus*, *B. subtilis*, *S. aureus*, *E. faecalis*, *S. marcescens*, *E. coli*, *Y. enterocolitica*, *S. typhi*, *A. baumannii*, *K. pneumonia*, *K. aeruginosa* | Effect from synergy (against *B. cereus*, *B. subtilis* and *S. aureus*) to indifference | [70] |
| *Rosmarinus officinalis* | Not determined | Ciprofloxacin | Microdilution (checkerboard): combinations of nine ratios of EOs and ciprofloxacin were tested | Reference strains of *S. aureus*, *K. pneumoniae* | Against *S. aureus*: antagonism at all ratios<br>Against *K. pneumoniae*: synergy at seven ratios, antagonism at the others | [21] |
| *Thymus maroccanus*, *Thymus broussonetii* | Not determined | Chloramphenicol | Microdilution | *E. coli*, *E. aerogenes*, *K. pneumoniae*, *Salmonella enterica* serotype *Typhimurium*, *P. aeruginosa* | Enhancement of chloramphenicol activity against resistant isolates | [53] |

EO: Essential oil; MRSA: Methicillin-resistant *Staphylococcus aures*; MSSA: Methicillin-susceptible *S. aureus*.

**Table 2. Studies on combinations between essential oils from different plant families and antibiotics (cont.).**

| EO | Major components | Antibiotics | Method | Bacterial model | Effect | Ref. |
|---|---|---|---|---|---|---|
| *Lamiaceae (cont.)* | | | | | | |
| *Thymus vulgaris* | Not determined | Ciprofloxacin | Microdilution (checkerboard): combinations of nine ratios of EOs and ciprofloxacin were tested | *S. aureus* ATTC 25923, *K. pneumoniae* NCTC 9633 | Against *S. aureus*: synergy at two ratios, antagonism at the others<br>Against *K. pneumoniae*: synergy at four ratios, antagonism at the others | [21] |
| *Zataria multiflora* | Thymol (38.7%), carvacrol (15.3%) and rho-cymene (10.2%) | Vancomycin | Microdilution | Clinical isolates of MRSA, MSSA | Synergy with vancomycin | [68] |
| *Lauraceae* | | | | | | |
| *Aniba rosaeodora* | Linalool (60.1%), limonene (19.2%), geraniol (7.8%), cymene (4.1%) and α-pinene (3.2%) | Gentamicin | Checkerboard | Reference strains of *B. cereus*, *B. subtilis*, *S. aureus*, *E. faecalis*, *S. marcescens*, *E. coli*, *Y. enterocolitica*, *S. typhi*, *A. baumannii*, *K. pneumonia*, *P. aeruginosa* | Synergy against all tested strains | [70] |
| *Myrtaceae* | | | | | | |
| *Melaleuca alternifolia* | Not determined | Ciprofloxacin | Microdilution (checkerboard): combinations of nine ratios of EOs and ciprofloxacin were tested | Reference strains of *S. aureus*, *K. pneumoniae* | Against *S. aureus*: antagonism at all ratios<br>Against *K. pneumoniae*: synergy at three ratios, antagonism at the others | [21] |
| *M. alternifolia* | Terpinen-4-ol (30.3%), γ-terpinene (16.3%), caryophyllene (15%), 2-carene (8.3%), eucalyptol (4.5%) and α-terpineol (4.4%) | Gentamicin | Checkerboard | Reference strains of *Bacillus cereus, B. subtilis, S. aureus, E. faecalis, S. marcescens, E. coli, Yersinia enterocolitica, Salmonella typhi, Acinetobacter baumannii, K. pneumonia, P. aeruginosa* | Effect from slight synergy (against *S. aureus*, *E. coli*, *S. marcescens* and *Y. enterocolitica*) to indifference | [70] |

EO: Essential oil; MRSA: Methicillin-resistant *Staphylococcus aures*; MSSA: Methicillin-susceptible *S. aureus*.

where the fractional inhibitory concentration index was 0.11. EOs of both *A. rosaeodora* and *P. graveolens* were characterized by high content of terpenalcohols – 73 and 63%, respectively. The authors hypothesized that just these compounds are responsible for the synergistic interactions with gentamicin, whose mechanism of action is interruption of protein synthesis by binding the 30S subunit of the bacteria ribosome. Likewise, terpenalcohols may facilitate internalization of gentamicin by interaction with bacterial cellular membranes.

The purpose of the study by Fadli *et al.* was to find efflux pump inhibitors among EOs from Moroccan plants that may increase the activity of antibiotics against resistant isolates [53]. The EOs evaluated by them demonstrated activity against strains of *E. coli, E. aerogenes, K. pneumoniae, S. enterica* serotype *Typhimurium* and *P. aeruginosa,* which increased in the presence of efflux pump inhibitor PAβN. Furthermore, the results indicated that EOs of *Thymus maroccanus* and *T. broussonetii* at sub-inhibitory concentrations enhanced susceptibility of resistant strains to chloramphenicol. These findings support the idea regarding the presence of efflux pump inhibitor activity in these EOs (Figure 2) [53].

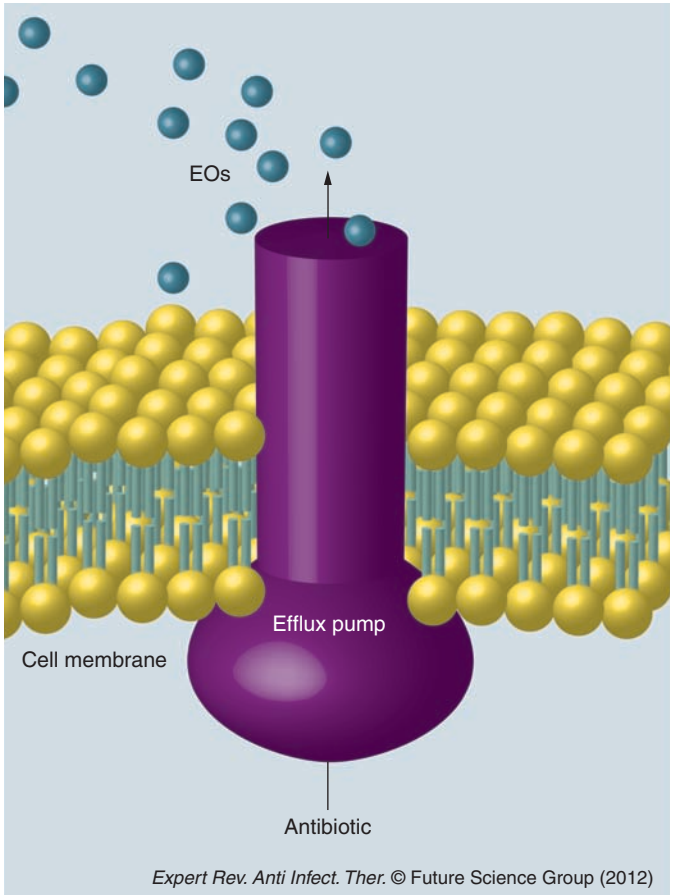


**Figure 2. Efflux pump inhibitor activity of essential oils.**
EOs inhibit efflux pumps that remove an antibiotic from the cell making it inactive; inhibition of efflux pumps restores the activity of an antibiotic.
EO: Essential oil.
Data taken from [20,53].

### *Combinations of plant constituents & antibiotics*

There are only a few studies that have examined the activity of isolated phytoconstituents in combination with antibiotics, but the results of these studies are crucial in understanding the mechanism of beneficial interactions. Lorenzi *et al.*, using a microdilution method, not only demonstrated a significant reduction of multidrug-resistance in *E. aerogenes*, *E. coli*, *P. aeruginosa* and *A. baumannii* after action of *Helichrysum italicum* EO, but also found that one of the components of this EO, geraniol, significantly increased the efficacy of β-lactams (ampicillin and penicillin), quinolones (norfloxacin) and chloramphenicol [20]. Geraniol was revealed to be a potent efflux pump inhibitor. The results demonstrated that geraniol had more potent antibacterial action compared with PAβN in an *acrAB* mutant strain of *E. aerogenes*, suggesting different targets of action in these compounds, which should be further evaluated.

Another study on efflux pump inhibitor activity of plant components was conducted by Smith *et al.*, who isolated an efflux inhibitor ferruginol from the cones of *Chamaecyparis lawsoniana* [71]. Ferruginol inhibited activity of three resistance pumps in *S. aureus* – quinolone (NorA), tetracycline (TetK) and erythromycin (MsrA). The authors observed an 80-fold increase in the activity of oxacillin in the presence of ferruginol, which restored sensitivity in the resistant strain. Efflux pump inhibitor activity was demonstrated in the study of ethidium bromide efflux at 10 µM ferruginol (2.86 µg/ml) in the *S. aureus* strain possessing the NorA efflux pump. This experiment resulted in 40% inhibition of ethidium bromide efflux.

Summarizing the above mentioned studies, it is worth emphasizing that although many EOs and their components have a high level of antibacterial activity [20,22,23,53,68], precautions should be taken when combining them with antibiotics because of the presence of a rather large number of antagonistic interactions [21] that should not be ignored [72]. These findings demonstrate the necessity for systematic studies of interactions between EOs and antibiotics to reveal undesirable combinations. Combinations of EOs with antibiotics should only be chosen for treatment when synergistic or, at least, indifferent interactions are documented *in vitro*.

### *Incorporating EOs into polymeric nanoparticles*

An increase in the activity of EOs can be achieved by transporting them directly to the site of infection. For this purpose it was proposed that EOs should be incorporated into polymeric nanoparticles. This approach not only directly transports EOs to microbial cells, but also the small size of the particles increases the contact area-to-volume ratio of the antimicrobial agent and thus enhances its activity many times over. Interactions of EO components with polymeric nanoparticles were studied by several researchers [73–75].

Chen *et al.* prepared chitosan nanoparticles grafted with either of two components of EOs, eugenol (phenylpropene, which is the component of clove, cinnamon, basil and other EOs) and carvacrol (monoterpenoid phenol, the component of oregano, thyme and other oils) [73]. Antibacterial activity of nanoparticles was

studied against *E. coli* and *S. aureus*. The authors demonstrated that the grafted eugenol and carvacrol conferred antioxidant activity to the chitosan nanoparticles. In addition, the antibacterial activity of these grafted nanoparticles was better or equal to the activity of unmodified chitosan nanoparticles. Furthermore, cytotoxicity of eugenol-grafted chitosan nanoparticles and carvacrol-grafted chitosan nanoparticles in the 3T3 mouse fibroblast model was significantly lower than cytotoxicity of pure EOs [73].

Chitosan nanoparticles grafted with thymol of five different sizes (189, 167, 134, 35 and 21 nm) were synthesized by Hu *et al.* [74]. The authors revealed that thymol-loaded water-soluble chitosan nanoparticles had stronger antibacterial activity than thymol itself. In addition, with the decrease in size, these nanoparticles showed a stronger antimicrobial effect on Gram-positive bacteria and fungi. The minimal inhibitory concentrations of nanoparticles were as low as 0.00313–0.00157% (w/v) against *S. aureus* and *Bacillus subtilis*.

Iannitelli *et al.* encapsulated carvacrol into PLGA nanoparticles and studied their ability to alter the properties of preformed bacterial staphylococcal biofilms [75]. The hydrodynamic diameter of carvacrol-loaded polymeric nanoparticles was approximately 210 nm, which allowed their diffusion through mucus layers of surfaces of anatomical sites. Alteration of bacterial biofilms was studied by using rheological tests that revealed considerable reduction in the elasticity and mechanical stability of biofilms. These changes might facilitate penetration of antimicrobial agents into the deep layers of bacterial biofilms.

The studies described above demonstrate that preparation of polymer nanoparticles grafted with EO components is beneficial in order to achieve an increase in antibacterial and antioxidant activity, reduction of toxic effects, and enhancement of bacterial biofilm penetration for other antimicrobial agents. Another application of EOs can be their incorporation into polymeric nanoparticles and combinations of EOs with metallic nanoparticles; however, despite great interest shown by researchers towards nanoparticles as an alternative approach to antibiotic use, EO metallic nanoparticle combinations have not yet been studied.

### *Combinations of EOs & bacteriophages*

Bacteriophages also represent a natural source of antimicrobial agents as an alternative approach to antibiotic treatment. Many studies have demonstrated great potential of bacteriophages in combating bacterial infections such as MDR bacteria [76–78].

Significant beneficial effects were demonstrated in the interactions between bacteriophages and antibiotics [16], and between bacteriophages and metallic nanoparticles [79], however, there is a lack of studies on interactions between bacteriophages and EOs. One of the applications of bacteriophage–EO combinations is to reduce bacterial contamination of food products. Viazis *et al.* studied survival of entero-hemorrhagic *E. coli* O157:H7 in a food model of whole baby romaine lettuce and baby spinach leaves after exposure to bacteriophage cocktail, BEC8, alone or in combination with the EO component *trans*-cinnamaldehyde (TC), depending on bacterial concentrations and temperature of incubation [80]. Results of the work showed that when a low bacterial concentration ($10^4$ colony-forming units/ml) was exposed to BEC8 or TC individually at 23 or 37°C there were no survivors after 24 h. However, an increase of bacterial concentration to $10^5$ or $10^6$ colony-forming units/ml and/or a decrease of incubation temperature led to a decrease in both phages and EO activities. By contrast, when BEC8 and TC were applied together, there were no survivors after 10 min at all temperature regiments and bacterial concentrations.

These findings indicate high mutually enhancing antibacterial effects of bacteriophages and EOs [80], which are worth further investigation with different bacterial strains and EOs. The mechanism of the EO–bacteriophage combining effects on bacteria have not been investigated well. The most obvious is inhibition of bacterial growth by action on different targets in bacterial cells. Bacteriophages can destroy bacterial cells by two mechanisms: after replication ('lysis from within'); or by adherence of a sufficiently high number of phage particles to the cell and its lysis through alteration of the membrane electric potential, and/or the activity of cell wall degrading enzymes ('lysis from without') [81]. EOs may contribute to both processes: by alteration of bacterial cell membranes and facilitating bacteriophage entry into the cell; and by simultaneous action on the cell membranes together with bacteriophages (Figure 3).

## Expert commentary & five-year view

The wide spread of MDR bacteria causing different infectious processes, observed worldwide, leads to an urgent need for the re-assessment of methods used for coping with drug-resistance. Multicomponent chemical compositions and alternative mechanisms of action renewed interest in the antimicrobial properties of natural antimicrobial compounds, such as plant EOs.

Complexity of EOs is their advantage and disadvantage simultaneously. Multicomponent composition gives benefit in overcoming bacterial resistance; however, at the same time, this creates problems with standardization of EOs as medical preparations and slows their scientific and practical implementation. For now, EOs are mainly used in folk medicine and are not included in standard treatment schemes of any infections. Identification of EO components and creation of medical preparations based on this will make it possible to broaden practical use of plant products.

Many EOs have demonstrated high levels of *in vitro* and *in vivo* activity against different types of bacteria regardless of the presence of resistance to antibiotics, including documented activity against MRSA [42,48,52,82,83], VRE [48,59,84] and other MDR bacteria. The mechanisms of action of EOs on MDR bacteria has not been well studied and are hypothesized to be the same as for usual antibiotic-sensitive strains: disruption of cell membrane; inhibition of proton motive force with leakage of ions and metabolites; and inhibition of synthesis of some enzymes. Bacterial resistance to traditional antibiotics is caused by enzyme modification, changes in binding sites or targets of action, efflux of the antibiotic from the bacterium or changes in the metabolic activity of the bacterial cell. In turn, activity of EOs against MDR

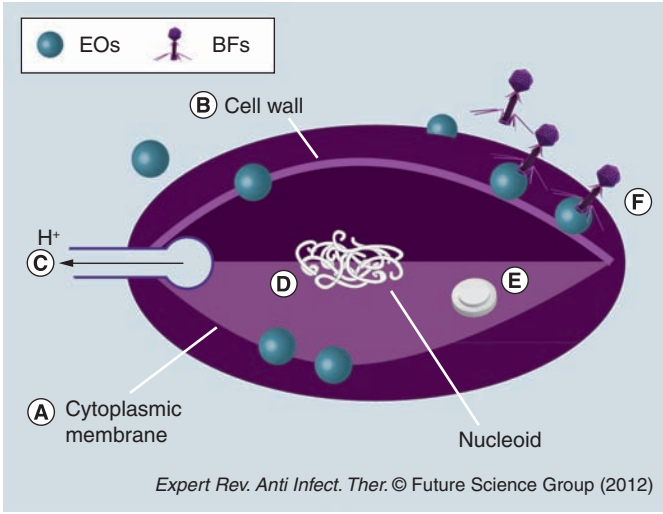


**Figure 3. Possible mechanisms of antibacterial action of essential oils alone and in combination with bacteriophages.** Mechansims of action may include **(A)** disintegration of cytoplasmic membrane and interaction with membrane proteins (ATPases and others), **(B)** disturbance of the outer membrane of Gram-negative bacteria with the release of lipopolysaccharides, **(C)** destabilization of the proton motive force with leakage of ions, **(D)** coagulation of the cell content, **(E)** inhibition of enzyme synthesis, and **(F)** damage to bacterial cell membrane caused by EOs, which either facilitates penetration of bacteriophages with subsequent replication inside the bacterial cell and its lysis, or is supported by simultaneous action of bacteriophages and EOs on the cell membranes.
BF: Bacteriophage; EO: Essential oil.
Data taken from [81,102]

bacteria can be explained by simultaneous action on different targets; and this action does not depend on target modifications leading to antibiotic ineffectiveness. Some EOs demonstrated antiplasmid activity, which has great potential in coping with bacterial resistance and merits further detailed investigation.

Furthermore, EOs not only demonstrated activity against MDR bacteria themselves, but also showed potential for the development of synergistic combinations that increases the antibacterial effect of antibiotics. EOs may alter the antibiotic efflux pumps and this helps to restore the activity of antibiotics that are losing their clinical application, or to prolong the use of presently effective antibiotics. However, it is worth mentioning that despite high activity of EOs and antibiotics, some EO–antibiotic combinations showed antagonistic effects, the mechanisms of which are not understood and need further study.

Bacteriophages are thought to be another alternative to antibiotic treatment, and some combinations between bacteriophages and EOs have also shown promising activity against bacteria [80]. However, such studies devoted to antibacterial potential of bacteriophage-EO combinations are very scarce; the mechanisms of these interactions are poorly understood. Understanding the potential of interactions between bacteriophages and EOs in combating bacterial resistance, their dose- and time-dependent effects along with a deeper understanding of the mechanism of combined action can be planned for the next 5 years.

Further research should also be performed during the next 5 years in order to better understand the mechanisms of action of EOs against MDR bacteria, especially those that have recently emerged, such as vancomycin-intermediate *S. aureus* and vancomycin-resistant *S. aureus*, through better investigation of antiplasmid activity of EOs and the mechanisms of EO interactions with antibiotics, and the resultant production of clinically applicable forms of such combined drugs.

In summary, the antibacterial application of EOs is not a new topic, but ineffectiveness of traditional antibiotics puts a new focus on this area, and the presence of many advantages in EOs, both in the mechanisms of action and complex healing properties, makes them promising agents in coping with MDR bacteria either alone or as a support for traditional antibiotic treatment.

**Financial & competing interests disclosure**

*The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.*

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## Key issues

- The high prevalence of multidrug-resistant (MDR) bacteria has increased interest in alternative methods of antibacterial treatment; among them, the use of essential oils (EOs) and their constituents with antimicrobial properties elicits wide interest.
- EOs have a multicomponent chemical composition and alternative mechanisms of action, including the ability to affect many bacterial structures simultaneously, which enables their activity both against antibiotic-sensitive and antibiotic-resistant isolates. Antiplasmid activity of EOs has great potential in combating MDR bacteria and in restricting their distribution.
- Different combinations of EOs and traditional antibiotics demonstrated an enhancing effect, which is important in restoring activity of inactive antibiotics and prolonging the activity of presently highly efficient drugs. One of the explanations for the beneficial interactions between antibiotics and EOs is the efflux pump inhibitor activity of EOs.
- Studies have shown that components of EOs can be successfully incorporated into polymeric nanoparticles; this not only increases their activity but also reduces possible toxic effects of EOs and allows the possibility for direct delivery of EOs into the site of infection.
- Combinations of bacteriophages and EOs have demonstrated promising beneficial effects and represent another alternative to antibiotic treatment that merits further investigation.
- Future studies should also be directed towards better understanding the mechanism of action of EOs against MDR bacteria and to the development of pharmaceutical formulations appropriate for the treatment of local and systemic infections.

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