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REVIEW

Synergism between natural products and antibiotics against infectious diseases

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Abstract

Antibiotics have been effective in treating infectious diseases, but resistance to these drugs has led to the emergence of new and the reemergence of old infectious diseases. One strategy employed to overcome these resistance mechanisms is the use of combination of drugs, such as β-lactams together with β-lactamase inhibitors. Several plant extracts have exhibited synergistic activity against microorganisms. This review describes in detail, the observed synergy and mechanism of action between natural products including flavonoids and essential oils and synthetic drugs in effectively combating bacterial, fungal and mycobacterial infections. The mode of action of combination differs significantly than that of the same drugs acting individually; hence isolating a single component may lose its importance thereby simplifying the task of pharma industries.

Keywords: Infectious diseases; Antibiotic resistance; Natural products; Resistance modifying agents; Synergy

Introduction

Infectious diseases are caused by bacteria, viruses, parasites and fungi, and it is due to a complex interaction between the pathogen, host and the environment. The discovery of antibiotics had eradicated the infections that once ravaged the humankind. But their indiscriminate use has led to the development of multidrug-resistant pathogens. Around 90–95% of *Staphylococcus aureus* strains worldwide are resistant to penicillin (Casal et al., 2005) and in most of the Asian countries 70–80% of the same strains are methicillin resistant (Chambers, 2001). There are considerable reports on the progress of resistance to the last line of antibiotic defense, which has led to the search for reliable methods to control vancomycin-resistant *Enterococci* (VRE) and *S. aureus* (VRSA), and methicillin-resistant *S. aureus* (MRSA). In addition, the synergy between tuberculosis and the AIDS epidemic, along with the surge of multidrug-resistant isolates of *Mycobacterium tuberculosis*, has reaffirmed it as a primary health threat. Multidrug-resistant TB (MDRTB) is associated with high death rates (50–80%), spanning within a relatively short period of time (4–16 weeks) from diagnosis to death (WHO, 2004). In developing countries, MDRTB has increased in incidence and it interferes with TB control programs.

Plant-derived antibacterials are always a source of novel therapeutics. A quick look at the way nature, especially plants, are tackling the issue of infection will provide a deeper understanding of the methodology, which needs to be adopted for the design and development of novel highly effective antiinfectious agents in general, and antimycobacterials in particular. The scarcity of infective diseases in wild plants is in itself an indication of the successful defense mechanisms

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developed by them. Plants are known to produce an enormous variety of small-molecule (MW < 500) antibiotics – generally classified as 'phytoalexins'. Their structural space is diverse having terpenoids, glycosteroids, flavonoids and polyphenols. Be that as it may, it is interesting to note that most of these small molecules have weak antibiotic activity – several orders of magnitudes less than that of common antibiotics produced by bacteria and fungi. In spite of the fact that plant-derived antibacterials are less potent, plants fight infections successfully. Hence, it becomes apparent that plants adopt a different paradigm – "synergy" – to combat infections. A case in study to reiterate this view is the observation on the combined action of berberine and 5′-methoxyhydnocarpin, both of which are produced by berberry plants. Berberine, a hydrophobic alkaloid that intercalates into DNA, is ineffective as an antibacterial because it is readily extruded by pathogen – encoded multidrug resistance pumps (MDRs). Hence, the plant produces 5′-methoxyhydnocarpin that blocks the MDR pump (Stermitz et al., 2000). This combination is a potent antibacterial agent (Lewis and Ausubel, 2006). Using this cue, Ball et al. (2006) reported that covalently linking berberine to INF_{55}, an inhibitor of MDR, results in a highly effective antibiotic that readily accumulates in bacteria.

This paper introduces and provides examples of synergistic interactions of the secondary metabolites of plants with antibiotics in the treatment of infectious diseases. The understanding of the molecular mechanisms of synergy would pave a new strategy for the treatment of infectious diseases, overcome drug-resistant pathogens, and decrease the use of antibiotics and hence the side effects created by them.

Synergy towards bacterial infection

The development of antibiotic resistance can be natural (intrinsic) or acquired and this can be transmitted within same or different species of bacteria. Natural resistance is achieved by spontaneous gene mutation and the acquired resistance is through the transfer of DNA fragments like transposons from one bacterium to another. Bacteria gains antibiotic resistance due to three reasons namely: (i) modification of active site of the target resulting in reduction in the efficiency of binding of the drug, (ii) direct destruction or modification of the antibiotic by enzymes produced by the organism or, (iii) efflux of antibiotic from the cell (Sheldon, 2005). One strategy employed to overcome these resistance mechanisms is the use of combination of drugs. Inhibitors of β-lactamases have been long known and they are administered with antibiotics as co-drugs. The most successful strategy that has been adopted to overcome the resistance to penicillinase is by administering clavulinic acid, with the drugs sulbactam and tazobactam (Lee et al., 2003b). But the frequent use of clavulanate has led to the emergence of resistant bacterial strains (Blasquez et al., 1993; Enright et al., 2002). The appearance of extended spectrum β-lactamase and resistance against IMP-1 (a new β-lactam), cephalosporins and carbapenems have further necessitated the need for developing new β-lactamase inhibitors (Chaibi et al., 1999).

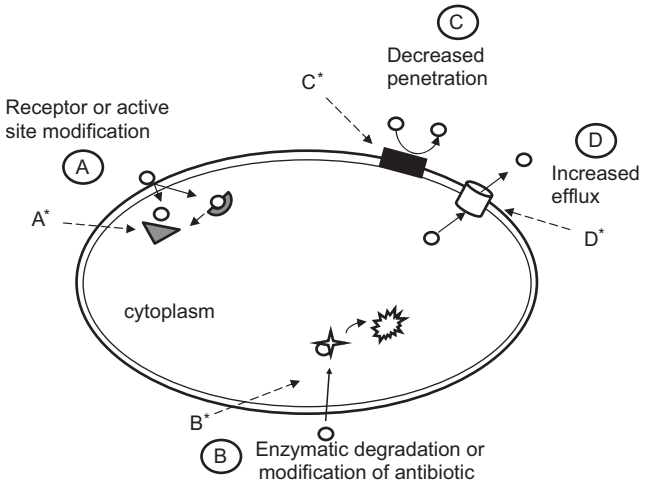

Fig. 1. Plant secondary metabolites as modifiers of multidrug resistance mechanisms. – antibiotic drug, – receptor, – modified receptor, – efflux pump, – enzyme, -degradation of the drug. (A*) Corilagin, tellimagrandin I, diterpene 416 and compound P inhibits PBP 2a, a modified receptor; (B*) EGCg inhibits the β-lactamase; (C*) thymol, carvacrol, gallic acid increases the outer membrane permeability; and (D*) EGCg, 5′-methoxyhydnocarpin, reserpine, carnosic acid and isopimarane derivatives inhibit the efflux pumps. See the text for details.

The secondary metabolites from plant are good sources for combination therapy. As shown in Fig. 1, there are a wide range of phytochemicals which act as multidrug resistance modifiers depicted and their mechanism of action is discussed in the following sections.

Receptor or active site modification

For selective antimicrobial action the target site plays a vital role. Introduction of mutations in the target site alters it, leading to a reduction in the activity of the drug towards the microbe. Two examples of receptor (target) modification are (a) mutations in RNA polymerase and DNA gyrase, rendering rifamycins and quinolones inactive (Heep et al., 2000; Willmott and Maxwell, 1993), and (b) modification in the structural confirmation of penicillin-binding proteins (PBPs) resulting in the

development of penicillin resistance. The most important example of a target change is the production of PBP2a, an altered transpeptidase.

β-Lactam antibiotics (BLA) are highly specific inhibitors of the metabolism of peptidoglycan and they target the membrane bound D,D-peptidase domain of the PBPs (Ghysen, 1994). These peptidases cross link the bacterial peptidoglycan cell wall which maintain the integrity of the latter (Berger-Bächi and Rohrer, 2002). *S. aureus* acquires resistance to all penicillins and cephalosporins with the acquisition of the gene mecA (Enright et al., 2002), which is carried on a large genetic element called the staphylococcal cassette chromosome mec (SCC mec). This is acquired by a parasexual horizontal transfer from a coagulase-negative *Staphylococcus* sp. (Ito et al., 2003). The house keeping PBPs are BLA sensitive whereas the MecA (PBP 2a) have reduced affinity to BLA (Lu et al., 1999). A number of BLAs, including modified cephalosporins (Vouillamoz et al., 2004), carbapenems (Kurazono et al., 2004) and trinem (Ferrari et al., 2003) have been designed with enhanced activity against PBP2a.

Another approach to overcome resistance is to include inhibitors of the PBP2a in the treatment strategy. A number of reports are available listing the synergistic interactions of BLA with natural compounds to overcome resistant microorganisms. The later includes catechins (*Camellia sinesis*) (Takahashi et al., 1995), EGCg (Epigallocatechin gallate) from green tea (Suresh et al., 1997), tellimagrandin I and rugosin B from rose red (*Rosa canina*) (Shiota et al., 2000), baicalin from *Scutellaria amoena* (Liu et al., 2000) and corilagin from *Arctostaphylos uva-ursi* (Shimizu et al., 2001). Corilagin, a polyphenol from *Arctostaphylos uva-ursi* is found to markedly reduce the Minimum Inhibitory Concentration (MIC) of β-lactams in MRSA. Shimizu et al. (2001) suggest that there are two possibilities regarding the mechanism of action of corilagin, namely inhibition of PBP2a activity or inhibition of its production. They later reported that the PBP2a of MRSA cells grown in the presence of corilagin or tellimagrandin I lost its ability to bind to BOCILLIN FL, a fluorescent-labeled benzylpenicillin (Shiota et al., 2004).

Studies through reverse transcription-PCR and a semiquantitative PBP2a latex agglutination assays indicted that, EGCg did not suppress either the mRNA expression of PBP2a or its production. But the synergy between EGCg and BLA was achieved since both directly or indirectly attacked the same target site namely, peptidoglycan present on the cell wall (Yam et al., 1998; Zhao et al., 2001). EGCg was found to synergistically enhance the activity of carbapenems against MRSA but the mechanism of action has not been studied (Hu et al., 2002). Nicolson et al. (1999) have shown that diterpene derivative 416 potentiated the activity of methicillin by significantly reducing the expression of PBP2a.

There is a wide list of phytochemicals which act as inhibitors and a few of them are glycosylated flavones suppressing topoisomerase IV activity (Bernard et al., 1997), myricetin inhibiting DnaB helicase (Griep et al., 2007), allicin inhibiting RNA synthesis (Feldberg et al., 1988) and compounds from the plant *Polygonum cuspidatum* inhibiting bacterial DNA primase (Hegde et al., 2004). These phytochemicals when used in combination with other classes of antibiotics have the potential to either inhibit the modified targets or exhibit a synergy by blocking one or more of the other targets in the metabolic pathway. Table 1 lists the synergy observed between natural products and commercial antibiotics against bacteria.

Enzymatic degradation and modification of the drug

Bacterial cells spend a considerable amount of energy to resist antibiotics. One way the cells achieve active drug resistance is by the synthesis of enzymes that selectively target and destroy or modify the antibiotics. The various enzymatic strategies that lead to antibiotic inactivation are through hydrolysis, group transfer or redox mechanisms (Wright, 2005). Hydrolytically susceptible chemical bonds (such as ester or amide bonds) are cleaved by enzymes that are expressed by the resistant organisms. The modification of the active group in the drug through acylation, phosphorylation, glycosylation, nucleotidylation or ribosylation by the organism could make the former innocuous. Redox mechanism involves the oxidation–reduction of the antibiotics leading to the formation of inactive compound (Wright, 2005).

β-Lactamases are one such family of enzymes that cleave the β-lactam ring of cephalosporins and penicillins. They act through the serine residue in the active site of the enzyme or through the activation of the Zn^{2+} center (Bush, 1998, 2002). Inhibitors of β-lactamases have long been known. The combination of ampicillin and sulbactam inhibits β-lactamase and increases the spectrum of activity of the former. Zhao et al. (2002) have confirmed that EGCg inhibits the penicillinase produced by *S. aureus* thereby restoring the activity of penicillin. It acts in a dose-dependent manner, with 50% inhibition at a concentration of 10 μg/ml. The combination of ampicillin and sulbactam is effective but is not powerful enough against MRSA and strains producing β-lactamases. When they are further combined with EGCg, the MIC_{90} of this combination is reduced to 4 mg/ml from an initial value of 16 mg/ml (Hu et al.,

Table 1. Synergism between natural products and antibiotics against bacterial infection

Natural product	Antibiotics	Microorganisms	Mechanism of action	References
Carnosic acid	Tetracycline	Tet (K) possessing strains	Inhibit the MDR pumps, Tet (K) and Msr (A)	Oluwatuyi et al. (2004)
Carnosol	Erythromycin	Msr (A)		
Epigallocatechin-gallate (EGCg)	Ampicillin/sulbactam	MSSA β-lactamase producing *S. aureus*	Inhibits β-lactamase	Hu et al. (2001)
EGCg	Penicillin	Penicillinase producing *S. aureus*	Inhibits penicillinase	Zhao et al. (2001)
	Ampicillin			
EGCg	Carbapenems	MRSA	–	Hu et al. (2002)
EGCg	β-Lactam	MSSA, MRSA	EGCg directly binds to the peptidoglycan and inhibits cell wall	Yoshida et al. (1990)
EGCg	Tetracycline	*S. aureus* with Tet (K) MDR pump	Blocks MDR efflux pumps	Roccaro et al. (2004)
Tea catechin	Oxacillin	MRSA	–	Takahashi et al. (1995)
Totatrol	Methicillin	MSSA, MRSA	PBP 2a production	Pao et al. (1998)
Berberine (*Berberis* plant)	5′-Methoxyhydnocarpin	Nor (A) mutant	Inhibits Nor (A) MDR pump	Smith et al. (2005)
Green tea extract	Levofloxacin	*Escherichia coli* 0157 in gnotobiotic mouse model	–	Isogai et al. (2001)
Craneberry juice extract Blueberry, Grape seed and oregano extract	–	*Helicobacter pylori*	–	Vattem et al. (2005)
Oregano and cranberry extract	Lactic acid	*Vibrio parahemolyticus*	–	Lin et al. (2005)
Isoflavone Bidwillon B from *Erythrina variegata*	Mupirocin	*MRSA*	Bidwillon B and mupirocin inhibited the incorporation of thymidine, uridine, glucose and isoleucine	Sato et al. (2004)
α-Mangostin	Vancomycin	*MRSA and Vancomycin enterococci*	–	Sakagami et al. (2005)
	Gentamycin			
Aqueous crude khat extracts	Tetracycline	*Streptococcus sanguis, Fusobacterium nucleatum*	–	Al-hebshi et al. (2006)
Corilagin from *Arctostaphylos uva-ursi*	β-Lactams such as oxacillin , cefmetazole	MRSA	Inhibits PBP2a production or activity	Shimizu et al. (2001)
Baicalin	β-Lactam antibiotics	MRSA	Inhibits β-lactamase	Liu et al. (2000)
Tellimagrandin I from rose red tree,	β-Lactams	MRSA	–	Shiota et al. (2000)
Rugosin B from rose red tree	β-Lactams	MRSA	–	Shiota et al. (2000)
Diterpenes from *Lycopus europaeus*	Tetracycline	*S. aureus* possessing Tet (K), Msr (A) MDR pumps	Blocks MDR pumps	Gibbons et al. (2003)
	Erythromycin			
A penta-substituted	Erythromycin	*S. aureus* possessing Tet (K),	Blocks MDR pumps	Marquez et al. (2005)

Table 1. (*continued*)

Natural product	Antibiotics	Microorganisms	Mechanism of action	References
pyridine from *Jatropha elliptica*		Nor (A) MDR pumps		
Pomegranate extract	Ciprofloxacin chloramphenicol, gentamicin, ampicillin, tetracycline, and oxacillin	*MRSA*	Blocks Nor (A) pump	Braga et al. (2005)
		MSSA		
Myricetin	Amoxicillin/clavulanate, ampicillin/sulbactam and cefoxitin	Extended-spectrum -lactamases (ESBL) producing *K. pneumoniae*	–	Lin et al. (2005)
Isopimaric acid from *Pinus nigra*	Reserpine	MRSA	Blocks Nor (A) pump	Simonetti et al. (2004)
Totarol, ferulenol (from *Ferula communis*) and plumbagin (from *Plumbago zeylanica*)	Isonicotinic acid hydrazide (INH)	*Mycobacterium intracellulare*, *M. smegmatis, M. xenopei* and *M. chelonei*	–	Mossa et al. (2004)
Erybraedin A or eryzerin C isolated from the roots of *Erythrina zeyheri*,	Vancomycin	Vancomycin-resistant enterococci (VRE) and MRSA	–	Sato et al. (2004)
Butylated hydroxyanisole, (BHA) green tea		*S. mutans*, non-susceptible *E. coli* and *C. albicans*	–	Shiota et al. (2004)
Sophoraflavanone G	Vancomycin hydrochloride, fosfomycin, methicillin, cefzonam, gentamicin, minocycline and levofloxacin	MRSA	–	Sakagami et al. (1998)
Essential oil – 1,8 cineol, linalool, alpha-terpineol and terpinen-4-ol from *Melaleuca leucodendron* and oil from *Ocimum gratissimum*	Antibiotics	Bacterial species	–	Jedlickova et al. (1992)
Novoimanin from *Hypericum perforatum* L.	Ampicillin, kanamycin, fusidic acid and rifocin	*Staphlococcus aureus* 209	–	Avenirova et al. (1975)

2001). The potent synergy between these concoctions could possibly have clinical use.

Reduced accumulation of the antibiotic within the bacterial cell

Reduced accumulation of the antibiotic inside the microorganism could be because of two reasons namely decreased permeability of the drug through the outer membrane of the cell or, the efflux of the accumulated drug out of the cell.

Decreased outer membrane permeability

Cells of Gram-negative bacteria are surrounded by an additional membrane (outer membrane, OM), which provide them with a hydrophilic surface and functions as a permeability barrier for many external hydrophobic agents including detergents, hydrophobic dyes and antibiotics (Helander et al., 1997a; Vaara, 1992, 1999; Nikaido and Vaara, 1985). This barrier is due to the presence of lipopolysaccharide (LPS) molecules in the outer leaflet (Nikaido, 2003; Nikaido and Vaara, 1985), which makes up to 75% of the total membrane surface

and forms specific contacts with integral outer membrane proteins (Omp), such as porins (Alexander and Rietschel, 2001; Bos and Tommassen, 2004). Bacterial lipoproteins anchor the OM to the periplasmic peptidoglycan layer (Brade et al., 1999). Divalent cations are tightly associated with the anionic membrane-proximal regions of the LPS molecules, strengthening the structure (Vaara, 1992). Some Gram-negative bacteria are known to contain glycosphingolipids instead of LPS in their OM (Kawahara et al., 1991).

Bivalent cations contribute to the stability of the OM by creating electrostatic interactions between the proteins and LPS (Leive, 1965; Vaara, 1981, 1999). EDTA is a chelator which sequesters these ions. Treatment with EDTA releases a large proportion of LPS from the OM, exposing the phospholipids and creating a hydrophobic pathway (Leive, 1965). EDTA has been reported to potentiate the activity of cell wall degrading agents including lysozyme, nisin and biocides (Leive, 1965; Vaara, 1981; Walsh et al., 2003a, b). In addition, there are a wide range of permeabilizers such as polycationic polymyxin B nonapeptide, which interact with and disorganize the anionic LPS thereby sensitizing the bacteria to hydrophobic antibiotics (Vaara and Vaara, 1983a, b). Essential oils such as thymol and carvacrol as membrane permeabilizers (Fig. 1) have been studied by Helander and co-workers (Helander et al., 1998). Magnesium chloride disrupts the activity of EDTA and polyethylenimine, but it has no effect on the activity of carvacrol or thymol. This indicates that essential oils neither chelate nor intercalate with LPS by replacing the divalent cations which stabilize the OM (Helander et al., 1997a; Vaara, 1992).

Active efflux

The development of multidrug resistance pumps (MDRs) is one of the defense mechanisms employed by bacteria against the accumulation of antimicrobial drugs inside the cell. These efflux pumps either use ATP hydrolysis or ion gradient to expel the antibiotics. They are grouped into five major classes namely, the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily (Veen and Konings, 1998; Veen et al., 1996), the major facilitator superfamily (MFS) (Pao et al., 1998), the small multidrug resistance family (SMR) (Paulsen et al., 1996), the resistance-nodualtion-cell division RND superfamily (Saier et al., 1994) and the multidrug and toxic compound extrusion (MATE) family (Brown et al., 1999). Of these the RND, SMR and MATE classes are unique to prokaryotes (Lynch, 2006).

The efflux pumps of *S. aureus* Qac A (MFS family), Smr (SMR family) and Nor A (MFS family) have been well characterized. The Nor A efflux pump is responsible for fluoroquinolone resistance (Yoshida et al., 1990); Qac A is responsible for acriflavine and ethidium bromide resistance (Littlejohn et al., 1992) and Tet (K) and Msr (A) transporters are specific to tetracycline and macrolide efflux (Renau et al., 1999). Renau et al. (1999) have developed the first broad-spectrum RND pump-inhibitor, MC-$_{207,110}$ (phenylalanyl-arginyl-β-naphthylamide), which potentiates the activity of levofloxacin, particularly the RND pumps against wild-type *P. aeruginosa*. Although these compounds are not effective antimicrobial agents by themselves, they reverse the resistance by blocking the efflux pumps.

Secondary metabolites of plants have shown to possess considerable activity against Gram-positive bacteria but not against Gram-negative species or yeast. In Gram-negative species, the outer membrane is a fairly effective barrier for amphiphatic compounds (Lewis and Lomovskaya, 2001). A set of multidrug resistance pumps (MDRs) extrude amphiphatic toxins across the outer membrane (Lewis, 2001; Nikaido, 1999). Tegos et al. (2002) have shown that MDR inhibitors MC $_{207,110}$ and INF $_{271}$ dramatically increase the effectiveness of a set of 11 plant antimicrobials (e.g. Rhein, resveratrol, gossypol, berberine) against Gram-negative bacteria. By contrast, certain plant-derived natural products can modulate MDR. For example carnosic acid (from *Rosmarinus officinalis*) (Oluwatuyi et al., 2004), and a penta substituted pyridine (from *Jatropha elliptica*) (Marquez et al., 2005), act as inhibitor of the Nor A efflux pump and restore the level of intracellular drug concentration.

Two isopirmarane diterpenes from the *Lycopus europaeus* enhance the activities of tetracycline and erythromycin against two strains of *S. aureus*. Otherwise these strains are highly resistant to these antibiotics due to the presence of multidrug efflux pumps, Tet (K) and Msr (A) (Gibbons et al., 2003). EGCg increases the accumulation of tetracycline in *S. aureus* strains by inhibiting the Tet (K) and Tet (B) efflux pumps (Roccaro et al., 2004). EGCg also enhances the activity of norfloxacin against a Nor A harboring *S. aureus* strain (Gibbons et al., 2004). Isoflavones isolated from *Lupinus argenteus* act in synergy with norfloxacin against a mutant of *S. aureus* by inhibiting the MDR pump. Reserpine, a plant alkaloid potentiates the activity of fluoroquinolones (Schmitz et al., 1998) and tetracycline against multidrug-resistant *S. aureus* strains (Gibbons and Udo, 2000). Reserpine has been shown to inhibit LmrA, the MDR ABC efflux system of *L. lactis* (Marquez et al., 2005), but unfortunately bacterial resistance to this natural product has been observed (Ahmed et al., 1993). The calcium channel antagonist verapamil, another known inhibitor of P-gp, also inhibits several bacterial ABC efflux pumps, including LmrA (Lee et al., 2003a; Pasca et al., 2004; Choudhuri et al., 2002).The efflux pump inhibitors from natural sources discussed so far can be co-administered with the antibiotic to decrease the degree of resistance of the

bacteria to the drugs, reverse the acquired resistance of the microorganism or reduce the emergence of resistant bacterial strains (Marquez et al., 2005).

Synergy and MDRTB therapy

Tuberculosis has established itself as a primary health threat. Few new agents are in development today for treating TB, and none has been designed specifically to shorten the treatment regimen and provide the breakthrough in therapy that is sorely needed if the epidemic is to be brought under control. Drug design targeting the latency stage and synergistic interaction between the various drug candidates might prove to be good alternatives.

Antimycobacterial treatment has always been a combination therapy. Today's TB treatment, which dates back to 1970s, is long and burdensome, requiring at least 6 months of multidrug chemotherapy. Novel targets are being identified alongside developing better drugs for known targets. Synergistic interaction between these drug like molecules is also gaining sufficient attention from the researchers (Chen et al., 2006; Vinogradova et al., 1999). Combination studies with natural products from plants and synthetic drugs are limited to few reports. Totarol, ferulenol and plumbagin were observed to increase the potency of isonicotinic acid hydrazide by fourfold against *Mycobacterium* sp. (Mossa et al., 2004). A napthoquinone 7-methyljuglone, isolated from the roots of *Euclea natalensis* in combination with isoniazid or rifampicin resulted in a four- to sixfold reduction in the MIC of the synthetic drugs (Bapela et al., 2006). An aqueous extract from *Cuminum cyminum* seeds produced a 35% enhancement of rifampicin levels in rat plasma. This activity was due to a flavonoid glycoside, 3′,5-dihydroxyflavone-7-*O*-*β*-D-galacturonide 4′-*O*-*β*-D-glucopyranoside, found in the natural product. The altered bioavailability profile of rifampicin could be attributed to the permeation enhancing effect of this glycoside (Sachin et al., 2007).

Antifungal agents and synergism

Fungi have higher number of chromosomes and complex nuclear membrane, cell organelles and cell wall composition. Since the last three decades, the rate of death every year due to fungal infections has risen significantly. With the increased use of antifungal agents there is an increase in the number and variety of fungal strains resistant to these drugs. Also the present antifungal therapeutics is often toxic. Alternative therapy needs to be developed to suppress the emergence of antifungal resistance. This can be achieved by the use of combinations of existing agents or the development of new, safer and effective agents primarily from plant sources which can exhibit synergy with drugs. Table 2

Table 2. Combination of natural products and synthetic drugs to combat fungal infection

Natural products	Synthetic drugs	Fungal species	References
Allium sativum	Ketaconazole	*T. rubrum*, *T. Erinacei* and *T. soudanense*	Pyun and Shin (2006)
Essential oil fraction of *P. graveolens* and its main components, geraniol and citronellol	Ketaconazole	*Aspergillus niger* and *A. flavus*	Shiota et al. (2000)
Essential oil from *Agastache rugosa* and its main component, estragole	Ketaconazole	*T. erinacei, T. mentagrophytes, T. rubrum, T. schoenleinii* and *T. soudanense*	Shin and Kang (2003)
Euphorbia characias latex	Ketoconazole	*Candida albicans*	Giordani et al. (2001)
Scopoletin, vanillin, 4-hydroxy-3-methoxycinnamaldehyde, and + Pinoresinol isolated from *Melia azedarach* L.fruits		*Fusarium verticillioides*	Carpinella et al. (2005)
Santolina oil	Clorimazole	*Candida albicans*	Suresh et al. (1997)
Anethole	Miconazole Amphotericin B	*Candida albicans*	Lee and Kin (1999)
Essential oil from *Thymus vulgaris* thymol chemotype	Amphotericin B	*Candida albicans*	Giordani et al. (2004)

lists the reported synergy observed between natural products and drugs towards fungal species.

Scopoletin, a hydroxycoumarin isolated from the fruits of *Melia azedarach* L. enhances the effect of two synthetic drugs namely, mancozeb and carboxin against *Fusarium verticillioides* (Carpinella et al., 2005). Synergistic interaction between EGCg and antimycotics such as amphotericin B and fluconazole has been reported against *C. albicans*. EGCg possibly attacks the cell membrane and causes cell lysis (Toyoshima et al., 1993). Amphotericin B below the minimum fungicidal concentration (MFC) is known to enhance the permeability of catechin through the fungal membrane, thereby increasing its uptake into the cell (Hirasawa and Takada, 2004). A few herbal essential oils (Shin and Lim, 2004) particularly estragole, an oil from *Agastache rugresa* (Shin and Kang, 2003), Tea tree (*Melaleuca alternifolia*) oil (Hammer et al., 2000) and volatile oils from Allium plants and *Euphorbia characigs* (Giordani et al., 2004) have demonstrated significant synergism with ketaconazole against certain fungal species.

In a recent study by Han (2007), a synergistic effect of grape seed extract (GSE) with amphotericin B was observed in both *in vitro* and in murine model of disseminated candidiasis due to *Candida albicans*. Mice treated with combination of amphotericin B and GSE or amphotericin B alone survived 62.4 and 38.4 days, respectively. The combination therapy reduced more than 75% of amphotericin B required to achieve the same level of inhibition.

In vitro evaluation of synergy

The accurate prediction of synergy between commercial drugs or between a drug and a natural product based upon the results of *in vitro* testing is very crucial. A number of methods are used to detect synergy. However, the checkerboard and time-kill curve methods are the two most widely used techniques and the former is a relatively easy test to perform (White et al., 1996). The checkerboard is prepared in microtiter plate for multiple combinations of two antimicrobial agents in concentrations equal to, above, and below their minimal inhibitory concentrations for the microorganism that is being tested. Each row (x axis) in the plate will contain the same diluted concentration of the first antimicrobial compound; while the concentration in each subsequent row will be half this value. Similarly each column (y axis) in the plate will contain the same diluted concentration of the second antimicrobial compound; while the concentration in each subsequent column will be half this value. The drugs combination in which the growth is completely inhibited is taken as effective MIC for the combination.

The time-kill method assesses the bactericidal activity of the individual as well as different concentration of the combination of drugs as a function of time. It is a labor intensive and time-consuming process (White et al., 1996). Tubes containing individual compounds and combination of compounds with concentrations ranging from one-quarter to twice the MIC for the bacterial strain of interest (NCCLS, 1987) are prepared. The tubes are inoculated with about 5×10^5 colony forming units/ml of the strain, and they are incubated overnight. Aliquots of the samples from 0 h of incubation (reflecting the initial inoculum) and 24 h of incubation (reflecting exposure of bacteria to the compound) are plated onto agar plates. Synergy is defined as a 100-fold or greater decrease in colony count at 24 h by the combination of agents with reference to the starting inoculum and also when compared to the most active single agent (Saiman, 2007).

E test is another method of recent origin. It consists of two plastic strips coated with a continuous gradient of each of the compound on one side. For evaluation of synergy, one compound strip is placed onto an agar plate for 1 h and then removed, and the second compound strip is placed on top of the gradient left behind by the first. The MIC of the combination is taken as the value at which the two inhibition zones intersect. If the use of the E strip could be standardized for testing the synergy of drugs and the results obtained could be demonstrated to be similar to those determined by established methods, this new test method would represent an attractive alternative to the labor-intensive procedures. Further, this method could be performed on a routine basis in a clinical microbiology laboratory (White et al., 1996). The standardization of these techniques for routine laboratory testing is the need because of the common use of combination therapies against the growing numbers of multiple drug-resistant strains.

Analysis of the synergy data

In all the above methods the interaction between the two antimicrobial agents is estimated by calculating the fractional inhibitory concentration of the combination (FIC) index. The FIC of each drug is calculated by dividing the concentration of the compound present in that well in combination where complete inhibition of growth of the microorganism is observed by the MIC of that compound alone to inhibit the microorganism. The FIC of the combination is then the sum of these two individual FIC values. When the FIC index of the combination is equal to or less than 0.5, the combination is termed as synergistic; when FIC index falls between 0.5 and 4.0, it indicates 'no interaction' between the agents, and a value above four indicates antagonism between the two compounds (Odds, 2003).

A convenient graphical way of representing the results of combination studies is by the use of an 'isobologram',

introduced by Loewe and Muischnek (1926). It is independent of the mechanism of action, makes no assumption about the behavior of each compound. So it is applicable to multiple component mixtures. Combination of drugs X and Y that shows inhibition of the growth of the organism are represented in a graph using rectangular coordinates as (x,y) for the respective doses. In this format, the dose of drug X alone as (a) and drug Y alone as (b) are represented along the axes as (a,0) and (0,b). The straight line connecting these points is called the 'line of additivity'. This line provides a convenient means for visually discriminating additive from non-additive interactions on the basis of whether or not the coordinate of the combination falls on (additive), below (superadditive) or above (subadditive) this line. The determination requires statistical evaluation (Tallarida et al., 1989) because the technique obtains the individual or combination of doses as random variables from the dose response data and there is always an error involved in the estimation (Tallarida and Raffa, 1996). If synergy is occurring, the dose of the combination needed to produce the same effect will be less than the sum of the individual components and then the curve will be concave. In antagonism, the dose of combination will be greater than expected and the curve will be convex. The 'isobole' method has been well explained with several examples by Williamson (2001).

Synergistic interactions in other therapies

The successful use of combinations of plant extracts is not only observed in antiinfective therapy, but also seen in the treatment of several disorders including cancer, HIV, inflammatory, stress-induced insomnia, osteoarthritis and hypertension (Williamson, 2001). Conventional medicine applies the "silver bullet" method, where single target therapy is employed. The recent trend has been the "herbal shotgun" method like Ayurveda, where multitargeted approach of the herbals and drugs is used. Today illness such as cancer, AIDS, hypertension, etc., are successfully treated with combination of 3–5 synthetic drugs. Cannabis extract is found to act in synergy as antispastic agent in mice than tetrahydrocannabinol at an equivalent dose (Baker et al., 2000; cit. at Wagner, 2006). Ginkgolide A and B has been seen to act in synergy in the inhibition of PAF-induced thrombocyte aggregation (Wagner, 2001, 2006) In the case of cancer chemotherapy, the molecular targets for the phytochemicals is diverse hence it necessitates the need to understand the degree of its interaction with synthetic drugs. Multitargeted therapy approach involving the application of phytochemicals or phytoextracts and synthetic drugs as anticancer agents has been detailed in a review (Hemaiswarya and Doble, 2006).

Conclusions

Before prescribing an antibiotic treatment the guidelines usually suggest that a specimen containing the suspected organism is sent for culture and sensitivity. Microbiology departments, for their part, use *in vitro* sensitivity of isolates taken from patients with bacterial infections to recommend which antibiotic(s) to prescribe or to use as an empirical guide for treatment in other situations. There are a number of reports available on the different antibiotic combinations tested *in vitro* and applied to clinical scenario. But there are no reports on the use of natural products and synthetic drug combinations used in the clinical settings. As discussed in the review, there is plenty of hope for the purified natural products to be used in combination with antibiotics as antiinfective drugs. EGCg, demonstrates a synergistic behavior with antibiotics by destroying the β-lactamase activity as well as by acting on the peptidoglycan of the cell wall. The safe consumption of tea for thousands of years indicates its low toxicity. EGCg, the principal constituent of tea, is absorbed through the digestive tract and distributed to many organs in animals and humans. This indicates the high bioavailability of EGCg which could enhance the activity of antibiotic under *in vivo* conditions. Thus the undesirable side effects of antibiotics on human and animal health could be possibly reduced by replacing at least in part the synthetic substances by negligibly toxic, highly specific antimicrobial compounds.

Several reports are available that describe the action of secondary metabolites from plants as antimicrobial agents. While the screening of natural compounds for antimicrobial activity is by itself a research area of major significance, the development of compounds with resistance modifying action is of interest since currently there are no known agents presently in use in clinics. In order to select a compound that could act in synergism with a drug it is necessary to understand the complete molecular mechanism of the drug action in the presence and absence of the natural compound. The problems that still need to be addressed are stability, selectivity and bioavailability of these natural products, and any adverse herb–drug interaction. To overcome multidrug resistance in the antimicrobial therapy a combination of drugs has to be used. The maximum benefit can be achieved when the pharmacokinetics of natural product and the antibiotic combination match. This does not mean that pharmacokinetic profiles for both agents should be identical. The optimal ratio and dosing regimens should be explored for higher efficacy and decreased toxicological profiles. Animal models with engineered strains lacking the particular resistant genotype can be used to very precisely define the pharmacokinetic and pharmacodynamic targets followed by regulated clinical trials. Even *in vitro* screening procedures for drug combination

are time-consuming process which should be speeded up to achieve quick breakthroughs in combination therapy. Techniques such as isobologram can be used successfully to demonstrate regions of synergy between drug combinations from other regimes.

The recent developments in genomics, proteomics and metabolomics have created a new platform to distinguish the synergistic efficacy of phytoextracts and for the determination of their mode of action. By the application of the ''-omic'' technologies it should be possible to detect the mechanism of action as the gene/protein expression profiles of the combination of drugs can be entirely different from the ones induced by the single drugs. This may lead to new phyto-based paradigms towards the use of complex plant mixtures in medicine (Ulrich-Merzenich et al., 2007).

As seen from this review, the number of natural compounds acting in synergy with synthetic drugs towards fungal and Mycobacterium species are minimal. This could be due to limited understanding of the mechanism of action of drugs against these organisms or insufficient screening of natural compounds. So research should be focused towards this direction to identify more natural compounds which exhibit synergistic behavior.

References

Ahmed, M., Borsch, C.M., Neyfakh, A.A., Schuldiner, S., 1993. Mutants of the *Bacillus subtilis* multidrug transporter Bmr with altered sensitivity to the antihypertensive alkaloid reserpine. J. Biol. Chem. 268, 11086–11089.

Alexander, C., Rietschel, E.T., 2001. Bacterial lipopolysaccharides and innate immunity. J. Endotoxin Res. 7, 167–202.

Al-hebshi, N., Al-haroni, M., Skaug, N., 2006. *In vitro* antimicrobial and resistance-modifying activities of aqueous crude khat extracts against oral microorganisms. Arch. Oral Biol. 51, 183–188.

Avenirova, E.L., Ashmarin, I.P., Movchan, N.A., Lapina, I.K., 1975. Combination of novoimanin with antibiotics with a different mechanism of action. Antibiotiki 20, 636–639.

Baker, D., Pryce, G., Coxford, J.L., Brown, P., Huffmann, I.W., Pertwee, R.G., Layward, L., 2000. Cannabinoids control spasticity and tremor in an animal model of multiple sklerosis. Nature 404, 84–87.

Ball, A.R., Casadei, G., Samosorn, S., Bremner, J.B., Ausubel, F.M., Moy, T.I., 2006. Conjugating berberine to a multidrug resistance pump inhibitor creates an effective antimicrobial. ACS Chem. Biol. 1, 594–600.

Bapela, N.B., Lall, N., Fourie, P.B., Franzblau, S.G., Van Rensburg, C.E., 2006. Activity of 7-methyljuglone in combination with antituberculous drugs *against Mycobacterium tuberculosis*. Phytomedicine 13, 630–635.

Berger-Bächi, B., Rohrer, S., 2002. Factors influencing methicillin resistant in staphylococci. Arch. Microbiol. 178, 165–171.

Bernard, F.X., Sablé, S., Cameron, B., Provost, J., Desnottes, F., Crouzet, J., Blanche, F., 1997. Glycosylated flavones as selective inhibitors of topoisomerase IV. Antimicrob. Agents Chemother. 41, 992–998.

Blasquez, J., Baquero, M.R., Canton, I., Alos, I., Baquero, F., 1993. Characterization of a new TEM-type β-lactamase resistant to clavulanate, sulbactam, and tazobactam. Antimicrob. Agents Chemother. 37, 2059–2063.

Bos, M.P., Tommassen, J., 2004. Biogenesis of the Gram-negative bacterial outer membrane. Curr. Opin. Microbiol. 7, 610–616.

Brade, H., Opal, S.M., Vogel, S.N., Membrane, D.C., 1999. In: Morrison (Ed.), Endotoxin in Health and Disease. Marcel Dekker, Inc., New York and Basel, pp. 31–38.

Braga, L.C., Leite, A.A.M., Xavier, K.G.S., Takahashi, J.A., Bemquerer, M.P., Chartone-Souza, E., Nascimento, A.M.A., 2005. Synergic interaction between pomegranate extract and antibiotics against *Staphylococcus aureus*. Can. J. Microbiol./Rev. Can. Microbial. 51, 541–547.

Brown, M.H., Paulsen, I.T., Skurray, R.A., 1999. The multidrug efflux protein Nor M is a prototype of a new family of transporters. Mol. Microbiol. 31, 393–395.

Bush, K., 1998. Metallo-β-lactamases: a class apart. Clin. Infect. Dis. 27, S48–S53.

Bush, K., 2002. The impact of β-lactamases on the development of novel antimicrobial agents. Curr. Opin. Investig. Drugs 3, 1284–1290.

Carpinella, M., Ferrayoli, C.G., Palacios, S.M., 2005. Antifungal synergistic effect of scopoletin, a hydroxycoumarin isolated from *Melia azedarach* L. fruits. J. Agric. Food Chem. 53, 2922–2927.

Casal, M., Vaquero, M., Rinder, H., Tortoli, E., Grosset, J., Rusch-Gerdes, S., Gutierrez, J., Jarlier, V., 2005. Microbial Drug Resist. 11, 62–67.

Chaibi, E.B., Sirot, D., Paul, G., Labia, R., 1999. Inhibitor-resistant TEM β-lactamase: phenotypic, genetic and biochemical characteristics. J. Antimicrob. Chemother. 43, 447–458.

Chambers, H.F., 2001. The changing epidemiology of *Staphylococcus aureus*. Emerg. Infect. Dis. 7, 178–182.

Chen, P., Gearhart, J., Protopopova, M., Einck, L., Carol, A., 2006. Synergistic interactions of SQ109, a new ethylene diamine, with front-line antitubercular drugs *in vitro*. J. Antimicrob. Chemother. 58, 332–337.

Choudhuri, B.S., Bhakta, S., Barik, R., Basu, J., Kundu, M., Chakrabarti, P., 2002. Overexpression and functional characterization of an ABC (ATP-binding cassette) transporter encoded by the genes drrA and drrB of *Mycobacterium tuberculosis*. Biochem. J. 367, 279–285.

Enright, M.C., Robinson, D.A., Randle, G., Feil, E.J., Grundmann, H., Spratt, B.G., 2002. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc. Natl. Acad. Sci. USA 99, 7687–7692.

Feldberg, A.R.S., Chang, S.C., Kotik, T.N., Nadler, M., Neuwirth, Z., Sundstrom, D.C., Thompson, H.N., 1988. *In vitro* mechanism of inhibition of bacterial cell growth by allicin. Antimicrob. Agents Chemother. 32, 1763–1768.

Ferrari, L., Iavarone, L., Braggio, S., Di Modugno, E., 2003. *In vitro* and *invivo* pharmacokinetics-pharmacodynamics of GV143253A, a novel trinem. Antimicrob. Agents Chemother. 47, 2471–2480.

Ghysen, J.M., 1994. Molecular structures of penicillin-binding proteins and β-lactamases. Trends Microbiol. 2, 372–380.

Gibbons, S., Oluwatuyi, M., Kaatz, G.W., 2004. A novel inhibitor of multidrug efflux pumps in *Staphylococcus aureus*. J. Antimicrob. Chemother. 48, 1968–1973.

Gibbons, S., Oluwatuyi, M., Veitch, N.C., Gray, A.I., 2003. Bacterial resistance modifying agents from *Lycopus europaeus*. Phytochemistry 62, 83–87.

Gibbons, S., Udo, E.E., 2000. The effect of reserpine, a modulator of multidrug efflux pumps, on the *in vitro* activity of tetracycline against clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA) possessing the Tet(K) determinant. Phytother. Res. 14, 139–140.

Giordani, R., Regli, P., Kaloustian, J., Mikail, C., Abou, L., Portugal, H., 2004. Antifungal effect of various essential oils against *Candida albicans*. Potentiation of antifungal action of amphotericin B by essential oil from *Thymus vulgaris*. Phytother. Res. 18, 990–995.

Giordani, R., Trebaux, J., Masi, M., Regli, P., 2001. Enhanced antifungal activity of ketaconaole by *Euphorbia characias* latex against *Candida albicans*. J. Ethnopharmacol. 78, 1–5.

Griep, M.A., Blood, S., Larson, M.A., Koepsell, S.A., Hinrichs, S.H., 2007. Myricetin inhibits *Escherichia coli* DnaB helicase but not primase. Bioorg. Med. Chem. 15, 7203–7208.

Hammer, K.A., Carson, C.F., Riley, T.V., 2000. *In vitro* activities of ketaconazole, econazole, miconazole, and *Melaleuca alternifolia* (Tea tree) oil against *Malassezia* species. Antimicrob. Agents Chemother. 44, 467–469.

Han, Y., 2007. Synergic effect of grape seed extract with amphotericin B against disseminated candidiasis due to *Candida albicans*. Phytomedicine 14, 733–738.

Heep, M., Rieger, U., Beck, D., Lehn, N., 2000. Mutations in the beginning of the rpoB gene can induce resistance to rifamycins in both *Helicobacter pylori* and *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. 44, 1075–1077.

Hegde, V.R., Pu, H., Patel, M., Black, T., Soriano, A., Zhao, W., Gullo, V.P., Chan, T.-M., 2004. Two new bacterial DNA primase inhibitors from the plant *Polygonum cuspidatum*. Bioorg. Med. Chem. Lett. 14, 2275–2277.

Helander, I.M., Alakomi, H.-L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E.J., Gorris, L.G.M., Von Wright, A., 1998. Characterization of the action of selected essential oil components on Gram-negative bacteria. J. Agric. Food Chem. 46, 3590–3595.

Helander, I.M., Von Wright, A., Mattila-Sandholm, T., 1997a. Potential of lactic acid bacteria and novel antimicrobials against Gram-negative bacteria. Trends Food Sci. Technol. 8, 146–150.

Hemaiswarya, S., Doble, M., 2006. Potential synergism of natural products in the treatment of cancer. Phytother. Res. 20, 239–249.

Hirasawa, M., Takada, K., 2004. Multiple effects of green tea catechin on the antifungal activity of antimycotics against *Candida albicans*. J. Antimicrob. Chemother. 53, 225–229.

Hu, Z.-Q., Zhao, W.-H., Asano, N., Yoda, Y., Hara, Y., Shimamura, T., 2002. Epigallocatechin gallate synergistically enhances the activity of carbapenems against methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 46, 558–560.

Hu, Z.-Q., Zhao, W.-H., Hara, Y., Shimamura, T., 2001. Epigallocatechin gallate synergy with ampicillin/sulbactam against 28 clinical isolates of methicillin-resistant *Staphylococcus aureus*. J. Antimicrob. Chemother. 48, 361–364.

Isogai, E., Isogai, H., Hirose, K., Hayashi, S., Oguma, K., 2001. *Invivo* synergy between green tea extract and levofloxacin against enterohemorrhagic *Escherichia coli* O157 infection. Curr. Microbiol. 42, 248–251.

Ito, T., Okuma, K., Ma, X.X., Yuzawa, H., Hiramatsu, K., 2003. Insights on antibiotics resistance of *Staphylococcus aureus* from its whole genome: genome island SCC. Drug Resist. Updat. 6, 41–52.

Jedlickova, Z., Mottl, O., Sery, V., 1992. Antibacterial properties of the Vietnamese cajeput oil and ocimum oil in combination with antibacterial agents. J. Hyg. Epidemiol. Microbiol. Immunol. 36, 303–309.

Kawahara, K., Seydel, U., Matsuura, M., Danbara, H., Rietschel, E.T., Zähringer, U., 1991. Chemical structure of glycosphingolipids isolated from *Sphingomonas paucimobilis*. FEBS Lett. 292, 107–110.

Kurazono, M., Ida, T., Yamada, K., Hirai, Y., Maruyama, T., Shitara, E., Yonezawa, M., 2004. In vitro activities of ME1036 (CP5609), a novel parenteral carbapenem, against methicillin-resistant staphylococci. Antimicrob. Agents Chemother. 48, 2831–2837.

Lee, E.-W., Huda, M.N., Kuroda, T., Mizushima, T., Tsuchiya, T., 2003a. EfrAB, an ABC multidrug efflux pump in *Enterococcus faecalis*. Antimicrob. Agents Chemother. 47, 3733–3738.

Lee, N., Yuen, K.Y., Kumana, C.R., 2003b. Clinical role of β-lactam/β-lactamase inhibitor combinations. Drugs 63, 1511–1524.

Lee, S.H., Kin, C.J., 1999. Selective combination effect of anethole to antifungal activities of miconazole and amphotericin B. Yakhak Hoeji 43, 228–232.

Leive, L., 1965. Release of lipopolysaccharide by EDTA treatment of *E. coli*. Biochem. Biophys. Res. Comm. 21, 290–296.

Lewis, K., 2001. In search of natural substrates and inhibitors of MDR pumps. J. Mol. Microbiol. Biotechnol. 3, 247–254.

Lewis, K., Ausubel, F.M., 2006. Prospects for plant derived antibacterials. Nat. Biotechnol. 24, 1504–1507.

Lewis, K., Lomovskaya, O., 2001. Drug efflux. In: Lewis, K., Salyers, A., Taber, H., Wax, R. (Eds.), Bacterial Resistance to Antimicrobials: Mechanisms, Genetics, Medical Practice and Public Health. Marcel Dekker, Inc., New York, NY, pp. 61–90.

Lin, R.-D., Chin, Y.P., Lee, M.H., 2005. Antimicrobial activity of antibiotics in combination with natural flavonoids against clinical extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae*. Phytother. Res. 19, 612–617.

Littlejohn, T.G., Paulsen, I.T., Gillespie, M.T., Tennent, J.M., Midgley, M., Jones, I.G., Purewal, A.S., Skurray, R.A., 1992. Substrate specificity and energetics of antiseptic and disinfectant resistance in *Staphylococcus aureus*. FEMS Microbiol. Lett. 74, 259–265.

Liu, I.X., Durham, D.G., Richards, R.M., 2000. Baicalin synergy with β-lactam antibiotics against methicillin-resistant *Staphylococcus aureus* and other β-lactam-resistant strain of *S. aureus*. J. Pharm. Pharmacol. 52, 361–366.

Loewe, S., Muischnek, H., 1926. Nauyn-Schmiedeberg's Arch. Exp. Path. Pharmacol. 114, 313–326.

Lu, W.P., Sun, Y., Bauer, M.D., Paule, S., Koenigs, P.M., Kraft, W.G., 1999. Penicillin-binding protein 2a from methicillin-resistant *Staphylococcus aureus*: kinetic characterization of its interactions with β-lactams using methicillin-resistant: kinetic characterization of its interactions with β-lactams using electrospray mass spectrometry. Biochemistry 38, 6537–6546.

Lynch, S.A., 2006. Efflux systems in bacterial pathogens: an opportunity for therapeutic intervention? An industry view. Biochem. Pharmacol. 71, 946–956.

Marquez, B., Neuville, L., Moreau, N.J., Genet, J.P., Santos, A.F., de Andrade, M.C.C., Sant'Ana, A.E.G., 2005. Multidrug resistance reversal agent from *Jatropha elliptica*. Phytochemistry 66, 1804–1811.

Mossa, J.S., El-Feraly, F.S., Muhammad, I., 2004. Antimycobacterial constituents from *Juniperus procera, Ferula communis* and *Plumbago zeylanica* and their *in vitro* synergistic activity with isonicotinic acid hydrazide. Phytother. Res. 2, 934–937.

NCCLS, National Committee for Clinical Laboratory Standards, 1987. Methods for Determining Bactericidal Agents. NCCLS Document M26-P, vol. 7. National Committee for Clinical Laboratory Standards, Villanova, PA.

Nicolson, K., Evans, G., O'Toole, P.W., 1999. Potentiation of methicillin activity against methicillin-resistant *Staphylococcus aureus* by diterpenes. FEMS Microbiol. Lett. 179, 233–239.

Nikaido, H., 1999. Microdermatology: cell surface in the interaction of microbes with the external world. J. Bacteriol. 181, 4–8.

Nikaido, H., 2003. Molecular basics of bacterial outer membrane permeability revisited. Microbiol. Mol. Biol. Rev. 4, 593–656.

Nikaido, H., Vaara, M., 1985. Molecular basis of bacterial outer membrane permeability. Microbiol. Rev. 49, 1–32.

Odds, F.C., 2003. Synergy, antagonism, and what the chequerboard puts between them. J Antimicrob. Chemother. 52, 1.

Oluwatuyi, M., Kaatz, G.W., Gibbons, S., 2004. Antibacterial and resistance modifying activity of *Resmarinus officinalis*. Phytochemistry 65, 3249–3254.

Pao, S.S., Paulsen, I.T., Saier Jr., M.H., 1998. Major facilitator superfamily. Microbiol. Mol. Biol. Rev. 62, 1–34.

Pasca, M.R., Guglierame, P., Arcesi, F., Bellinzoni, M., De Rossi, E., Riccardi, G., 2004. Rv2686c-Rv2687c-Rv2688c, an ABC fluoroquinolone efflux pump in *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. 48, 3175–3178.

Paulsen, I.T., Skurray, R.A., Tam, R., Saier Jr., M.H., Turner, R.J., Weiner, J.H., Goldberg, E.B., Grinius, L.L., 1996. The SMR family: a novel family of multidrug efflux proteins involved with the efflux of lipophilic drugs. Mol. Microbiol. 19, 1167–1175.

Pyun, M.-S., Shin, S., 2006. Antifungal effects of the volatile oils from *Allium* plants against *Trichophyton* species and synergism of the oils with ketaconazole. Phytomedicine 13, 394–400.

Renau, T.E., Leger, R., Flamme, E.M., Sangalang, J., She, M.W., Yen, R., Gannon, C.L., Griffith, D., Chamberland, S., Lomovskaya, O., Hecker, S.J., Lee, V.J., Ohta, T., Nakayama, K., 1999. Inhibitors of efflux pumps in *Pseudomonas aeruginosa* potentiate the activity of fluoroquinolone antibacterial leuofloxacin. J. Med. Chem. 42, 4928–4931.

Roccaro, A.S., Blanco, A.R., Giuliani, F., Rusciano, D., Enea, V., 2004. Epigallocatechin gallate enhances the activity of tetracycline in Staphylococci by inhibitory its efflux from bacterial cells. Antimicrob. Agents Chemother. 48, 1968–1973.

Sachin, B.S., Sharma, S.C., Sethi, S., Tasduq, S.A., Tikoo, M.K., Tikoo, A.K., Satti, N.K., Gupta, B.D., Suri, K.A., Johri, R.K., Qazi, G.N., 2007. Herbal modulation of drug bioavailability: enhancement of rifampicin levels in plasma by herbal products and a flavonoid glycoside derived from *Cuminum cyminum*. Phytother. Res. 212, 157–163.

Saier Jr., M.H., Tam, R., Reizer, A., Reizer, J., 1994. Two novel families of bacterial membrane proteins concerned with nodulation, cell division and transport. Mol. Microbiol. 11, 841–847.

Saiman, L., 2007. Clinical utility of synergy testing for multidrug-resistant *Pseudomonas aeruginosa* isolated from patients with cystic fibrosis: 'the motion for'. Paediatr. Respir. Rev. 8, 249–255.

Sakagami, Y., Iinuma, M., Piyasena, K.G.N.P., Dharmarane, H.R.W., 2005. Antibacterial activity of α-mangostin against vancomycin resistant Enterococci (VRE) and synergism with antibiotics. Phytomedicine 12, 203–208.

Sakagami, Y., Mimura, M., Kajimura, K., Yokoyama, H., Linuma, M., Tanaka, T., Ohyama, M., 1998. Anti-MRSA activity of sophoraflavanone G and synergism with other antibacterial agents. Lett. Appl. Microbiol. 27, 98–100.

Sato, M., Tanaka, H., Oh-Uchi, T., Fukai, T., Etoh, H., Yamaguchi, R., 2004. Antibacterial activity of phytochemicals isolated from *Erythrina zeyheri* against vancomycin-resistant enterococci and their combinations with vancomycin. Phytother. Res. 18, 906–910.

Schmitz, F., Fluit, A., Luckefahr, M., Engler, B., Hofmann, B., Verhoef, J., Heiz, ., Hadding, U., Jones, M., 1998. The effect of reserpine, an inhibitor of multidrug efflux pumps, on the in-vitro activities of ciprofloxacin, Sparfloxacin and moxifloxacin against clinical isolates of *Staphyloccocus aureus*. J. Antimicrob. Chemother. 42, 807–810.

Sheldon, A.T., 2005. Antibiotic resistance: a survival strategy. Clin. Lab. Sci. Summer. 18, 170–180.

Shimizu, M., Shiota, S., Mizushima, T., Ito, H., Hatano, T., Yoshida, T., Tsuchiya, T., 2001. Marked potentiation of activity of β-lactams against methicillin-resistant *Staphylococcus aureus* by corilagin. Antimicrob. Agents Chemother. 45, 3198–3201.

Shin, S., Kang, C.-A., 2003. Antifungal activity of the essential oil of *Agastache rugosa* Kuntze and its synergism with ketaconazole. Lett. Appl. Microbiol. 36, 111–115.

Shin, S., Lim, S., 2004. Antifungal effects of herbal essential oils alone and in combination with ketoconazole against *Trichophyton* sp. J. Appl. Microbiol. 97, 1289–1296.

Shiota, S., Shimizu, M., Mizushima, M., Ito, H., Hatano, T., Yoshida, T., Tsuchiya, T., 2000. Restoration of effectiveness of β-lactams on methicillin resistant *Staphylococcus aureus* by tellimagrandin I from rose red. FEMS Microb. Lett. 185, 135–138.

Shiota, S., Shimizu, M., Sugiyama, J., Morita, Y., Mizushima, T., Tsuchiya, T., 2004. Mechanisms of action of corilagin and tellimagrandin I that remarkably potentiate the activity of β-lactams against methicillin-resistant *Staphylococcus aureus*. Microbiol. Immunol. 48, 67–73.

Simonetti, G., Simonetti, N., Villa, A., 2004. Increased microbicidal activity of green tea (*Camellia sinensis*) in combination with butylated hydroxyanisole. J. Chemother. 16, 122–127.

Smith, E., Williamson, E., Zloh, M., Gibbons, S., 2005. Isopimaric acid from *Pinus nigra* shows activity against multidrug-resistant and EMRSA strains of *Staphylococcus aureus*. Phytother. Res. 19, 538–542.

Stermitz, F.R., Lorenz, P., Tawara, J.N., Zenewicz, L.A., Lewis, K., 2000. Synergy in a medicinal plant. Antimicrobial action of berberine potentiated by 5-methoxy hydnocarpin, a multidrug pump inhibitor. Proc. Natl. Acad. Sci. USA 97, 1433–1437.

Suresh, B.S., Dhanaraj, S.A., Elangosriram, K., Chinnaswamy, K., 1997. Anticandidal activity of *Santolina chamaecyparissus* volatile oil. J. Ethnopharmacol. 55, 151–159.

Takahashi, O., Cai, Z., Toda, M., Hara, Y., Shimamura, T., 1995. Appearance of antibacterial activity of oxacillin against methicillin-resistant *Staphylococcus aureus* (MRSA) in the presence of catechin. Kansenshogaku Zasshi 69, 1126–1134.

Tallarida, R.J., Porreca, F., Cowan, A., 1989. Statistical analysis of drug–drug and site–site interactions with isobolograms. Life Sci. 45, 947–961.

Tallarida, R.J., Raffa, R.B., 1996. Testing for synergism over a range of fixed ratio drug combinations: replacing the isobologram. Pharmacol. Lett. 58, 23–28.

Tegos, G., Stermitz, F.R., Lomovskaya, O., Lewis, K., 2002. Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. Antimicrob. Agents Chemother. 46, 3133–3141.

Toyoshima, Y., Okuba, S., Toda, M., Hara, Y., Shimamura, T., 1993. Effect of catechin on the ultrastructure of *Trichophyton mentagrophytes*. Kansenshogaku Zasshi 68, 295–303.

Ulrich-Merzenich, G., Zeitler, H., Jobst, D., Panek, D., Vetter, H., Wagner, H., 2007. Application of the '-Omic-' technologies in phytomedicine. Phytomedicine 14, 70–82.

Vaara, M., 1981. Increased outer membrane resistance to ethylenediaminetetraacetate and cations in novel lipid A mutants. J. Bacteriol. 148, 426–434.

Vaara, M., 1992. Agents that increase the permeability of the outer membrane. Microbiol. Rev. 56, 395–411.

Vaara, M., 1999. Lipopolysaccharide and the permeability of the bacterial outer lipopolysaccharide and the permeability of the bacterial outer membrane. In: Brade, H., Opal, S.M., Vogel, S.N., Morrison, D.C. (Eds.), Endotoxin in Health and Disease. Marcel Dekker, New York and Basel, pp. 31–38.

Vaara, M., Vaara, T., 1983a. Polycationic sensitize enteric bacteria to antibiotics. Antimicrob. Agents Chemother. 24, 107–113.

Vaara, M., Vaara, T., 1983b. Polycations as outer membrane destabilizing agents. Antimicrob. Agents Chemother. 24, 114–122.

Vattem, D.A., Lin, Y.-T., Ghaedian, R., Shetty, K., 2005. Cranberry synergies for dietary management of *Helicobacter pylori* infections. Process Biochem. 40, 1583–1592.

Veen, H.W.V., Konings, W.N., 1998. The ABC family of multidrug transporters in microorganism. Biochim. Biophys. Acta 1365, 31–36.

Veen, H.W.V., Venema, K., Bolhuis, H., Oussenko, I., Kok, J., Poolman, B., Driessen, A.J.M., Konings, W.N., 1996. Multidrug resistance mediated by a bacterial homolog of the human multidrug transporter MDR1. Proc. Natl. Acad. Sci. USA 93, 10668–10672.

Vinogradova, T.I., Aleksandrova, A.E., Antonenkova, E.V., Elokhina, V.N., Nakhmanovich, A.S., 1999. Design and study of new agents having antitubercular activity: the original compound perchlosone as a potent agent of etiotropic therapy for tuberculosis. My p Probl. Tuberk. 3, 45–47.

Vouillamoz, J., Entenza, J.M., Hohl, P., Moreillon, P., 2004. LB11058, a new cephalosporin with high penicillin-binding protein 2a affinity and activity in experimental endocarditis due to homogeneously methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 48, 4322–4327.

Wagner, H., 2001. Trends and challenges in phytomedicine. In: Yaniv, Z., Bachrach, U. (Eds.), Handbook of Medicinal Plants. Haworth Medical Press. Inc., Bindhamton, UK, pp. 3–28 (Chapter 1).

Wagner, H., 2006. Multitarget-therapy, the future of treatment for more than just functional dyspepsia. Phytomedicine 13 (SV), 122–129.

Walsh, S.E., Maillard, J.-Y., Russell, A.D., Catrenich, C.E., Charbonneau, D.L., Bartolo, R.G., 2003a. Development of bacterial resistance to several biocides and effects on antibiotic susceptibility. J. Hosp. Infect. 55, 98–107.

Walsh, S.E., Maillard, J.-Y., Russell, A.D., Catrenich, C.E., Charbonneau, D.L., Bartolo, R.G., 2003b. Activity and mechanisms of action of selected biocidal agents on Gram-positive and Gram-negative bacteria. J. Appl. Microbiol. 94, 240–247.

White, R.L., Burgess, D.S., Manduru, M., Bosso, J.A., 1996. Comparison of three different *in vitro* methods of detecting synergy: time-kill, checkerboard and E-test. Antimicrob. Agents Chemother. 40, 1914–1918.

WHO, 2004. WHO project: ICP BCT 001. 2004 Monitoring of antimicrobial resistance. Report of an Intercountry Workshop, Vellore, Tamil Nadu, India, 14–17 October 2003. World Health Organization, New Delhi, March 2004.

Williamson, E.M., 2001. Synergy and other interactions in phytomedicines. Phytomedicine 8, 401–409.

Willmott, C.J., Maxwell, A., 1993. A single point mutation in the DNA gyrase A protein reduces binding of fluoroquinolones

to the gyrase-DNA complex. Antimicrob. Agents Chemother. 37, 126–127.

Wright, G.D., 2005. Bacterial resistance to antibiotics: enzymatic degradation and modification. Adv. Drug Deliv. Rev. 57, 1451–1470.

Yam, Y.S., Hamilton-Miller, J.M.T., Shah, S., 1998. The effect of a component of tea (*Camellia sinensis*) on methicillin resistance, PBP2' synthesis, and β-lactamase production in *Staphylococcus aureus*. J. Antimicrob. Chemother. 42, 211–216.

Yoshida, H., Bogaki, M., Nakamura, S., Ubukata, K., Konno, M., 1990. Nucleotide sequence and characterization of *Staphylococcus aureus* norA gene, which confers resistance to quinolones. J. Bacteriol. 172, 6942–6949.

Zhao, W.-H., Hu, Z.-Q., Hara, Y., Shimamura, T., 2002. Inhibition of penicillinase by epigallocatechin-gallate resulting in restoration of antibacterial activity of penicillin against penicillinase-producing *Staphylococcus aureus*. Antimicrob. Agents Chemother. 46, 2266–2268.

Zhao, W.-H., Hu, Z.-Q., Okuba, S., Hara, Y., Shimamura, T., 2001. Mechanism of synergy between epigallocatechin-gallate and β-lactams against methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 45, 1737–1742.