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REVIEW ARTICLE

Synergy between essential oil components and antibiotics: a review

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Abstract

With the increase in antibiotic-resistant bacteria and the lack of new antibiotics being brought onto the market, alternative strategies need to be found to cope with infections resulting from drug-resistant bacteria. A possible solution may be to combine existing antibiotics with phytochemicals to enhance the efficacy of antibiotics. A group of phytochemicals that is said to have such effects, according to *in vitro* studies, is essential oils (EOs) and their components. Amongst others, EOs containing carvacrol, cinnamaldehyde, cinnamic acid, eugenol and thymol can have a synergistic effect in combination with antibiotics. Several modes of action have been put forward by which antibiotics and the essential oil components may act synergistically, such as by affecting multiple targets; by physicochemical interactions and inhibiting antibacterial-resistance mechanisms. Many reported assays show additivity or moderate synergism, indicating that EOs may offer possibilities for reducing antibiotic use.

Keywords

antibiotic, essential oil, FIC, synergy

History

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Introduction

Since the discovery of penicillin in 1928 the use of antibiotics, originally developed for human health care, has spread to animal therapeutics, agriculture and to industrial applications (Fleming, 1929; Goldman, 2004; Sengelov et al., 2003; World Health Organization, 2012). With the continual emergence of new bacterial strains resistant to antibiotics, the efficacy of antibiotics has dropped and antibiotic resistance has become a global public health issue; when infections become resistant to first-line medicines more expensive therapies must be used (ECDC/EMEA, 2009; World Health Organization, 2012). Antibiotic resistance causes an estimated 400 000 infections and 25 000 deaths annually in the European Union and an estimated EUR 1.5 billion in extra health care costs (Bush et al., 2011; ECDC/EMEA, 2009). In some regions of the world infections occur which are resistant to all known antibiotics (Udwadia et al., 2011).

Research into novel antibiotics has decreased to such an extent that nowadays hardly any novel antibiotics are developed to market (Lewis, 2012). This is mainly due to a change that occurred in the 1970s, where the focus shifted from research and development of new antibiotics to modifying existing classes, mainly for reasons of financial efficiency (ECDC/EMEA, 2009; Nordberg et al., 2005). Older, previously discarded drugs are once again being used even though they have significant side effects (Boucher et al., 2009). In 2011, academics and industry collaborated on a priority list for approaches to resolve the antimicrobial-resistance crisis. Amongst the potential strategies proposed are the development of alternatives to antibiotics and the discovery or development of adjuvants (Bush et al., 2011). One possibility is to combine antibiotics with other non-antibiotic drugs (Ejim et al., 2011), for example some antipsychotic and anti-inflammatory drugs improve the antibiotic efficacy *in vitro* (Lehtinen, 2007; Mazumdar, 2009). Another possibility is to combine antibiotics with adjuvants or antimicrobials selected from the reservoir of bioactive compounds in nature (Bush et al., 2011). Phytochemicals may represent promising adjuvants for antibiotics and an overview of synergism between plant metabolites and antibiotics has been provided by Hemaiswarya et al. (2008). A group of phytochemicals which featured only briefly in that review are the plant essential oils (EOs), which are oily aromatic liquids extracted from plants (Guenther, 1948). An overview of the influence of essential oils on multidrug-resistant bacteria has been produced by Kon & Rai (2012).

This review aims to provide an overview of studies investigating the use of EOs to enhance the efficacy of antibiotics. Current knowledge on the modes of action of the EO constituents and synergy with antibiotics are presented and possible mechanisms by which they interact are discussed. This paper is limited to EOs that occur naturally in foods, are found in over-the-counter antiseptics or body care products or that have been used as medicines by indigenous

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peoples historically because it is assumed that these will probably have the fewest practical and legal obstacles to their use.

Essential oils

Essential oils with antibacterial properties

Essential oils are oily aromatic liquids extracted from plant material by expression, fermentation, enfleurage, extraction or distillation (Van de Braak & Leijten, 1999). The extraction method most often used is distillation (Guenther, 1948). Their function in plants is likely antibacterial, antifungal, insecticidal and antiviral defense, and their strong flavor makes plants less palatable for herbivores (Guenther, 1948). The biological effects of EOs have been reviewed (Bakkali et al., 2008). Although there are over 3000 EOs known, approximately 300 are currently in use, chiefly in fragrances (Van de Braak & Leijten, 1999). EOs have been distilled for more than 2000 years, but their widespread use dates back to the 16th century. Over the past 200 years their medicinal purposes have become secondary to their commercial uses, such as flavoring in foods and as fragrances in cosmetics and perfumes (Guenther, 1948). There is renewed interest in the antimicrobial properties of phytochemicals (reviewed by Gibbons (2008)) and EOs in particular, which has led to the production of a limited number of food preservatives in which they play a significant role (Burt, 2004). The potential for use of EOs to complement or replace antibiotics in animal feeds has been reviewed by Franz et al. (2010).

Whole EOs tend to vary in their exact composition due to factors such as seasonal variation, climate, subspecies and even the oil-extraction method (Santoyo et al., 2006). This has consequences for their application and their antibacterial activity. The complexity also hampers a thorough understanding of the exact mechanism of antibacterial action of the EOs, since all interactions between separate components should be taken into account (Bassole & Juliani, 2012; Van Vuuren & Viljoen, 2011). Two of the most studied EOs are oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) oil. They both are primarily made up of carvacrol, thymol, γ-terpinene and ρ-cymene (Burt, 2004; Burt et al., 2005). The most important components of cinnamon (*Cinnamomum zeylanicum*) oil, another well-studied EO, are cinnamic acid and *trans*-cinnamaldehyde (Woehrlin et al., 2010) and the most important antimicrobial component of clove (*Syzygium aromaticum* or *Eugenia aromaticum*) oil is eugenol (Moon et al., 2011). Tea tree oils have good antibacterial properties too and have long been used as antiseptics by indigenous Australasian peoples; depending on the plant species the constituents vary greatly (Christoph et al., 2000; Harkenthal et al., 1999). The main components of Australian tea tree (*Melaleuca alternifolia*) oil are terpinen-4-ol, γ- and α-terpinene, and 1,8-cineole (Carson et al., 2006; Christoph et al., 2000). New Zealand white tea tree (*Kunzea ericoides*) (kanuka) oil has α-pinene as its most important constituent (Christoph et al., 2000). The main components of New Zealand red tea tree (*Leptospermum scoparium*) (manuka) oil are calamenene and leptospermone (Christoph et al., 2000). The structures of the EO components mentioned are presented in Figure 1.

Antibacterial mechanisms of EO components

There is a large amount of data available on the mode of action of EOs and their components. An extended description is beyond the scope of this review but the reader is referred to an excellent review by Hyldgaard et al. (2012). Interactions between individual EO components have been reviewed by Bassolé & Juliani (2012). The most frequently reported mechanisms are summarized in Table 1. Disruption of bacterial membranes contributes to the antibacterial properties of most EOs. Damage to membrane proteins (e.g. enzymes), cell content leakage, depletion of the motive proton force and coagulation of the cytoplasm are also

Figure 1. Chemical structure of selected EO components.

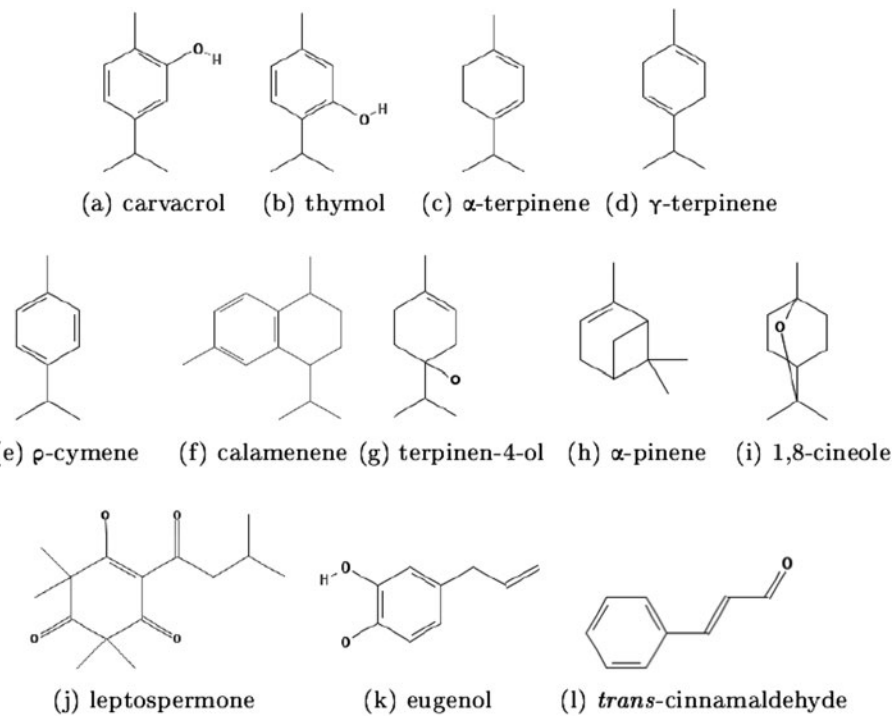

Table 1. Mechanisms of antibacterial action of EOs and their components.

EO or component	Mode of action	References
Oregano	Reduction in lipase and coagulase activity, enzyme inhibition	Carneiro de Barros et al., 2009
Carvacrol	Membrane disruption, inhibition of ATPase activity, membrane destabilization, leakage of cell ions, fluidization of membrane lipids, reduction of proton motive force	Di Pasqua et al., 2007; Gill & Holley, 2006a,b; Ultee et al., 2002
Thymol	Membrane disruption with potential intracellular targets, citrate metabolic pathway disruption	Di Pasqua et al., 2007, 2010; Trombetta et al., 2005
ρ-Cymene	Membrane disruption	Ultee et al., 2002
Cinnamaldehyde	Membrane disruption by inhibiting ATPase activity	Gill & Holley, 2004, Gill & Holley, 2006a,b.
Cinnamic acid	Membrane disruption	Hemaiswarya & Doble, 2010; Chen *et al.*, 2011
Eugenol	Membrane disruption by inhibiting ATPase activity, possible efflux pump blocker, reduction of several virulence factors at sub inhibitory concentrations	Bolla et al., 2011; Di Pasqua et al., 2007; Gill & Holley, 2006a,b; Hemaiswarya & Doble, 2009; Qiu et al., 2010
Melaleuca tea tree	Inhibition of membrane-located metabolic events leading to inhibition of respiration and increased membrane permeability	Cox et al., 2001
γ-terpinene	Membrane disruption	Oyedemi et al., 2009

common effects (Gill & Holley 2006b; Helander et al., 1998; Ultee et al., 2002). Although the site of action of individual EO components has been established in many cases, the mechanism itself is often still not completely understood; filling these gaps in scientific knowledge will assist in finding synergistic combinations and avoiding antagonistic ones (Hyldgaard et al., 2012).

A proposed specific target for EO components also exists, namely the inhibition of efflux pumps that are responsible for antibiotic resistance (Kollanoor Johny et al., 2010; Shahverdi et al., 2007). Although efflux pump inhibitors are often thought to be large, alkaloidal and lipophilic molecules, it is also proposed that efflux pumps can be inhibited through membrane disruption and inhibition of metabolic pathways (Gibbons, 2008). Inhibiting the production or activity of enzymes is also a target for EOs. Several examples have been described: oregano oil reduces lipase and coagulase activity of *Staphylococcus aureus* (Carneiro de Barros et al., 2009); eugenol reduced the production or activity of staphylococcal enterotoxin A and B, toxic shock syndrome toxin 1 and α-haemolysin, in *S. aureus* (Qiu et al., 2010); and the carbonyl group on cinnamaldehyde may bind to proteins to inhibit the function of bacterial amino acid decarboxylases (Wendakoon & Sakaguchi, 1993, 1995).

The spectrum of bacterial targets affected by EOs reflects the potential of these compounds but also requires caution. It is very likely that these effects are actually a result of the initial mode of bacterial membrane destabilization activity of EOs. In addition, many of the reports describe the effects in a very limited number of bacterial strains. In order to truly apply these compounds as either therapeutic or as a food preservative, especially when used in combination with other compounds based on synergistic action, they should be tested against a larger number of strains to determine the usefulness of these compounds.

Bacterial resistance to EO components

An important question regarding the use of EOs as new therapeutics is whether bacterial resistance, comparable to induced resistance toward antibiotics, could arise when these compounds are used on a large scale. Some bacteria have intrinsic tolerance to EO components; *Pseudomonas* spp. was shown to tolerate tea tree oil and its main components through the expression of the MexAB-oprM efflux pump (Papadopoulos et al., 2008). However, to what extent bacteria may acquire resistance to EO components has not yet been broadly investigated. A study with tea tree oil showed the level of resistance gained by Gram-positive strains to be very low compared to that of the antibiotic rifampicin; single-step mutants resistant to tea tree oil were not detected at 2× minimum inhibitory concentration (MIC) or 3× MIC for seven *S. aureus* isolates, including a methicillin-resistant *S. aureus* (MRSA) (Greay & Hammer, 2011; Hammer et al., 2008). For a *S. epidermidis* strain no mutants were detected at 2× MIC and an *Enterococcus faecalis* strain showed no mutants at 1× MIC (Hammer et al., 2008). In contrast, resistance frequencies for rifampicin at 8× MIC were at least 10^{-7}–10^{-8} for all isolates (Hammer et al., 2012), indicating the relative incapability of these bacteria to become resistant to tea tree oil.

A study involving passaging bacteria up to 50 times with oregano oil led to a four-fold increase in the MIC (from 200 to 800 mg/L) for *Morganella morganii* and *Proteus mirabilis*, whilst the MICs for *Serratia marcescens* and *Pseudomonas aeruginosa* were unchanged (Becerril et al., 2012). In contrast, passaging the same strains 50 times in cinnamon oil produced no increase in the MICs (Becerril et al., 2012). Exposing *Salmonella typhimurium* to repeated passaging in sub-lethal concentrations of *O. vulgare* oil or carvacrol apparently revealed a doubling of the MIC (Luz et al., 2012), which is actually a small increase compared to the resistance development against conventional antibiotics in similar set-ups. A *S. aureus* strain exposed to sublethal concentrations of rosemary oil or 1,8-cineole for 18 h in meat broth showed no decrease in sensitivity to these antimicrobials (Gomes Neto et al., 2012). The apparent low level of induction of resistance towards EOs could be due to the fact that EOs do not attack a single specific target but can have multiple modes of antibacterial action; the presence of several components with antibacterial activity may hamper the induction of

resistance (Becerril et al., 2012). Finally, if a membrane destabilizing mode of action is considered for EOs, it may simply be difficult to develop a resistance mechanism protecting such a large bacterial target since changing membranic structures and/or composition is likely unbeneficial for the viability of the bacteria. However, the example of the efflux pump-related tolerance of *Pseudomonas* spp. toward EOs show that an effective defense against EOs is possible. Although the initial observations indicate that induction of resistance towards EOs is low, more studies using different EOs and under different conditions are required to convincingly confirm these observations. In addition, more studies are required that address the induction of resistance towards single EO components.

The presence of sub-MIC concentrations of EO while passaging bacteria can influence the bacterial sensitivity to antibiotics (Fadli et al., 2011; Kon & Rai, 2012). Passaging *S. marcescens* in oregano oil increased the MICs for tetracycline and nalidixic acid two- to three-fold and increased the MICs for chloramphenicol, minocycline and ciprofloxacin to a smaller extent (Becerril et al., 2012). In contrast, passaging *P. mirabilis* in oregano oil increased the sensitivity to ampicillin eight-fold. *M. morganii* and *P. aeruginosa* were unaffected by passaging in oregano oil and repeated exposure to cinnamon oil had no significant effect on the MICs for all four bacterial strains and all antibiotics tested (Becerril et al., 2012). Passaging of three *Staphylococcus* strains up to 22 times in sub-MIC concentrations of tea tree oil or its major component terpinen-4-ol resulted in no significant changes in susceptibility to tea tree oil and no cross-protection to amoxicillin, ciprofloxacin, gentamicin, tetracycline and vancomycin (Hammer et al., 2012). A similar lack of significantly increased MICs for ciprofloxacin, kanamycin and ampicillin were found for *Escherichia coli* isolates after serial subculture over six days with tea tree oil or its major constituent terpinen-4-ol (Hammer et al., 2012). However, a study analyzing the effect on the MICs for mupirocin, fusidic acid, chloramphenicol, linezolid and vancomycin against 10 strains of MRSA and methicillin-susceptible *S. aureus* (MSSA) found some significant increases in the MICs after three days habituation to tea tree oil (McMahon et al., 2008). These studies indicate that EOs can indeed induce some resistance to antibiotics, but the levels of induction are still relatively low. Since the exact mechanism inducing resistance was not described either, it would be very interesting to determine which actual resistance mechanism is induced using EOs as this could also provide information on the antibacterial target of EOs. Exposing a strain of *S. aureus* to rosemary oil or 1,8-cineole overnight actually increased bacterial sensitivity to lactic acid, heat and sodium chloride over a four-hour period in a time-kill assay (Gomes Neto et al., 2012). Interestingly, a component of peppermint oil is reported to preferentially kill bacteria carrying a resistance plasmid (Schelz et al., 2006).

Antibiotics

Mode of action of antibiotics

Current knowledge on targets and modes of action of antibiotics are summarized in Table 2. Mainly, they inhibit either protein synthesis or target specific sites on the cell wall, although downstream targets can also be affected through the primary mode of action (ECDC/EMEA, 2009; Kohanski et al., 2010; Nordberg et al., 2005). The mechanisms by which bacteria have become resistant to these antibiotic mechanisms have been reviewed by Van Hoek et al. (2011).

Bacterial resistance to antibiotics

Antibiotics inhibit bacterial growth and many disrupt an essential cellular function that leads to the rapid death of bacteria. Not all antibiotics are effective against all types of bacteria due to the inherent differences between classes of bacteria. For instance, the structural difference between Gram-negative and Gram-positive bacteria means that there is a different susceptibility pattern because the sites where antibiotics can exert their effects are divergent (ECDC/EMEA, 2009). Gram-positive species possess a thick peptidoglycan layer outside the cell membrane, whereas Gram-negative species have a thin peptidoglycan layer enveloped by an outer membrane. The saccharide part of the lipopolysaccharide in the outer membrane provides a hydrophilic surface that forms a barrier against many hydrophobic substances, including hydrophobic antibiotics (Nikaido, 1994), such as penicillin and EOs. Situated in the membrane are diffusion channels (porins) through which small hydrophilic molecules, such as ampicillin, can enter the cell (Hemaiswarya et al., 2008; Lehtinen et al., 2007; Nikaido, 1994, 2001).

Resistance to one antibiotic can mean that a whole class of antibiotics becomes ineffective, or even several classes (ECDC/EMEA, 2009). Antibiotic resistance can be intrinsic or acquired and the mechanisms can be divided into different categories: (i) enzymes produced by the bacterium cause direct destruction or modification of the antibiotic (e.g. bacteria that produce beta lactamases (ESBLs) which cleave penicillin and related beta lactams); (ii) active site modification occurs so that there is inefficient binding of the antibiotic (e.g. MRSA where the target penicillin-binding protein is modified); (iii) a reduced amount of antibiotic is present due to the removal, or efflux, from the cell (e.g. in *Pseudomonas* spp.) or (iv) production of an alternative target that is resistant to inhibition by the antibiotic (metabolic by-pass), e.g. an overproduction of the target enzyme in trimethoprim-resistant *E. coli* (Huovinen, 2001; Sheldon, 2005). An inherent mechanism of resistance to antimicrobials in Gram-negative bacteria is the expression of efflux pumps which actively remove certain toxic molecules from the cell and renders them less susceptible to detergents and antibiotics (Lehtinen et al., 2007; Ma et al., 1994; Nikaido, 2001).

Assay methods used for detecting interactions between antibiotics and EOs

Different methods of testing for synergism can sometimes produce different conclusions for the same combination of antibiotics, but there is generally a fair degree of agreement between the methods, which have been compared by White et al., (1996). An interaction between antimicrobials is additive when the combined effect is equal to the sum of the individual substances (Bhat & Ahangar, 2007). Additivity is sometimes referred to as indifference because there is no

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Table 2. Mechanisms of antibacterial action of major antibiotic classes and bacterial resistance mechanisms.

Class	Antibiotics	Mode of action	Antibiotic resistance mechanisms
Aminoglycosides	Amikacin, gentamicin, kanamycin, trobramycin, streptomycin	Protein synthesis inhibitors with 30S ribosomes as primary target	Active efflux, decreased permeability, ribosome alteration, aminoglycoside modification enzymes
ß-Lactams	Penicillins (e.g. amoxicillin), ampicillin, oxacillin) cephalosporins (e.g. ceftiofur, ceftazidime, ceftriaxone), carbapenems	Cell-wall synthesis inhibitors with penicillin binding sites as primary target	Beta-lactamases, altered penicillin-binding proteins
Fluoroquinolones	Ciprofloxacin, nalidixic acid, gemifloxacin, levofloxacin, sarafloxacin, norfloxacin	DNA synthesis inhibitors with primary target being topoisomerase II and IV	Loss of porins, efflux pumps, DNA gyrase/topoisomerase IV mutations
Glycopeptides and glycolipopeptides	Vancomycin, teicoplanin	Cell-wall synthesis inhibitors by targeting peptidoglycan units	Modified peptidoglycan precursors (low-binding affinity)
Lipopeptides	Daptomycin, polymyxin B	Cell-wall synthesis inhibitors by targeting cell membrane	Spontaneous mutations
Lincosamides	Lincomycin, clindamycin	Protein synthesis inhibitors by targeting 50S ribosomes	rRNA methylases, efflux, inactivating genes
Macrolides	Erythromycin, azythromycin	Protein synthesis inhibitors by targeting 50S ribosomes	(as for lincosamides)
Phenicols	Chloramphenicol, florfenicol	Protein synthesis inhibitors by targeting 50S ribosomes	Acetylation by acetyltransferases, phosphotransferases, target site mutation, permeability barrier, efflux
Rifamycins	Rifamycins, rifapentine, rifampin	RNA synthesis inhibitor by targeting DNA-dependent RNA polymerase	Mutations in the rpoB gene that encodes for RNA polymerase
Streptogramins	Dalfopristin, pristinamycin, quinupristin	Protein synthesis inhibitors by targeting 50S ribosomes	(as for lincosamides)
Tetracyclines	Doxycycline, tetracycline, minocycline	Protein synthesis inhibitors by targeting 30S ribosomes	Efflux pumps, ribosomal protection protein, enzymatic inactivation
Trimethoprim-sulfamethoxazole	Co-trimoxazole	Folic acid synthesis inhibitors	Permeability barrier and/or efflux pumps; naturally insensitive target enzymes; regulational changes in target enzymes; mutational changes in target enzymes; acquired resistance by drug-resistant target enzymes

Adapted and compiled from Huovinen (2001); ECDC/EMEA (2009); Kohanski et al. (2010); Nordberg et al. (2005); Telenti et al. (1993); Van Hoek et al. (2011).

interaction between the tested antimicrobials (White et al., 1996). An effect is said to be synergistic when the combined effect is greater than the sum of the effects of the two individual substances. When the combined effect is smaller than that of the sum of the individual substances, it is termed antagonism (Bhat & Ahangar, 2007).

Checkerboard method

The most common *in vitro* technique for testing for synergy between antimicrobial substances is the checkerboard technique, so called because the concentrations of one substance are arranged horizontally and the other vertically in a microwell plate. The dilutions that are tested are based on the MIC of the substances, usually ranging from a few steps below the expected MIC, to concentrations twice the expected MIC. Measurements are usually made at one time point and therefore do not give a dynamic view of the antimicrobial interactions (Pillai et al., 2005).

The results of the checkerboard assay are interpreted by plotting an isobologram (Figure 2) or calculating the fractional inhibitory concentration (FIC) index for the two antimicrobials. A full description of the methodology and interpretation of isobolograms and FIC indices is beyond the scope of this review and the reader is referred to other works covering this in more detail (Bhat & Ahangar, 2007; White et al., 1996). A FIC index of 0.5 or less is said to be synergistic; between 0.5 and 1 is said to be additive (EUCAST, 2000). When a more stringent discrimination between additive and synergistic effects is required, synergism is said to occur only if there is a FIC index value of less than 0.5 (Sanders et al., 1993). A FIC index between 1 and 2 denotes indifference and a FIC index greater than 2 denotes antagonism, although some studies would say that antagonism requires a FIC index of 4 (EUCAST, 2000; White et al., 1996).

Time-kill method

The time-kill assay involves measuring the number of viable bacteria present in liquid medium in the presence of a particular combination of antibacterials at different time points. Although time-kill curves are not widely used to study antibacterial interactions, these can be considered a clinically-relevant model if the concentrations used represent those achieved at the site of an infection (Pillai et al., 2005). Due to the fact that microbial colonies have to be counted at numerous time-points, the time-kill curve is labor intensive and limits the number of concentrations and combinations that can be tested (White et al., 1996).

E-test

Although not widely used, the Epsilometer or E-test method is less laborious than the time-kill and the checkerboard method (White et al., 1996) and standard test strips for antibiotics are commercially available (AB Biodisk, Solna, Sweden). In the E-test, two plastic strips are coated with a continuous gradient of the two antimicrobials. The strips are placed at 90° to each other on an agar plate coated with a bacterial lawn, intersecting at the respective MICs of the individual antimicrobials. Where the two inhibition zones intersect is the value taken for the MIC of the combination (White et al., 1996) (Figure 3). If the compounds interact synergistically, bacterial growth in the lower right quadrant will be inhibited and if they antagonize each other the growth will be inhibited in the upper left quadrant (Figure 3). Measurements are often only carried out at one time-point and therefore give a static view of the antibacterial interaction. The E-test investigates inhibition of growth, whereas in the time-kill assay bacterial death can be one of the endpoints investigated (Pillai et al., 2005).

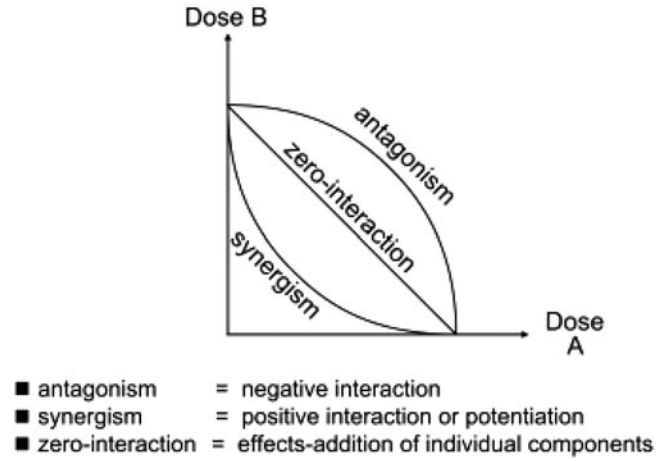

Figure 2. Isobologram method of determining synergy.
Reprinted from Wagner & Ulrich-Merzenich (2009) with permission from Elsevier.

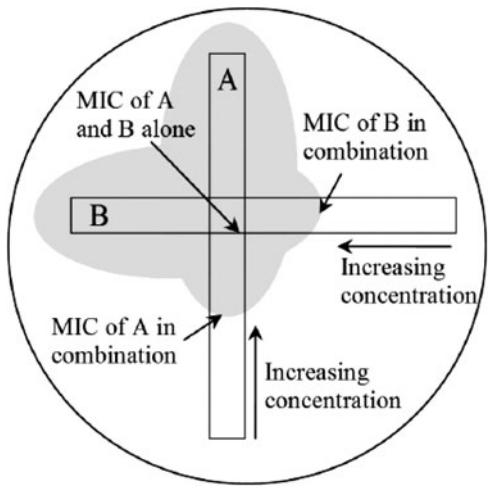

Figure 3. Diagram of strip-placement for the E-test synergy assay.
Reprinted from White et al. (1996), with permission from ASM.

Other methods

The methods described above rely on a fixed end point after approximately 24 h of incubation. To obtain real-time results, Lehtinen & Lilius (2007) developed an immunometric checkerboard assay with green fluorescent protein luciferase readout. Rastogi et al. (1994) used a method in which bacterial growth is monitored radiometrically as a function of ^{14}C-labeled CO_2 release. Growth index and the ''x/y

Table 3. *In vitro* synergy between EOs and antibiotics.

Essential oil	Antibiotic	Bacterial species*	Method	Synergy established†	References
Australian tea tree (*Melaleuca alternifolia*)	Ciprofloxacin	*S. aureus* (1.58–7.7) *K. pneumoniae* (0.73–1.85)	Method similar to checkerboard (FIC index & isobologram)	– 0/–	van Vuuren et al., 2009
	Gentamicin	*A. baumannii* (0.5) *B. subtilis* (0.5) *B. cereus* (0.5–2.0; 0.52) *E. coli* (0.49) *S. marcescens* (0.49) *S. aureus* (0.49; 0.50 >2) *Y. enterocolitica* (0.49)	Checkerboard (FIC index)	0/+	LaPlante, 2007; Rosato et al., 2010
	Tobramycin	*E. coli* (0.37) *S. aureus* (0.62)	Time-kill curves	+	D'Arrigo et al., 2010
	Vancomycin	*S. aureus* (>0.5)	Checkerboard (FIC index)	0	LaPlante, 2007
Cinnamon (*Cinnamomum zeylanicum*)	Amikacin	*A. baumanii* (0.05)	Checkerboard (FIC index)	++	Guerra et al., 2012
	Gentamicin	*A. baumanii* (0.5)	Checkerboard (FIC index)	+	Guerra et al., 2012
	Imipenem	*A. baumanii* (2)	Checkerboard (FIC index)	0	Guerra et al., 2012
	Meropenem	*A. baumanii* (1.5)	Checkerboard (FIC index)	0	Guerra et al., 2012
Cinnamon/Cassia (*Cinnamomum burmannii*)	Gentamicin	*S. epidermidis* (0.14–0.22)	Checkerboard (FIC index)	++	Nuryastuti et al., 2009
Clove (*Syzygium aromaticum*)	Ampicillin	> 10 strains (0.38–0.75)	Checkerboard (FIC index), time-kill curves	+	Moon et al., 2011
	Gentamicin	> 10 strains (0.38–0.75)	Checkerboard (FIC index), time-kill curves	+	Moon et al., 2011
Coriander (*Coriandrum sativum*)	Cefoperazone	*A. baumanii* (0.75–1.00)	Checkerboard (FIC index)	0	Duarte et al., 2012
	Chloramphenicol	*A. baumanii* (0.05–0.31)	Checkerboard (FIC index)	++	Duarte et al., 2012
	Ciprofloxacin	*A. baumanii* (0.28–0.38)	Checkerboard (FIC index)	++	Duarte et al., 2012
	Gentamicin	*A. baumanii* (0.25–0.38)	Checkerboard (FIC index)	++	Duarte et al., 2012
	Piperacillin	*A. baumanii* (0.63–1.00)	Checkerboard (FIC index)	0	Duarte et al., 2012
	Tetracycline	*A. baumanii* (0.19–0.31)	Checkerboard (FIC index)	++	Duarte et al., 2012
Curry plant (*Helichrysum italicum*)	Chloramphenicol	*E. aerogenes* *A. baumanii* *P. aeruginosa* *E. coli*	Fold reduction in MIC	+	Lorenzi et al., 2009
Lemon (*Citrus limon*)	Amikacin	*A. baumanii* (0.04)	Checkerboard (FIC index)	++	Guerra et al., 2012
	Gentamicin	*A. baumanii* (0.5)	Checkerboard (FIC index)	+	Guerra et al., 2012
	Imipenem	*A. baumanii* (2)	Checkerboard (FIC index)	0	Guerra et al., 2012
	Meropenem	*A. baumanii* (2)	Checkerboard (FIC index)	0	Guerra et al., 2012
Lemongrass (*Cymbopogon citratus*)	Kanamycin	*S. typhimurium* (0.28–1.00)	Checkerboard (FIC index)	++	Shin, 2005
	Streptomycin	*S. typhimurium* (0.31–0.67)	Checkerboard (FIC index)	+	Shin, 2005
Lippia sidoides	Amikacin	*S. aureus* *P. aeruginosa*	Change in inhibition zone in the presence of EO vapour	0/+	Veras et al., 2012
	Gentamicin	*S. aureus* *P. aeruginosa*	Change in inhibition zone in the presence of EO vapour	0/+	Veras et al., 2012

(*continued*)

Table 3. Continued.

Essential oil	Antibiotic	Bacterial species*	Method	Synergy established†	References
	Neomycin	*S. aureus* *P. aeruginosa*	Change in inhibition zone in presence of EO vapour	0/+	Veras et al., 2012
Mentha piperita	Ciprofloxacin	*S. aureus* (0.75–1.40) *K. pneumonia* (0.68–2.24)	Method similar to checkerboard (FIC index & isobologram)	0	Van Vuuren et al., 2009
Oregano (*Origanum vulgare*)	Amoxicillin	*E. coli* (0.75)	Checkerboard (FIC index)	0	Si et al., 2008
	Ceftiofur	*E. coli* (0.63)	Checkerboard (FIC index)	0	Si et al., 2008
	Ceftriaxone	*E. coli* (0.63)	Checkerboard (FIC index)	0	Si et al., 2008
	Doxycycline	*E. coli* (0.38)	Checkerboard (FIC index)	++	Si et al., 2008
	Florfenicol	*E. coli* (0.38)	Checkerboard (FIC index)	++	Si et al., 2008
	Gentamicin	*A. baumannii* (0.65) *E. coli* (0.65) *S. aureus* (0.31, 0.51)	Checkerboard (FIC index & isobologram)	+	Rosato et al., 2010
	Gentamicin	*B. cereus* (0.28) *B. subtilis* (0.33)	Checkerboard (FIC index)	++	Rosato et al. 2010
	Gentamicin	*S. marcescens* (0.65) *Y. enterocolitica* (0.63)	Checkerboard (FIC index)	0	Rosato et al. 2010
	Kanamycin	*E. coli* (1.5)	Checkerboard (FIC index)	0	Si et al., 2008
	Levofloxacin	*E. coli* (0.5)	Checkerboard (FIC index)	+	Si et al., 2008
	Lincomycin	*E. coli (0.75)*	Checkerboard (FIC index)	0	Si et al., 2008
	Maquindox	*E. coli* (0.5)	Checkerboard (FIC index)	+	Si et al., 2008
	Polymyxin	*E. coli* (0.75)	Checkerboard (FIC index)	0	Si et al., 2008
	Sarafloxacin	*E. coli* (0.38)	Checkerboard (FIC index)	++	Si et al., 2008
Peppermint (*Mentha piperita*)	Ampicillin	*E. coli* (1.0)	Checkerboard (FIC index)	0	Schelz et al., 2006
	Erythromycin	*E. coli* (1.0)	Checkerboard (FIC index)	0	Schelz et al., 2006
	Gentamicin	*E. coli* (1.25)	Checkerboard (FIC index)	0	Schelz et al., 2006
	Oxytetracycline	*E. coli* (0.5)	Checkerboard (FIC index)	+	Schelz et al., 2006
Rose geranium (*Pelargonium graveolens*)	Ciprofloxacin	*K. pneumonia* (0.38) *P. mirabilis* (0.38) *S. aureus* (0.5)	Checkerboard (FIC index)	+/ ++	Malik et al., 2011
	Norfloxacin	*S. aureus* (0.37–0.38) *B. cereus* (0.5) *B. subtilis* (0.5) *E. coli* (0.5)	Checkerboard (FIC index)	+	Rosato et al., 2007
Rosemary (*Rosmarinus officinalis*)	Ciprofloxacin	*S. aureus* (1.03–1.30) *K. pneumoniae* (0.28–1.0)	Method similar to Checkerboard (FIC index & isobologram)	0/+	Van Vuuren et al., 2009
Shiraz oregano (*Zataria multiflora*)	Vancomycin	*Methicillin resistant* *S. aureus* (0.32) *Methicillin susceptible* *S. aureus* (0.19)	Checkerboard (FIC index)	++	Mahboubi et al., 2010
Thyme (*Thymus broussonetii*)	Ciprofloxacin	*E. coli* (0.37) *Salmonella sp.* (0.56) *Ent. cloacae* (0.5) *K. pneumoniae* (0.62) *V. cholera* (0.14)	Checkerboard (FIC index)	+ 0 0 0 ++	Fadli et al., 2012

(*continued*)

Table 3. Continued.

Essential oil	Antibiotic	Bacterial species*	Method	Synergy established†	References
		P. aeruginosa (0.14)		++	
		B. subtilis (0.26)		+	
		B. cereus (0.15)		++	
		M. luteus (0.26)		++	
		S. aureus (0.5)		+	
	Cefixime	*E. coli* (0.5)	Checkerboard	+	Fadli et al., 2012
		Salmonella sp. (0.18)	(FIC index)	++	
		Ent. cloacae (1)		0	
		K. pneumoniae (1)		0	
		V. cholera (0.62)		0	
		P. aeruginosa (0.5)		+	
		B. subtilis (1)		0	
		B. cereus (0.5)		+	
		M. luteus (1)		0	
		S. aureus (0.5)		+	
	Gentamicin	*E. coli* (0.37)	Checkerboard	+	Fadli et al., 2012
		Salmonella sp. (0.62)	(FIC index)	0	
		Ent. cloacae (0.5)		+	
		K. pneumoniae (0.62)		0	
		V. cholera (0.28)		++	
		P. aeruginosa (0.28)		++	
		B. subtilis (0.09)		++	
		B. cereus (0.12)		++	
		M. luteus (0.12)		++	
		S. aureus (0.5)		+	
	Pristinamycin	*E. coli* (0.37)	Checkerboard	+	Fadli et al., 2012
		Salmonella sp. (0.5)	(FIC index)	+	
		Ent. cloacae (0.5)		+	
		K. pneumoniae (0.5)		+	
		V. cholera (0.15)		++	
		P. aeruginosa (0.75)		0	
		B. subtilis (0.37)		+	
		B. cereus (0.37)		+	
		M. luteus (0.31)		+	
		S. aureus (0.5)		+	
Thyme (*Thymus magnus*)	Norfloxacin	*S. aureus*	Checkerboard, isobologram	+/0	Shin & Kim, 2005
Thyme (*Thymus maroccanus*)	Ciprofloxacin	*E. coli* (0.12)	Checkerboard	++	Fadli et al., 2012
		Salmonella sp. (0.37)	(FIC index)	+	
		Ent. cloacae (0.37)		+	
		K. pneumoniae (0.37)		+	
		V. cholera (0.14)		++	
		P. aeruginosa (0.15)		++	
		B. subtilis (0.09)		++	
		B. cereus (0.15)		++	
		M. luteus (0.28)		+	
		S. aureus (0.26)		+	
	Cefixime	*E. coli* (0.5)	Checkerboard	+	Fadli et al., 2012
		Salmonella sp. (0.18)	(FIC index)	++	
		Ent. cloacae (1)		0	
		K. pneumoniae (1)		0	
		V. cholera (0.31)		+	
		P. aeruginosa (0.75)		0	
		B. subtilis (0.62)		0	
		B. cereus (0.75)		0	
		M. luteus (1)		0	
		S. aureus (0.18)		++	
	Gentamicin	*E. coli* (0.28)	Checkerboard	+	Fadli et al., 2012
		Salmonella sp. (0.75)	(FIC index)	0	
		Ent. cloacae (0.19)		++	
		K. pneumoniae (0.5)		+	
		V. cholera (0.75)		0	
		P. aeruginosa (0.18)		++	
		B. subtilis (0.5)		+	
		B. cereus (0.25)		++	
		M. luteus (0.75)		0	
		S. aureus (0.5)		+	

(*continued*)

Table 3. Continued.

Essential oil	Antibiotic	Bacterial species*	Method	Synergy established†	References
	Pristinamycin	*E. coli* (0.5) *Salmonella sp.* (0.75) *Ent. cloacae* (0.5) *K. pneumoniae* (0.5) *V. cholera* (0.62) *P. aeruginosa* (0.75) *B. subtilis* (0.31) *B. cereus* (0.28) *M. luteus* (0.25) *S. aureus* (0.62)	Checkerboard (FIC index)	+ 0 + + 0 0 + ++ ++ 0	Fadli et al., 2012
Thyme (*Thymus quinquecostatus*)	Norfloxacin	*S. aureus*	Checkerboard, isobologram	+/0	Shin & Kim, 2005
Thyme (*Thymus vulgaris*)	Ciprofloxacin	*S. aureus* (0.80–2.59) *K. pneumonia* (0.71–1.40)	Method similar to checkerboard (FIC index & isobologram)	0	Van Vuuren et al., 2009

*Number between parentheses is the FIC index.
†Interactions have been classified as follows: ++ clear synergy; + possible synergy (either borderline synergy (FIC index = 0.5) or different methods in the same study showed additivity and synergism); 0 additivity; − antagonism.

quotient'' are used for analyzing synergistic effects. The antimicrobials are used at sublethal concentrations, to which precultures with a specific growth index are added. Resulting x/y quotients with a value of 1 indicate additivity, less than 0.5 indicates synergism and greater than 2 indicates antagonism (Rastogi et al., 1994). Some studies compare the MIC of an antibiotic in the presence and absence of a potential synergist (Abulrob et al., 2004). Another method of looking for synergy involves determining the highest sub-inhibitory concentration (SIC) for EO components (i.e. immediately below the MIC) and the breakpoint for resistance to the antibiotics by the Clinical Laboratory Standards Institute (CLSI, 2009) method. A combination of the two is classed as synergistic if the optical density of a bacterial suspension is significantly lower than after incubation with either of the antimicrobials alone (Kollanoor Johny et al., 2010).

Evidence for synergy between antibiotics and EOs

To improve the efficacy of antibiotics it is necessary to find methods of improving diffusion of antibiotics across bacterial membranes and/or to hinder the efflux pumps that are a general resistance mechanism in Gram-negative bacteria (Bolla et al., 2011). An overview of the published studies on interactions between EOs and antibiotics and between EO components and antibiotics is presented in Tables 3 and 4, respectively. The most frequently reported assay method is the checkerboard assay with calculation of the FIC index.

Mechanisms that can lead to pharmacological synergy are:
(1) multi-target effect in which compounds target different sites in the bacterial cell;
(2) pharmacokinetic or physicochemical effects (e.g. improvement of solubility or bioavailability); or
(3) targeting a specific resistance mechanism of bacteria (Hemaiswarya et al., 2008; Wagner & Ulrich-Merzenich, 2009).

The neutralization of adverse effect of drugs by adjuvants is not true synergy since the efficacy is not increased (Wagner & Ulrich-Merzenich, 2009).

Finding a synergistic combination by identifying antibacterials that bind to different targets may appear most productive. However, some reports show synergy between EOs and beta-lactam antibiotics, which both act on the cell membrane. For example, the oregano constituent thymol was synergistic with penicillin against *E. coli* (Gallucci et al., 2006) and thymol and carvacrol were synergistic with penicillin against *E. coli* and *S. typhimurium* (Palaniappan & Holley, 2010). However, in another study, penicillin combined with whole oregano oil or its constituent carvacrol was not synergistic against *E. coli* (Gallucci et al., 2006; Si et al., 2008). The differences in these results may lay in the use of different *E. coli* strains or the presence/absence of smaller components in the oregano oil.

Synergism via pharmacokinetic or physicochemical effects can be found for phenolic phytochemicals that do not possess any pharmacological effects themselves. An example is the improvement of sensitivity to carvacrol in *Bacillus cereus* by the presence of *p*-cymene, which is proposed to accumulate in bacterial membranes and distort their physico-chemical structure (Ultee et al., 2002). Examples of how EOs are suggested to interact synergistically with antibiotics are presented in Figure 4.

Interactions between EOs and antibiotics

Several studies have described synergistic or additive activity for existing antibiotics and whole EOs. Oregano (*O. vulgare*) oil in combination with doxycycline, florfenicol or sarafloxacin was shown to have synergistic effects against an ESBL-producing *E. coli* isolated from chickens (FICs 0.375–0.5) (Si et al., 2008). Although these antibiotics are not beta-lactams, this result may offer possibilities for finding a solution to the problem of beta-lactamase producing bacterial resistance.

Table 4. *In vitro* synergy between EO components and antibiotics.

Component	Antibiotic	Bacterial species*	Method	Synergy established†	References
Bergamottin/ coumarin epoxide	Norfloxacin	Methicillin resistant *S. aureus*, 4 strains	MIC measurement	++	Abulrob et al., 2004
Carvacrol	Ampicillin	*E. coli* (0.25) *K. oxytoca* (0.375) *S. typhimurium* (0.25) *S. aureus* (0.15)	Checkerboard (FIC index)	++	Palaniappan & Holley, 2010; Zhang et al., 2011
	Ampicillin	*S. typhimurium*	SIC/breakpoint	0	Kollanoor Johny et al., 2010
	Bacitracin	*E. coli* (0.25) *S. typhimurium* (0.25) *S. aureus* (0.25)	Checkerboard (FIC index)	++	Palaniappan & Holley, 2010
	Chloramphenicol	*S. typhimurium*	SIC/breakpoint	+	Kollanoor Johny et al., 2010
	Erythromycin	*E. coli* (1.0)	Checkerboard (FIC index)	0	Palaniappan & Holley, 2010
	Erythromycin	*S. typhimurium* (0.25) *S. pyogenes* (0.25)	Checkerboard (FIC index)	++	Palaniappan & Holley, 2010
	Nalidixic acid	*E. cloacae* (1.5) *K. oxytoca* (1.5)	Checkerboard (FIC index), time-kill curve	0	Choi et al., 2009
	Nalidixic acid	*E. coli* (0.25, 2.25)	Checkerboard (FIC index)	+/−	Choi et al., 2009
	Nalidixic acid	*S. derby* (0.25) *S. enteritidis* (0.28) *S. minnesota* (0.12) *S. typhimurium* (0.31)	Checkerboard (FIC index)	++	Choi et al., 2009
	Nitrofurantoin	*K. oxytoca* (0.15)	Checkerboard (FIC index)	++	Zhang et al., 2011
	Novobiocin	*E. coli* (0.63)	Checkerboard (FIC index)	0	Palaniappan & Holley, 2010
	Novobiocin	*S. typhimurium* (0.37)	Checkerboard (FIC index)	++	Palaniappan & Holley, 2010
	Penicillin	*E. coli* (2.0)	Checkerboard (FIC index)	−	Gallucci et al., 2006
	Penicillin	*E. coli* (0.37) *S. typhimurium* (0.37) *S. aureus* (0.11)	Checkerboard (FIC index)	++	Palaniappan & Holley, 2010
	Streptomycin	*S. typhimurium*	SIC/breakpoint	+	Kollanoor Johny et al., 2010
	Sulfamethoxazole	*S. typhimurium*	SIC/breakpoint	+	Kollanoor Johny et al., 2010
	Tetracycline	*S. typhimurium* (0.18)	Checkerboard (FIC index)	++	Palaniappan & Holley, 2010
	Tetracycline	*S. typhimurium*	SIC/breakpoint	+	Kollanoor Johny et al., 2010
trans-Cinnamaldehyde	Ampicillin	*S. typhimurium*	SIC/breakpoint	+	Kollanoor Johny et al., 2010
	Ampicillin	*E. coli* (0.37) *S. typhimurium* (0.25) *S. aureus* (0.25)	Checkerboard (FIC index)	++	Palaniappan & Holley, 2010
	Ampicillin	*K. oxytoca* (1.5)	Checkerboard (FIC index)	0	Zhang et al., 2011
	Bacitracin	*E. coli* (0.63)	Checkerboard (FIC index)	0	Palaniappan & Holley, 2010
	Bacitracin	*S. typhimurium* (0.24) *S. aureus* (0.24)	Checkerboard (FIC index)	++	Palaniappan & Holley, 2010
	Clindamycin	*C. difficile* (0.31)	Checkerboard (FIC index & isobologram)	++	Shahverdi et al., 2007
	Erythromycin	*E. coli* (0.24) *S.* Typhimurium (0.24)	Checkerboard (FIC index)	++	Palaniappan & Holley, 2010
	Erythromycin	*S. pyogenes* (1.0)	Checkerboard (FIC index)	0	Palaniappan & Holley, 2010
	Nitrofurantoin	*K. oxytoca* (1.05)	Checkerboard (FIC index)	0	Zhang et al., 2011
	Novobiocin	*E. coli* (0.24) *S. typhimurium* (0.24)	Checkerboard (FIC index)	++	Palaniappan & Holley, 2010
	Penicillin	*E. coli* (0.24)	Checkerboard (FIC index)	++	Palaniappan & Holley, 2010

(*continued*)

Table 4. Continued.

Component	Antibiotic	Bacterial species*	Method	Synergy established†	References
	Penicillin	S. *typhimurium* (0.63)	Checkerboard (FIC index)	0	Palaniappan & Holley, 2010
	Streptomycin	*S. typhimurium*	SIC/breakpoint	+	Kollanoor Johny et al., 2010
	Sulfamethoxazole	*S. typhimurium*	SIC/breakpoint	+	Kollanoor Johny et al., 2010
	Tetracycline	*E. coli* (0.37) *S. typhimurium* (0.37)	Checkerboard (FIC index)	++	Palaniappan & Holley, 2010
	Tetracycline	*S. typhimurium*	SIC/breakpoint	+	Kollanoor Johny et al., 2010
trans-Cinnamic acid	Amikacin	*E. aerogenes* (0.25) *E. coli* (0.14) *S. aureus* (0.18)	Checkerboard (FIC index & isobologram), time-kill curve	++	Hemaiswarya et al., 2010
	Amikacin	*M. tuberculosis* *M. avium*	Radiometric x/y quotient	++	Rastogi et al., 1994, 1998
	Amikacin	*P. aeruginosa* (0.51)	Checkerboard (FIC index & isobologram)	+	Hemaiswarya & Doble, 2010
	Ampicillin	*E. aerogenes* (0.51) *P. aeruginosa* (0.51)	Checkerboard (FIC index & isobologram)	+	Hemaiswarya & Doble, 2010
	Ampicillin	*E. coli* (0.26) *S. aureus* (0.38)	Checkerboard (FIC index & isobologram)	++	Hemaiswarya & Doble, 2010
	Ciprofloxacin	*E. aerogenes* (0.42) *P. aeruginosa* (0.49) *E. coli* (0.34) *S. aureus* (0.29)	Checkerboard (FIC index)	++	Hemaiswarya & Doble, 2010
	Clarithromycin	*M. avium*	Radiometric x/y quotient	++	Rastogi et al., 1994
	Erythromycin	*E. aerogenes* (1.2) *P. aeruginosa* (1.3)	Checkerboard (FIC index)	0	Hemaiswarya & Doble, 2010
	Erythromycin	*E. coli* (0.36) *S. aureus* (0.4)	Checkerboard (FIC index)	++	Hemaiswarya & Doble, 2010
	Oxacillin	*S. aureus* (1.0)	Checkerboard (FIC index)	0	Basri et al., 2008
	Rifampicin	*M. tuberculosis*	Combined sub-lethal concentrations	+	Chen et al., 2011
	Rifampicin	*M. tuberculosis*	Radiometric x/y quotient	0	Rastogi et al., 1998
	Sparfloxacin	*M. avium*	Radiometric x/y quotient	++	Rastogi et al., 1994
	Vancomycin	*E. aerogenes* (0.82) *P. aeruginosa* (0.76)	Checkerboard (FIC index)	0	Hemaiswarya & Doble, 2010
	Vancomycin	*E. coli* (0.42) *S. aureus* (0.36)	Checkerboard (FIC index)	++	Hemaiswarya & Doble, 2010
cis-Cinnamic acid	Rifampicin	*M. tuberculosis*	Combined sub-lethal concentrations	+	Chen et al., 2011
Eugenol	Ampicillin	*A.actinomycetemcomitans* (0.5) *F. nucleatum* (0.5) *S. anginosus* (0.5) *S. criceti* (0.38–0.5) *S. gordonii* (0.38–0.5) *S. mutans* (0.5) *S. sobrinus* (0.5) *P. intermedia* (0.5) *S. ratti* (0.75) *S. sanguinis* (0.5) *P. gingivalis* (0.38–0.5)	Checkerboard (FIC index), time-kill curve, SIC/breakpoint	+	Moon et al., 2011, Kollanoor Johny et al., 2010
	Ampicillin	*E. aerogenes* *E. coli* *P. vulgaris* *P. aeruginosa* *S. typhimurium*	Checkerboard	++	Hemaiswarya & Doble, 2009
	Ampicillin	*E. coli* (>0.5) *K. oxytoca* (1.12) *S. aureus* (1.0) *S. typhimurium* (>0.5)	Checkerboard (FIC index)	0	Palaniappan & Holley, 2010, Zhang et al., 2011
	Bacitracin	*E. coli* (>0.5) *S. aureus* (>0.5) *S. typhimurium* (>0.5)	Checkerboard (FIC index)	0	Palaniappan & Holley, 2010
	Chloramphenicol	*E. aerogenes* *E. coli* *P. vulgaris* *P. aeruginosa* *S. typhimurium*	Checkerboard	++	Hemaiswarya & Doble, 2009

(*continued*)

Table 4. Continued.

Component	Antibiotic	Bacterial species*	Method	Synergy established†	References
	Chloramphenicol	*S. typhimurium*	SIC/breakpoint	+	Kollanoor Johny et al., 2010
	Erythromycin	*E. aerogenes* *E. coli* *P. vulgaris* *P. aeruginosa* *S. typhimurium*	Checkerboard	++	Hemaiswarya & Doble, 2009
	Erythromycin	*E. coli* (1.1) *S. pyogenes* (1.0) *S. typhimurium* (0.63)	Checkerboard (FIC index)	0	Palaniappan & Holley, 2010
	Gentamicin	*A. actinomycetemcomitans* (0.5) *F. nucleatum* (0.5) *P. gingivalis* (0.38–0.5) *P. intermedia* (0.5) *S. anginosus (0.5)* *S. criceti* (0.38-0.5) *S. gordonii* (0.5) *S. mutans* (0.75) *S. ratti* (0.75) *S. sanguinis* (0.38–0.5) *S. sobrinus* (0.5)	Checkerboard (FIC index), time-kill curve	+	Moon et al., 2011
	Nitrofurantoin	*K. oxytoca* (1.1)	Checkerboard (FIC index)	0	Zhang et al., 2011
	Norfloxacin	*E. aerogenes* *E. coli* *P. vulgaris* *P. aeruginosa* *S. typhimurium*	Checkerboard	++	Hemaiswarya & Doble, 2009
	Novobiocin	*E. coli* (1.1)	Checkerboard (FIC index)	0	Palaniappan & Holley, 2010
	Novobiocin	*S. typhimurium* (0.4)	Checkerboard (FIC index)	+	Palaniappan & Holley, 2010
	Oxacillin	*E. aerogenes* *E. coli* *P. vulgaris* *P. aeruginosa* *S. typhimurium*	Checkerboard	++	Hemaiswarya & Doble, 2009
	Penicillin	*E. aerogenes* *E. coli* *P. vulgaris* *P. aeruginosa* *S. typhimurium*	Checkerboard	++	Hemaiswarya & Doble, 2009
	Penicillin	*E. coli* (0.16) *S. aureus* (1)	Checkerboard (FIC index)	+	Gallucci et al., 2006,
	Penicillin	*S. typhimurium* (>0.5) *E. coli* (>0.5) *S. aureus* (0.33)	Checkerboard (FIC index)	+	Palaniappan & Holley, 2010
	Polymyxin	*E. aerogenes* *E. coli* *P. vulgaris* *P. aeruginosa* *S. typhimurium*	Checkerboard	++	Hemaiswarya & Doble, 2009
	Rifampicin	*E. aerogenes* *E. coli* *P. vulgaris* *P. aeruginosa* *S. typhimurium*	Checkerboard	++	Hemaiswarya & Doble, 2009
	Streptomycin	S. *typhimurium*	SIC/breakpoint	0	Kollanoor Johny et al., 2010
	Sulfamethoxazole	S. *typhimurium*	SIC/breakpoint	+	Kollanoor Johny et al., 2010
	Tetracycline	*E. aerogenes* *E. coli* (0.16) *P. vulgaris* *P. aeruginosa* *S. typhimurium* (0.22)	Checkerboard	++	Hemaiswarya & Doble, 2009, Palaniappan & Holley, 2010
	Tetracycline	S. *typhimurium*	SIC/breakpoint	0	Kollanoor Johny et al., 2010
	Vancomycin	*E. aerogenes* *E. coli* *P. vulgaris*	Checkerboard	++	Hemaiswarya & Doble, 2009

(*continued*)

Table 4. Continued.

Component	Antibiotic	Bacterial species*	Method	Synergy established†	References
		P. aeruginosa *S. typhimurium*			
Geraniol	Ampicillin	*E. aerogenes*	Influence on MIC	–	Schelz et al., 2006
	Norfloxacin	*E. aerogenes*		–	
	Penicillin	*E. aerogenes*		0	
	Penicillin	*S. aureus* (1.0) *E. coli* (1.5)	Checkerboard (FIC index)	0 0	Gallucci et al., 2006
Menthol	Ampicillin	*E. coli* (2)	FIC Index	–	Schelz et al., 2006
	Erythromycin	*E. coli* (2)		–	
	Gentamicin	*E. coli* (1)		0	
	Oxytetracycline	*E. coli* (0.5)		+	
	Penicillin	*S. aureus* (1.0) *E. coli* (1.5)	Checkerboard (FIC index)	0 0	Gallucci et al., 2006
Thymol	Amikacin	*S. aureus* *P. aeruginosa*	Influence on inhibition zone in presence of EO vapour	+/0 0	Veras et al., 2012
	Ampicillin	*E. coli* (0.12) *S. typhimurium* (0.12) *S. aureus* (0.12)	Checkerboard (FIC index)	++	Palaniappan & Holley, 2010
	Ampicillin	*K. oxytoca* (0.75)	Checkerboard (FIC index)	0	Zhang et al., 2011
	Ampicillin	*S. typhimurium*	SIC/breakpoint	+	Kollanoor Johny et al., 2010
	Bacitracin	*E. coli* (0.56)	Checkerboard (FIC index)	0/+	Palaniappan & Holley, 2010
	Bacitracin	*S. typhimurium* (0.15) *S. aureus* (0.25)	Checkerboard (FIC index)	++	Palaniappan & Holley, 2010
	Chloramphenicol	*S. typhimurium*	SIC/breakpoint	+	Kollanoor Johny et al., 2010
	Erythromycin	*E. coli* (0.25) *S. typhimurium* (0.25) *S. pyogenes* (0.4)	Checkerboard (FIC index)	++	Palaniappan & Holley, 2010
	Gentamicin	*S. aureus* *P. aeruginosa*	Influence on inhibition zone in presence of EO vapour	+/0 0	Veras et al., 2012
	Neomycin	*S. aureus* *P. aeruginosa*	Influence on inhibition zone in presence of EO vapour	0 0	Veras et al., 2012
	Nitrofurantoin	*K. oxytoca* (0.55)	Checkerboard (FIC index)	0/+	Zhang et al., 2011
	Norfloxacin	*S. aureus*	Isobologram	++	Shin & Kim, 2005
	Novobiocin	*E. coli* (0.37) *S. typhimurium* (0.37)	Checkerboard (FIC index)	++	Palaniappan & Holley, 2010
	Penicillin	*E. coli* (0.15)	Checkerboard (FIC index)	++	Gallucci et al., 2006
	Penicillin	*E. coli* (0.2) *S. typhimurium* (0.13) *S. aureus* (0.18)	Checkerboard (FIC index)	++	Palaniappan & Holley, 2010
	Penicillin	*S. aureus* (3.98)	Checkerboard (FIC index)	–	Gallucci et al.,2006
	Streptomycin	*S. typhimurium*	SIC/breakpoint	+	Kollanoor Johny et al., 2010
	Sulfamethoxazole	*S. typhimurium*	SIC/breakpoint	+	Kollanoor Johny et al., 2010
	Tetracycline	*E. coli* (0.15) *S. typhimurium* (0.1)	Checkerboard (FIC index)	++	Palaniappan & Holley, 2010
	Tetracycline	*S. typhimurium*	SIC/breakpoint	0	Kollanoor Johny et al., 2010

*Number between parentheses is the FIC index.
†Interactions have been classified as follows: ++ clear synergy; + possible synergy (either borderline synergy (FIC index = 0.5) or different methods in the same study showed additivity and synergism); 0 additivity; – antagonism.

Oregano oil with gentamicin showed synergistic effects against *B. cereus, B. subtilis* and one strain of *S. aureus* (Rosato et al., 2010). In contrast, the combination with gentamicin against *E. coli, Acinetobacter baumannii* and another strain of *S. aureus* was less effective and more likely to be additive than synergistic; the isobologram method showed some synergism whilst the FIC index indicated an additive effect (Rosato et al., 2010). Oregano oil and gentamicin were additive against *Yersinia enterocolitica* (Rosato et al., 2010). Results for the combination of oregano oil with levofloxacin and maquindox against *E. coli* showed a FIC index of 0.5, which just classifies as synergy (Si et al., 2008). A study investigating thyme oil reported a synergistic effect in combination with ciprofloxacin against *S. aureus* and *Klebsiella pneumoniae* (Van Vuuren et al., 2009). However, with a FIC between 0.5 and 1.0, this combination

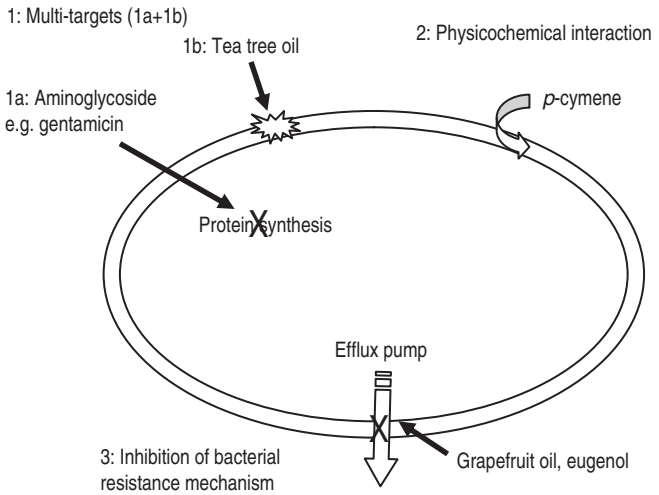

Figure 4. Mechanisms which may contribute to synergy between EO components and antibiotics (Hemaiswarya et al., 2008) with examples from this review: 1, multi-target effects: tea tree oil and aminoglycosides (D'Arrigo et al., 2010; Rosato et al., 2010); 2, physicochemically active adjuvants: *p*-cymene (Ultee et al., 2002); 3, inhibitors of bacterial resistance mechanisms: grapefruit oil, eugenol and thyme (Abulrob et al., 2004; Hemaiswarya & Doble, 2009).

would not be classed as synergistic by other researchers (*vide supra*).

Vancomycin is the one of the few antibiotics available to treat MRSA infections and resistance has already been reported (Mahboubi & Ghazian Bidgoli, 2010). Shiraz oregano (*Zataria multiflora*) EO showed synergy with vancomycin against MSSA and 12 clinical isolates of MRSA, although the FIC data for individual strains are not stated (Mahboubi & Ghazian Bidgoli, 2010). The composition of this oil (thymol 38.7%, carvacrol 15.3% and *p*-cymene 10.2%) is very similar to other oregano oils (Burt, 2004; Mahboubi & Ghazian Bidgoli, 2010).

Clove oil has been tested for synergy with ampicillin and gentamicin against a number of periodontic pathogens. Although a less stringent qualification for synergy was used than described above, FIC indices of less than 0.5 were found for ampicillin against *Streptococcus mutans*, *S. sobrinus* and *S. gordonii* and for gentamicin against *S. sanguinis*, *S. criteci* and *Porphyromonas gingivalis* (Moon et al., 2011).

Australian tea tree (*M. alternifolia*) oil combinations with aminoglycoside antibiotics have been investigated. Bacterial species for which synergism was found with gentamicin were *E. coli, Y. enterocolitica*, *S. marcescens* and one strain of *S. aureus* (Rosato et al., 2010). Against *A. baumannii*, *B. subtilis* and another strain of *S. aureus* the FIC index was borderline between additivity and synergism (Rosato et al., 2010). Tea tree oil with tobramycin also had a synergistic effect against *E. coli* and *S. aureus* (D'Arrigo et al., 2010). Aminoglycosides inhibit protein synthesis and tea tree oil damages the cytoplasmic membrane of bacteria; possibly this is an example of multi-target synergy. In contrast, tea tree oil was shown to have an additive/indifferent effect with the glycopeptide vancomycin against one clinical isolate of MRSA and an antagonistic effect against another (LaPlante, 2007).

Interactions between individual constituents of EOs and antibiotics

Most studies reported here have determined *in vitro* whether synergy exists and have not fully investigated the underlying mechanism. Since many EO compounds exhibit general perturbation effects on the cell membranes of bacteria (Helander et al., 1998; Ultee et al., 2002) and most antibiotics have specific targets in protein or DNA synthesis (Table 2), it seems likely that synergy in most cases may be due to multi-target effects. However, there are some indications for synergy between antibiotics and EO constituents in targeting bacterial resistance mechanisms, specifically the inhibition of efflux pumps (Kollanoor Johny et al., 2010; Lorenzi et al., 2009; Shahverdi et al., 2007). However, it is also proposed that efflux pumps can be inhibited through membrane disruption and inhibition of metabolic pathways (Gibbons, 2008). Two components of grapefruit oil reduced the MIC of norfloxacin against four strains of MRSA from above 100 μg/mL to between 5 and 10 μg/mL, although the FIC index was not calculated (Abulrob et al., 2004). The compounds were isolated and identified as bergamottin and coumarin epoxides; their mechanism of synergy is proposed to be the blocking of efflux pumps (Abulrob et al., 2004). It would be interesting if the hypothesis that EOs inhibit efflux pumps could be confirmed by further investigations.

Eugenol, a constituent of clove oil, was tested in combination with antibiotics representing eight of the antibiotic groups (described in Table 2) against *E. coli*, *E. aerogenes*, *P. vulgaris*, *P. aeruginosa* and *S. typhimurium*. Synergy was found for all groups of antibiotics tested including penicillin and chloramphenicol, but was most apparent for ampicillin, polymyxin B, norfloxacin, tetracycline, rifampicin and vancomycin (Hemaiswarya & Doble, 2009). Eugenol has long been an ingredient of dental root canal sealants (Manabe, 1987) and synergy between eugenol and antibiotics against a number of reference strains of cariogenic and periodontopathogenic bacteria has also been examined (Moon et al., 2011). Eugenol was synergistic with ampicillin against *S. criceti* and *S. gordonii* (FIC index = 0.375) and with gentamicin against *S. sanguinis* and *P. gingivalis* (FIC index = 0.375) (Moon et al., 2011).

The findings for interactions between eugenol and beta-lactam antibiotics against *E. coli* strains are varied. Gallucci et al. (2006) found a synergistic effect for the combination of penicillin with eugenol against an unspecified strain of *E. coli* (FIC index = 0.16). However, eugenol in combination with ampicillin, penicillin or erythromycin was shown to have only an additive effect against *E. coli* (FIC index = 0.5) by Palaniappan & Holley (2010). Findings of synergism between tetracycline and eugenol against *E. coli* (FIC index = 0.16) and *S. typhimurium* (FIC index = 0.22) (Palaniappan & Holley, 2010) are confirmed by other reports (Hemaiswarya & Doble, 2009).

In a study comparing several EO constituents as synergists for antibiotics against drug-resistant strains of *S. typhimurium*, *E. coli* and *S. aureus*, carvacrol and thymol were synergistic in more cases than eugenol (Palaniappan & Holley, 2010). The mechanism involved was purported to be increased ingress of antibiotics via permeabilized membranes and/or

inhibition of protective enzymes (Palaniappan & Holley, 2010). This difference in performance may be due to the ring of delocalized electrons present in carvacrol and thymol but lacking in eugenol (Figure 1), which is purported to assist in the positioning in the hydrophobic membranes (Veldhuizen et al., 2006). Relatively small differences in chemical structure between EO components can have significant effects on the ability to synergize with antibiotics. For example, carvacrol and thymol are structurally similar, differing only in the location of the hydroxyl group (Figure 1). However, carvacrol was synergistic in combination with both ampicillin and nitrofurantoin against *Klebsiella oxytoca* isolated from animal feed (FIC index 0.15–0.375) whilst thymol was indifferent (FIC index 0.55–0.75) (Zhang et al., 2011) (Table 4). Cinnamaldehyde, which contains a prop-2-enal side group to the benzene ring, was synergistic with fewer of the antibiotics than the phenols carvacrol and thymol (Palaniappan & Holley, 2010; Zhang et al., 2011), which could provide some initial indications on the mode of action of these EO components.

Some phenylpropanoids which are present in foods have antimicrobial properties, particularly cinnamic acid and related compounds (Hemaiswarya & Doble, 2010). Synergistic results were found for reference strains of *E. coli* and *S. aureus* when a combination of cinnamic acid with amikacin, ampicillin, ciprofloxacin, erythromycin or vancomycin was tested (FIC indices: 0.14–0.42 and 0.18–0.4, respectively). In contrast, for *P. aeruginosa* the only synergistic combination found (with ciprofloxacin) was borderline (FIC index = 0.49) (Hemaiswarya & Doble, 2010).

Two of the earliest studies on the use of EO components to enhance the efficacy of antibiotics were carried out using *Mycobacterium avium* and *M. tuberculosis* and reported that trans-cinnamic acid (which historically has been used to treat tuberculosis) with amikacin had a synergistic effect against both species when measured by radiometric growth index (Rastogi et al., 1994, 1998; Ryan, 1992). For *M. avium*, the mode of action is proposed to be related to the similarity between the chemical structures of trans-cinnamic acid and phenylalanine, a component of the *M. avium* outer wall (Rastogi et al., 1994), but this is excluded as a mechanism for *M. tuberculosis* (Rastogi et al., 1998). Interestingly, *cis*-cinnamic acid in combination with rifampicin was recently shown to give a greater effect against a clinical isolate of multiple drug resistant *M. tuberculosis* than *trans*-cinnamic acid, which is the isomer usually found in plants (Chen et al., 2011; Turner et al., 1993). The study measured the concentration of both antibacterials required to achieve a significant reduction in viable number of *M. tuberculosis*, individually and combined. The observed effect was reported to be synergistic although no FIC index was stated. Electron micrographs showed physical changes (roughening) of the exterior of the bacteria after 24 h treatment with cis-cinnamic acid (Chen et al., 2011).

In vivo and clinical synergy studies

To the authors' knowledge, no *in vivo* studies have been published on the use of EOs and their components for enhancing the efficacy of antibiotics as antibacterial therapy. However, a few studies on the use of EOs in wound care give an indication of the potential that combined therapy may have. For example, a clinical study was carried out to investigate an EO mixture containing mainly eucalyptus oil (a skin antiseptic containing α-pinene, 1,8-cineole, γ-terpinene and ρ-cymene (Tyagi & Malik, 2011)) as a treatment for necrotic ulcers of cancer patients on antibiotics. The EO treatment led to a reduction in inflammation and malodors, and in some cases to the complete reduction of the ulcers (Warnke et al., 2006). Though the modes of administration for the EO mixture and antibiotic were different, an interaction between the two substances at the site of infection cannot be ruled out. In a small study, in which tea tree (*M. alternifolia*) oil was used as a preliminary treatment against non-resistant *M. tuberculosis*, two patients inhaled the oil as an aerosol daily for 14 days. Before starting antibiotic therapy, coughing had eased and sputum cultures were negative for *M. tuberculosis* (Sherry et al., 2004). Although in these cases there was no simultaneous administration of antibiotic and EO, the physicians intend to investigate whether tea tree oil could contribute to TB treatment (Sherry et al., 2004).

Conclusions

The authors appreciate that most of the studies showing synergy reported here present results which represent moderate synergism, most likely due to membrane interactions of the EO compounds. Although it is conceivable that compounds with the largest effects are most likely to have clinical relevance, one should not disregard compounds which show smaller effects. In addition the availability, toxicology, organoleptic aspects and interaction of the compound with other components or drugs when used in a clinical setting are at least equally important when considering these compounds for clinical use or in foods or animal feeds. Furthermore, *in vitro* qualification of synergy versus additivity depends on the methodology used and the definitions of synergy. One should not put too much emphasis on the actual terminology based on an arbitrarily used definition of FIC < 0.5. Furthermore, studies showing no synergism are less likely to lead to a publication, indicating that more compounds could have beneficial effects than are reported in the literature so far. However, even if only additivity is observed these compounds still provide an interesting option to reduce the use of antibiotics. It is clear that a greater understanding of the exact mechanisms of action of EOs and their components is required before they can be put into use in this respect. The mode of administration will be an important factor to ensure EOs reach the location where bacterial infection occurs in high enough concentrations and thereby determining the efficacy of the EOs in combination with antibiotics. In addition, factors that can affect the efficacy of EOs *in vivo*, for example the presence of mucus or serum proteins, should be better documented. With this in mind, a topical application of EOs is most easily envisioned and could contribute to a reduction in antibiotic use for infections. However, use of EOs systemically, although challenging, should not be ruled out as a future application. The use of

EOs probably will not completely resolve the current antibiotic resistance problems but could play a part in the overall solution to reducing antibiotic use.

There are many EO components as yet untested for their potential to enhance the efficacy of antibiotics. The E-test assay may be useful for screening large number of antibiotic-component combinations, although the reliability of the assay can be limited with drugs with extremely low MICs (Arroyo et al., 2005). Even though the majority of studies use the checkerboard method for fine testing of combinations, studies take different approaches to the data analysis. Harmonization of techniques used for synergism studies would be a useful development.

Developing a sufficiently large supply of medicinal plant compounds may be an obstacle to developing new medicines from plants (McChesney, 2007). The supply must be large enough to span the period of initial mechanistic and feasibility studies and also be developed further to supply market needs should a drug evolve from it. Furthermore, the production chain should be sustainable and reliable (McChesney, 2007).

The use of components instead of whole EOs has two major benefits. First, the reproducibility should be better because of the batch-to-batch variability in EO composition. Second, true mechanistic studies on synergistic/additive effects with existing antibiotics are easier to perform. One important issue is that the general tendency in current research is that only the active and very abundant EO components are considered in the mechanistic studies. However, especially in regard to the inhibition of efflux pumps, components that are not actually antimicrobial themselves may provide the most interesting options to reactivate conventional antibiotics. This was clearly shown by the studies using grapefruit oil (Abulrob et al., 2004), but the enhancing effect of *p*-cymene on the activity of carvacrol (Ultee et al., 2002) also indicates that non-antibacterial components potentially can contribute significantly to the antibacterial activity of antibiotics.

The methods used to evaluate interactions between EOs and antibiotics differ widely and this makes comparison of the data difficult; the citation of FIC indices provides a relatively useful tool for comparison. The development of a more standardized method of serial passaging in sublethal concentrations of EO would enable better investigation of possible loss of sensitivity or cross-resistance.

In conclusion, with EO adjuvants, several resistant strains including ESBL-producers, MRSA, penicillin-resistant *S. aureus* and multidrug resistant *S. typhimurium* DT104 can be rendered sensitive to antibiotics (Abulrob et al., 2004; Kollanoor Johny et al., 2010; Kon & Rai, 2012; Lorenzi et al., 2009; Orhan et al., 2011; Si et al., 2008; Vinod et al., 2010). Generally, repeated passaging in EOs leads to no significant effects on bacterial sensitivity (Becerril et al., 2012; Gomes Neto et al., 2012; Hammer et al., 2012). With more knowledge of the mechanism underlying the synergism it may be possible to develop safe drug combinations and reduce the health impact of multi-drug resistance.

Declaration of interest

The authors have no conflicts of interest.

References

Abulrob A-N, Suller MTE, Gumbleton M, et al. (2004). Identification and biological evaluation of grapefruit oil components as potential novel efflux pump modulators in methicillin-resistant *Staphylococcus aureus* bacterial strains. Phytochemistry 65:3021–7.

Arroyo LA, García-Curiel A, Pachón-Ibañez ME, et al. (2005). Reliability of the E-test method for detection of colistin resistance in clinical isolates of *Acinetobacter baumannii*. J Clin Microbiol 43:903–5.

Bakkali F, Averbeck S, Averbeck D, Idaomar M. (2008). Biological effects of essential oils – a review. Food Chem Toxicol 46:446–75.

Basri DF, Zin NM, Bakar NS, et al. (2008). Synergistic effects of phytochemicals and oxacillin on laboratory passage-derived vancomycin-intermediate *Staphylococcus aureus* strain. J Med Sci 8:131–6.

Bassole IHN, Juliani HR. (2012). Essential oils in combination and their antimicrobial properties. Molecules 17: 3989–4006.

Becerril R, Nerin C, Gomez-Lus R. (2012). Evaluation of bacterial resistance to essential oils and antibiotics after exposure to oregano and cinnamon essential oils. Foodborne Pathogens Dis 9:699–705.

Bhat AS, Ahangar AA. (2007). Methods for detecting chemical-chemical interaction in toxicology. Toxicol Mech Meth 17:441–50.

Bolla J-M, Alibert-Franco S, Handzlik J, et al. (2011). Strategies for bypassing the membrane barrier in multidrug resistant gram-negative bacteria. FEBS Lett 585:1682–90.

Boucher HW, Talbot GH, Bradley JS, et al. (2009). Bad bugs, no drugs: No ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 48:1–12.

Burt S. (2004). Essential oils: their antibacterial properties and potential applications in foods – a review. Int J Food Microbiol 94:223–53.

Burt SA, Vlielander R, Haagsman HP, Veldhuizen EJA. (2005). Increase in activity of essential oil components carvacrol and thymol against *Escherichia coli* O157:H7 by addition of food stabilizers. J Food Prot 68: 919–26.

Bush K, Courvalin P, Dantas G, et al. (2011). Tackling antibiotic resistance. Nat Rev Microbiol 9: 894–6.

Carneiro de Barros J, Lúcia da Conceição M, Gomes Neto NJ, et al. (2009). Interference of *Origanum vulgare* L. essential oil on the growth and some physiological characteristics of *Staphylococcus aureus* strains isolated from foods. LWT – Food Sci Technol 42:1139–43.

Carson CF, Hammer KA, Riley TV. (2006). *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. Clin Microbiol Rev 19:50–62.

Chen Y, Huang S., Sun F, et al. (2011). Transformation of cinnamic acid from trans- to cis-form raises a notable bactericidal and synergistic activity against multiple-drug resistant *Mycobacterium tuberculosis*. Eur J Pharm Sci 43:188–94.

Choi J, Kang O, Lee Y, et al. (2009). Antibacterial activity of methyl gallate isolated from *Galla rhois* or carvacrol combined with nalidixic acid against nalidixic acid resistant bacteria. Molecules 14:1773–80.

Christoph F, Kaulfers P, Stahl-Biskup E. (2000). A comparative study of the in vitro antimicrobial activity of tea tree oils with special reference to the activity of β-triketones. Planta Med 66:556–60.

CLSI. (2009). Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically: Approved standard – 8th edition. CLSI document M07-A8. Wayne, PA: Clinical and Laboratory Standards Institute.

Cox SD, Mann CM, Markham JL, et al. (2001). Determining the antimicrobial actions of tea tree oil. Molecules 6:87–91.

D'Arrigo M, Ginestra G, Mandalari G, et al. (2010). Synergism and postantibiotic effect of tobramycin and *Melaleuca alternifolia* (tea tree) oil against *Staphylococcus aureus* and *Escherichia coli*. Phytomedicine 17:317–22.

Di Pasqua R, Betts G, Hoskins N, et al. (2007). Membrane toxicity of antimicrobial compounds from essential oils. J Agric Food Chem 55:4863–70.

Di Pasqua R, Mamone G, Ferranti P, et al. (2010). Changes in the proteome of *Salmonella enterica* serovar Thompson as stress adaptation to sublethal concentrations of thymol. Proteomics 10:1040–9.

Duarte A, Ferreira S, Silva F, Domingues FC. (2012). Synergistic activity of coriander oil and conventional antibiotics against *Acinetobacter baumanii*. Phytomedicine 19:236–8.

ECDC/EMEA. (2009). Technical report: the bacterial challenge: time to react. European Centre for Disease Prevention and Control/European Medicines Agency Joint Working Group, Stockholm.

Ejim L, Farha MA, Falconer SB, et al. (2011). Combinations of antibiotics and nonantibiotic drugs enhance antimicrobial efficacy. Nature Chem Biol 7:348–50.

EUCAST. (2000). Terminology relating to methods for the determination of susceptibility of bacteria to antimicrobial agents. European Committee for Antimicrobial Susceptibility Testing of the European Society of Clinical Microbiology and Infectious Diseases (EUCAST) definitive document E. Def 1.2,503–508, CMI, Vol. 6.

Fadli M, Chevalier J, Saad A, et al. (2011). Essential oils from Moroccan plants as potential chemosensitisers restoring antibiotic activity in resistant gram-negative bacteria. Int J Antimicrob Agents 38:325–30.

Fadli M, Saad A, Sayadi, S, et al. (2012). Antibacterial activity of *Thymus maroccanus* and *Thymus broussonetii* essential oils against nosocomial infection – bacteria and their synergistic potential with antibiotics. Phytomedicine 19:464–71.

Fleming A. (1929). On the antibacterial action of cultures of a *Penicillium*, with special reference to their use in the isolation of *B. influenzae*. Br J Exp Pathol 10:226–36.

Franz C, Baser KHC, Windisch W. (2010). Essential oils and aromatic plants in animal feeding – a European perspective: a review. Flavour Fragrance J 25:327–40.

Gallucci N, Casero C, Oliva M, et al. (2006). Interaction between terpenes and penicillin on bacterial strains resistant to beta-lactam antibiotics. Molec Med Chem 10:30–2.

Gibbons, S. (2008). Phytochemicals for bacterial resistance – strengths, weaknesses and opportunities. Planta Med 74:594–602.

Gill AO, Holley RA. (2004). Mechanisms of bactericidal action of cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *L. monocytogenes* and *Lactobacillus* sakei. Appl Environ Microbiol 70: 5750–5.

Gill AO, Holley RA. (2006a). Disruption of *E. coli*, *L. monocytogenes* and *L. sakei* cellular membranes by plant essential oils aromatics. Int J Food Microbiol 108:1–9.

Gill AO, Holley RA. (2006b). Inhibition of membrane bound ATPases of *Escherichia coli* and *Listeria monocytogenes* by plant oil aromatics. Int J Food Microbiol 3:170–4.

Goldman E. (2004). Antibiotic abuse in animal agriculture: exacerbating drug resistance in human pathogens. Human Ecol Risk Assess 10:121–34.

Gomes Neto NJ, Luz ID, Tavares AG, et al. (2012). *Rosmarinus officinalis* L. essential oil and its majority compound 1,8-cineole at sublethal amounts induce no direct and cross protection in *Staphylococcus aureus* ATCC 6538. Foodborne Pathogens Dis 9:1–6.

Greay SJ, Hammer KA. (2011). Recent developments in the bioactivity of mono- and diterpenes: anticancer and antimicrobial activity. Phytochem Rev, DOI 10.1007/s11101-011-9212-6 (1–6).

Guenther E. (1948). The essential oils. Vol. 1. New York: D. van Nostrand Company Inc.

Guerra FQS, Mendes JM, De Sousa JP, et al. (2012). Increasing antibiotic activity against a multidrug-resistant *Acinetobacter* spp. by essential oils of *Citrus limon* and *Cinnamomum zeylanicum*. Nat Prod Res 26:2235–38.

Hammer KA, Carson CF, Riley TV. (2008). Frequencies of resistance to *Melaleuca alternifolia* (tea tree) oil and rifampicin in *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis*. Int J Antimicrob Agents 32:170–3.

Hammer KA, Carson CF, Riley TV. (2012). Effects of *Melaleuca alternifolia* (tea tree) essential oil and the major monoterpene component terpinen-4-ol on the development of single- and multistep antibiotic resistance and antimicrobial susceptibility. Antimicrob Agents Chemother 56:909–15.

Harkenthal M, Reichling J, Geiss H, Saller R. (1999). Comparative study on the in vitro antibacterial activity of Australian tea tree oil, cajuput oil, niaouli oil, manuka oil, kanuka oil, and eucalyptus oil. Pharmazie 54:460–3.

Helander IM, Alakomi H-L, Latva-Kala K, et al. (1998). Characterization of the action of selected essential oil components on gram negative bacteria. J Agric Food Chem 46:3590–5.

Hemaiswarya S, Doble M. (2009). Synergistic interaction of eugenol with antibiotics against gram negative bacteria. Phytomedicine 16:997–1005.

Hemaiswarya S, Doble M. (2010). Synergistic interaction of phenylpropanoids with antibiotics against bacteria. J Med Microbiol 59: 1469–76.

Hemaiswarya S, Kruthiventi AK, Doble M. (2008). Synergism between natural products and antibiotics against infectious diseases. Phytomedicine 15:639–52.

Huovinen P. (2001). Resistance to trimethoprim-sulfamethoxazole. Clin Infect Dis 32:1608–14.

Hyldgaard M, Mygind T, Meyer RL. (2012). Essential oils in food preservation: mode of action, synergies and interactions with food matrix components. Front Microbiol 3:1–24.

Kohanski MA, Dwyer DJ, Collins JJ. (2010). How antibiotics kill bacteria: from targets to networks. Nat Rev Microbiol 8:423–35.

Kollanoor Johny AK, Hoagland T, Venkitanarayanan K. (2010). Effect of subinhibitory concentrations of plant-derived molecules in increasing the sensitivity of multidrug-resistant *Salmonella enterica* serovar Typhimurium DT104 to antibiotics. Foodborne Pathogens Dis 7:1165–70.

Kon KV, Rai MK. (2012). Plant essential oils and their constituents in coping with multidrug-resistant bacteria. Expert Rev Anti Infect Ther 10:775–90.

LaPlante KL. (2007). In vitro activity of lysostaphin, mupirocin, and tea tree oil against clinical methicillin-resistant *Staphylococcus aureus*. Diagn Microbiol Infect Dis 57:413–18.

Lehtinen J, Lilius E-M. (2007). Promethazine renders *Escherichia coli* susceptible to penicillin G: real-time measurement of bacterial susceptibility by fluoro-luminometry. Int J Antimicrob Agents 30:44–51.

Lewis K. (2012). Recover the lost art of drug discovery. Nature 485:439–40.

Lorenzi V, Muselli A, Bernardini AF, et al. (2009). Geraniol restores antibiotic activities against multidrug-resistant isolates from gram-negative species. Antimicrob Agents Chemother 53:2209–11.

Luz IS, Neto NJG, Tavares AG, et al. (2012). Evidence for lack of acquisition of tolerance in *Salmonella enterica* Typhimurium ATCC 14028 after exposure to subinhibitory amounts of *Origanum vulgare* L. essential oil and carvacrol. Appl Environ Microbiol 78:5021–4.

Ma D, Cook DN, Hearst JE, Nikaido H. (1994). Efflux pumps and drug resistance in gram negative bacteria. Trends Microbiol 2:489–93.

Mahboubi M, Ghazian Bidgoli F. (2010). Antistaphylococcal activity of *Zataria multiflora* essential oil and its synergy with vancomycin. Phytomedicine 17:548–50.

Malik T, Singh P, Pant S, et al. (2011). Potentiation of antimicrobial activity of ciprofloxacin by *Pelargonium graveolens* essential oil against selected uropathogens. Phytother Res 25:1225–8.

Manabe A, Nakayama S, Sakamoto K. (1987). Effects of essential oils on erythrocytes and hepatocytes from rats and dipalmitoyl phosphaditylcholine-liposomes. Japan J Pharmacol 44:77–84.

Mazumdar K, Dastidar SG, Park JH, Dutta NK. (2009). The anti-inflammatory non-antibiotic helper compound diclofenac: an antibacterial drug target. Eur J Clin Microbiol Infect Dis 28:881–91.

McChesney JD, Venkataraman SK, Henri JT. (2007). Plant natural products: back to the future or into extinction? Phytochemistry 68:2015–22

McMahon MA, Tunney MM, Moore JE, et al. (2008). Changes in antibiotic susceptibility in staphylococci habituated to sub-lethal concentrations of tea tree oil (Melaleuca alternifolia). Lett Appl Microbiol 47:263–8.

Moon SE, Kim HY, Cha JD. (2011). Synergistic effect between clove oil and its major compounds and antibiotics against oral bacteria. Arch Oral Biol 56:907–16.

Nikaido H. (1994). Prevention of drug access to bacterial targets: permeability barriers and active efflux. Science 264:382–8.

Nikaido H. (2001). Preventing drug access to targets: cell surface permeability barriers and active efflux in bacteria. Semin Cell Dev Biol 12:215–23.

Nordberg P, Monnet DL, Cars O. (2005). Antibacterial drug resistance: options for concerted action. Geneva, Switzerland: WHO Department of Medicines Policy and Standards, World Health Organization.

Nuryastuti T, Van der Mei HC, Busscher HJ, et al. (2009). Effect of cinnamon oil on icaA expression and biofilm formation by *Staphylococcus epidermidis*. Appl Environ Microbiol 75: 6850–5.

Orhan IE, Ozcelik B, Kan Y, Kartal M. (2011). Inhibitory effects of various essential oils and individual components against extended-spectrum beta-lactamase (ESBL) produced by *Klebsiella pneumoniae* and their chemical compositions. J Food Sci 76:538–46.

Oyedemi SO, Okoh AI, Mabinya LV, et al. (2009). The proposed mechanism of bactericidal action of eugenol, alpha-terpineol and

gamma-terpinene against *Listeria monocytogenes*, *Streptococcus pyogenes*, *Proteus vulgaris* and *Escherichia coli*. African J Biotechnol 8:1280–6.

Palaniappan K, Holley RA. (2010). Use of natural antimicrobials to increase antibiotic susceptibility of drug resistant bacteria. Int J Food Microbiol 140:164–8.

Papadopoulos CJ, Carson CF, Chang BJ, Riley TV. (2008). Role of the MexAB-OprM efflux pump of *Pseudomonas aeruginosa* in tolerance to tea tree (*Melaleuca alternifolia*) oil and its monoterpene components terpinen-4-ol, 1,8-cineole, and α-terpineol. Appl Environ Microbiol 74:1932–5.

Pillai SK, Moellering RC, Eliopoulos GM. (2005). Antimicrobial combinations. In: Lorian V, ed. Antibiotics in laboratory medicine. Philadelphia: Lippincott, Williams and Wilkins, 365–440.

Qiu J, Feng H, Lu J, et al. (2010). Eugenol reduces the expression of virulence-related exoproteins in *Staphylococcus aureus*. Appl Environ Microbiol 76:5846–51.

Rastogi N, Goh KS, Horgen L, Barrow WW. (1998). Synergistic activities of antituberculous drugs with cerulenin and trans-cinnamic acid against *Mycobacterium tuberculosis*. FEMS Immunol Med Microbiol 21:149–57.

Rastogi N, Goh KS, Wright EL, Barrow WW. (1994). Potential drug targets for *Mycobacterium avium* defined by radiometric drug-inhibitor combination techniques. Antimicrob Agents Chemother 38:2287–95.

Rosato A, Piarulli M, Corbo F, et al. (2010). In vitro synergistic antibacterial action of certain combinations of gentamicin and essential oils. Curr Med Chem 17:3289–95.

Rosato A, Vitali C, De Laurentis N, et al. (2007). Antibacterial effect of some essential oils administered alone or in combination with norfloxacin. Phytomedicine 14, 727–32.

Ryan F. (1992). The forgotten plague. Boston, MA: Little, Brown and Company.

Sanders CC, Sanders WE, Moland ES. (1993). Decimal assay for additivity of drugs permits delineation of synergy and antagonism. Antimicrob Agents Chemother 37:260–4.

Santoyo S, Cavero S, Jaime L, et al. (2006). Supercritical carbon dioxide extraction of compounds with antimicrobial activity from *Origanum vulgare* L.: determination of optimal extraction parameters. J Food Prot 69:369–75.

Schelz Z, Molnar J, Hohmann J. (2006). Antimicrobial and antiplasmid activities of essential oils. Fitoterapia 77:279–85.

Sengeløv G, Agersø Y, Halling-Sørensen B, et al. (2003). Bacterial antibiotic resistance levels in Danish farmland as a result of treatment with pig manure slurry. Environ Int 28:587–95.

Shahverdi AR, Monsef-Esfahani HR, Tavasoli F, et al. (2007). Trans-cinnamaldehyde from *Cinnamomum zeylanicum* bark essential oil reduces the clindamycin resistance of *Clostridium difficile* in vitro. J Food Sci 72:S55–8.

Sheldon AT. (2005). Antibiotic resistance: a survival strategy. Clin Lab Sci 18:170–80.

Sherry E, Reynolds M, Sivananthan S, et al. (2004). Inhalational phytochemicals as possible treatment for pulmonary tuberculosis: two case reports. Am J Infect Control 32, 369–70.

Shin S. (2005). Anti-*Salmonella* activity of lemongrass oil alone and in combination with antibiotics. Nat Prod Sci 11, 160–4.

Shin S, Kim JH. (2005). In vitro inhibitory activities of essential oils from two Korean *Thymus* species against antibiotic-resistant pathogens. Arch Pharmacal Res 28:897–901.

Si H, Hu J, Liu Z, Zeng Z-L. (2008). Antibacterial effect of oregano essential oil alone and in combination with antibiotics against extended-spectrum β-lactamase-producing *Escherichia coli*. FEMS Immunol Med Microbiol 53:190–4.

Telenti A, Imboden P, Marchesia F, et al. (1993). Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*. Lancet 341:647–50.

Trombetta D, Castelli F, Sarpietro MG, et al. (2005). Mechanisms of antibacterial action of three monoterpenes. Antimicrob Agents Chemother 49:2474–8.

Turner LB, Mueller-Harvey I, MacAllan AB. (1993). Light-induced isomerization and dimerization of cinnamic acid derivatives in cell walls. Phytochemistry 33:791–6.

Tyagi AK, Malik A. (2011). Antimicrobial potential and chemical composition of *Eucalyptus globulus* oil in liquid and vapour phase against food spoilage microorganisms. Food Chem 126:228–35.

Udwadia ZF, Amale RA, Ajbani KK, Rodrigues C. (2011). Totally drug-resistant tuberculosis in India. Clin Infect Dis 54:579–81.

Ultee A, Bennik MHJ, Moezelaar R. (2002). The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Appl Environ Microbiol 68:1561–8.

Van de Braak SAAJ, Leijten GCJJ. (1999). Essential oils and oleoresins: a survey in the Netherlands and other major markets in the European Union. Rotterdam, The Netherlands: CBI, Centre for the Promotion of Imports from Developing Countries.

Van Hoek AHM, Mevius D, Guerra B, et al. (2011). Acquired antibiotic resistance genes: an overview. Front Microbiol 2, 1–27.

Van Vuuren SF, Suliman S, Viljoen AM. (2009). The antimicrobial activity of four commercial essential oils in combination with conventional antimicrobials. Lett Appl Microbiol 48, 440–6.

Van Vuuren S, Viljoen A. (2011). Plant-based antimicrobial studies – methods and approaches to study the interaction between natural products. Planta Med 77:1168–82.

Veldhuizen EJA, Tjeerdsma-van Bokhoven JLM, Zweijtzer C, et al. (2006). Structural requirements for the antimicrobial activity of carvacrol. J Agric Food Chem 54:1874–9.

Veras HNH, Rodrigues FFG, Colares AV, et al (2012). Synergistic antibiotic activity of volatile compounds from the essential oil of *Lippia sidoides* and thymol. Fitoterapia 83:508–12.

Vinod NV, Shijina R, Dileep KV, Sadasivan C. (2010). Inhibition of beta-lactamase by 1,4-naphthalenedione from the plant *Holoptelea integrifolia*. Appl Biochem Biotechnol 160:1752–9.

Wagner H, Ulrich-Merzenich G. (2009). Synergy research: approaching a new generation of phytopharmaceuticals. Phytomedicine 16:97–110.

Warnke PH, Sherry E, Russo PAJ, et al. (2006). Antibacterial essential oils in malodorous cancer patients: clinical observations in 30 patients. Phytomedicine 13:463–7.

Wendakoon CN, Sakaguchi M. (1993). Combined effect of sodium chloride and clove on growth and biogenic amine formation of *Enterobacter aerogenes* in mackerel muscle extract. J Food Prot 56: 410–13.

Wendakoon CN, Sakaguchi M. (1995). Inhibition of amino acid decarboxylase activity of *Enterobacter aerogenes* by active components in spices. J Food Prot 58:280–3.

White RL, Burgess DS, Manduru M, Bosso JA. (1996). Comparison of three different in vitro methods of detecting synergy: time-kill, checkerboard, and E test. Antimicrob Agents Chemother 40:1914–18.

Woehrlin F, Fry H, Abraham K, Preiss-Weigert A. (2010). Quantification of flavoring constituents in cinnamon: high variation of coumarin in cassia bark from the German retail market and in authentic samples from Indonesia. J Agric Food Chem 58:10568–75.

World Health Organization. (2012). Antimicrobial resistance, fact sheet 194. Available from: http://www.who.int/mediacentre/factsheets/fs194/en/ [last accessed 31 May 2012).

Zhang D, Hu H, Rao Q, Zhao Z. (2011). Synergistic effects and physiological responses of selected bacterial isolates from animal feed to four natural antibacterials and two antibiotics. Foodborne Pathogens Dis 8:1055–62.