

ORIGINAL ARTICLE

Essential oils and metal ions as alternative antimicrobial agents: a focus on tea tree oil and silver

Wan-Li Low¹, Ken Kenward¹, Stephen T Britland^{1,2}, Mohd CIM Amin³ & Claire Martin^{1,2}

1 School of Pharmacy, University of Wolverhampton, Wolverhampton, UK

2 Research Institute in Healthcare Science, Faculty of Science and Engineering, University of Wolverhampton, Wolverhampton, UK

3 Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

Key words

Antimicrobial; Silver; Tea tree oil; Wound infection

Correspondence to

C Martin
School of Pharmacy
Faculty of Science and Engineering
University of Wolverhampton
Wulfruna Street
Wolverhampton WV1 1LY
UK
E-mail: claire.martin2@wlv.ac.uk

doi: 10.1111/iwj.12611

Low WL, Kenward K, Britland ST, Amin MCIM, Martin C. Essential oils and metal ions as alternative antimicrobial agents: a focus on tea tree oil and silver. *Int Wound J* 2017; 14:369–384

Abstract

The increasing occurrence of hospital-acquired infections and the emerging problems posed by antibiotic-resistant microbial strains have both contributed to the escalating cost of treatment. The presence of infection at the wound site can potentially stall the healing process at the inflammatory stage, leading to the development of a chronic wound. Traditional wound treatment regimes can no longer cope with the complications posed by antibiotic-resistant strains; hence, there is a need to explore the use of alternative antimicrobial agents. Pre-antibiotic compounds, including heavy metal ions and essential oils, have been re-investigated for their potential use as effective antimicrobial agents. Essential oils have potent antimicrobial, antifungal, antiviral, anti-inflammatory, antioxidant and other beneficial therapeutic properties. Similarly, heavy metal ions have also been used as disinfecting agents because of their broad spectrum activities. Both of these alternative antimicrobials interact with many different intracellular components, thereby resulting in the disruption of vital cell functions and eventually cell death. This review will discuss the application of essential oils and heavy metal ions, particularly tea tree oil and silver ions, as alternative antimicrobial agents for the treatment of chronic, infected wounds.

Introduction

The use of metal ions and essential oils as antimicrobial agents is of particular interest in topical wound management. Management of chronic wounds, such as varicose skin ulcers and burns, aims to induce rapid healing and minimise the extent of scarring. Infection of such wounds can not only delay the healing process but also lead to the development of a chronic wound with increased potential to develop into a systemic infection. Topical application of antimicrobial agents is common since effective concentrations may be difficult to achieve with systemic drugs as the effects of the wound trauma may impede delivery of the agent into the wound (1,2). The reduced concentration may also create selective pressure for antibiotic resistance.

The current problems posed by increasing antibiotic resistance in Gram-positive bacteria (methicillin-resistant *Staphylococcus aureus*, MRSA, Vancomycin-resistant enterococci, VRE) and Gram-negative bacteria (New Delhi metallo- β -lactamase-1 (NDM-1) positive *Escherichia*

Key Messages

- The use of broad spectrum, pre-antibiotic era antimicrobial agents, such as essential oils and metal ions, may be an alternative approach to tackling the growing problem of drug-resistant wound infections
- The aim of this review is to consider the evidence for the use of silver and tea tree oil (TTO) in tackling chronic wound infections, such as those associated with diabetic foot and burns
- The broad spectrum, multi-target mechanism of action of silver and TTO indicates that there may be a role for them in a clinical setting to both reduce the microbial load within the wound bed and speed up the healing process

coli, multidrug-resistant *Acinetobacter baumannii*, ciprofloxacin-resistant *Pseudomonas aeruginosa*) (3,4) have renewed interest in pre-antibiotic antibacterial agents, such as

metal ions and essential oil [tea tree oil (TTO)] (5,6). Alternative, non-antibiotic-based treatments are attractive due to their decreased side effects compared with synthetic drugs and their multiple target sites within the microorganisms, which may contribute to reduced development of resistant strains (7). Despite their usefulness and long history of use, these agents are mainly restricted to topical applications against infected wounds, skin burns, ulcers and fungal infections of the skin. Despite their effectiveness in treating topical infections, the complete healing of wounds, especially slow/non-healing wounds, may require repeated application or use of high concentrations that may have adverse effects on the patient. Current developments in wound treatment focus on approaches that will allow reduced concentration (using combined agents) or delivery via controlled release delivery systems to minimise potential side effects whilst maintaining bioavailability to achieving therapeutic effects (8–11).

The useful properties of alternative antimicrobial agents, together with advances in drug-delivery technologies, may be able to enhance and expand the medical applications of these agents (9,10,12). Combining these alternative antimicrobial agents with advanced drug-delivery systems aims to:

- Promote bioavailability of agent at microbiocidal concentrations.
- Reduce drug concentration to enhance safety and practicality of application.
- Minimise scarring and promote wound-healing processes.
- Reduce discomfort and pain in consideration of the patient's psychological needs.
- Decrease the frequency of dressing changes.

These aims would increase convenience, provide less opportunity for infection and/or reinfection of the wounds and ultimately reduce treatment costs.

Microorganisms and wound management

In healthy individuals, the skin supports a natural microflora comprising a balanced community of microorganisms, which rarely cause infection. However, a disturbance to the normal ratio of microflora or an exposure of subcutaneous tissue due to trauma may result in the pathogenic invasion by these microorganisms (7). Colonisation of wounds by these opportunistic pathogens is usually polymicrobial (12). The diversity and proliferation of the pathogens is influenced by various factors including the type, depth and location of the trauma as well as the host immune system response (7). The presence of microorganisms at a wound site does not confirm infection (13). Infection only occurs when the host immune system can no longer cope with the virulence factors expressed by the colonising microorganisms, thus triggering a series of systemic responses that delay the healing process (7,13).

The increasing occurrence of hospital infections and widespread emergence of resistant microorganisms contribute to escalating treatment costs. In addition, hypersensitivity reactions to antibiotics and the lack of access to new treatments within the health care industry makes the provision of sufficient support and care for patients difficult. Modern

lifestyles that frequently lack physical activity increase the possibility of developing various life-long (interconnected) health conditions, for example, diabetes, obesity and hypertension in old age. These underlying health conditions may influence the complexity and severity of wound healing. In addition, the growing size and longevity of the elderly population has increased the prevalence of wounds associated with these conditions, including slow/non-healing ulcers. The improvement in medical facilities has increased the number of patients surviving from complicated wounds, such as those caused by burns. Although rates of survival have improved, severely burned patients usually require extended stays in hospital, suffer from lowered immunity and extensive loss of skin. Such patients are prone to infection by both common wound pathogens as well as antibiotic-resistant microorganisms, which may further complicate the treatment regime (1).

The nature of burn wounds, and varicose ulcers in particular, may involve relatively lengthy treatment with antibiotics, which carries the attendant risk of selecting drug-resistant bacteria. Various approaches have been conducted to find the best method to treat and overcome this problem. The increasing incidence and broadening spectrum of pathogens resistant to antibiotics has refocused scientific interest on the use of alternative antimicrobial compounds (6,14). Alternative, non-antibiotic-based treatments are attractive because of their decreased side effects compared with synthetic drugs and their multiple target sites within the microorganisms, which may contribute to reduced development of resistant strains (7). Despite their usefulness and long history of use, these agents are mainly restricted to topical applications against infected wounds, skin burns, ulcers and fungal infections of the skin.

Microorganisms and the wound environment

In healthy individuals, the skin supports a natural microflora comprising a balanced community of microorganisms, which rarely cause infection. However, a disturbance to the normal ratio of microflora or an exposure of subcutaneous tissue because of trauma may result in pathogenic polymicrobial invasion by these microorganisms (7). The diversity and proliferation of the pathogens is influenced by various factors, including the type, depth and location of the trauma as well as the host immune system response (7). The presence of microorganisms at a wound site does not confirm infection (13). Infection only occurs when the host immune system can no longer cope with the virulence factors expressed by the colonising microorganisms, thus triggering a series of systemic responses that delay the healing process (7,13). Besides patient microflora, other sources of infection include those acquired directly or indirectly via air, other infected patients, health care workers, contaminated medical devices, hospital environment and external sources, such as visitors (15).

Wound healing mechanism

Wound healing is a complex cascading event involving cellular, enzymatic and biochemical pathways, spanning from the time the skin is damaged until the wound is completely healed (16,17). Maintenance of healthy wound site homeostasis is

thus important to maximise wound healing capacity, especially in immuno-compromised patients or those with underlying chronic health conditions. The events in wound healing have been divided into four main stages (10,18,19):

- i. Exudative – Haemostasis and clot formations occur, release of inflammatory mediators including cytokines, chemokines and growth factors
- ii. Resorptive – Inflammation occurs, signs of redness, swelling, heat, pain and possible loss of function, immune system reacts by sending neutrophils and macrophages into the wound site to remove any foreign body (e.g. bacteria) and inflammatory debris to facilitate conditions for angiogenesis
- iii. Proliferative – Wound closure is initiated by the migration of epithelial cells from the wound edge, angiogenesis occurs, simultaneously, a provisional matrix is formed and granulation tissue is also deposited
- iv. Regenerative – Re-epithelialisation occurs to allow full wound closure, development of scar, tissue remodelling occurs to re-establish functionality

In general, wound healing follows this sequence but, being non-linear, the stages may move forwards and backwards, based on intrinsic and extrinsic factors as well as the severity of the wound (19). The rate of wound healing differs between individuals and is influenced by factors such as type and depth of wound, age, nutrition, immune status, underlying health conditions as well as local microbial burden.

Microbial infection in particular retards wound healing by increasing the bio-burden at wound sites, thus stalling the normal process at the inflammatory phase. It is common for chronic wounds to become contaminated and colonised by microorganisms. Such colonisation by microorganisms, especially common skin commensals such as *Staphylococcus epidermidis* and *Corynebacterium* species, does not usually impair wound healing, provided the population remains below the critical threshold of approximately 10^5 cells per gram of wound tissue (20). Their presence and production of proteolytic enzymes may help to stimulate the activity of neutrophils, thereby encouraging an inflammatory response which may facilitate wound healing (21). Critical colonisation is defined as a condition in which bacterial infection exceeds the ability of the host's immune system to eliminate further proliferation and colonisation (20).

Cutaneous wounds offer a favourable (moist, warm, nutritious) environment to support bacterial growth and proliferation. Heavy microbial infection (above critical colonisation) retards wound healing by increasing the bio-burden at wound sites, which stalls the normal process at the inflammatory phase. When acute wounds become infected and reach critical colonisation by pathogenic microorganisms, the stimulated pro-inflammatory environment (due to microbial production of toxins, proteases or pro-inflammatory molecules) will stop the process of wound healing, and the site develops into a chronic wound (7,10,12,20).

Alternative antimicrobial agents

Although conventional antibiotics are regarded as effective antimicrobial agents, there is concern about their side effects

and the increasing incidence of microbial resistance to them (22). Antimicrobial agents are only effective until resistant strains of the target microorganisms begin to emerge (6,23). With conventional antibiotics, the emergence of resistance is mainly because of their action against a single target. This has led to re-examination of the use of other antimicrobial agents, such as metal ions and plant extracts, which often attack multiple target sites (6,22,24,25).

The application of essential oils to reduce bacterial growth and prevent decay is not a new idea. Plants synthesise aromatic secondary metabolites to protect against predation and prevent colonisation by plant pathogens (24). These aromatic compounds are divided into classes, including essential oils (primarily phenolics and/or terpenoids), alkaloids, lectins, polypeptides and polyacetylenes, all of which have different mechanisms of antimicrobial activity (24). Some examples of essential oils or plant extracts commonly used for their antimicrobial properties are TTO, ylang ylang, betel pepper, manuka, eucalyptus, arnica, lemon verbena, rosemary, green tea extract and cadendula (23,24). Although extensively practiced since ancient times, the use of natural extracts from plants as antimicrobial compounds declined after the development of synthetic antibiotics.

Metals (especially heavy metals) were used as disinfecting agents since ancient times. Silver, copper and gold, for example, have been used to treat diseases, disinfect wounds and water. Examples of metals commonly used in these applications include zinc, iron, bismuth, cobalt, magnesium, titanium, copper and the more extensively used heavy metal, silver. Despite having useful properties, treatment using metals is limited because excessive concentrations of metals, especially heavy metals, are toxic to human cells. Amongst the heavy metals, silver has a long history of use as an antimicrobial agent because of its relatively lower toxicity to human cells (26,27). Recently, in response to issues surrounding antibiotic resistance, topical application of silver compounds has increased in popularity (28).

Plant products as antimicrobial agent

Plants with medicinal properties have been used for the treatment of various diseases both in traditional and modern medicine. When faced with microbial invasion or attack leading to infection, plants have their own defence mechanisms, which rely on the production of compounds that interfere with the cellular and intracellular structure of microorganisms (29). These antimicrobial plant compounds that show effective antimicrobial activity are often secondary metabolites formed in aromatic plants. These aromatic compounds give the plants their characteristic strong odour (29) that can repel insects or herbivores. In addition, certain compounds give plants pigment or flavour; these may be irritants to other organisms and may flow out from injured plants to prevent colonisation by microorganisms (30,31). These secondary metabolites are mainly present as volatile compounds such as phenols or their oxygen-substituted derivatives, which are categorised into five major classes detailed in Table 1 (24). All plant organs, including buds, flowers, leaves, stems, seeds, fruits, twigs and branches, are able to synthesise these compounds.

Table 1 Summary of the major classes of active antimicrobial compounds from plants [summarised from references (24, 29, 39, 99–102)]

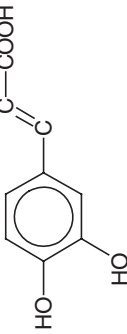

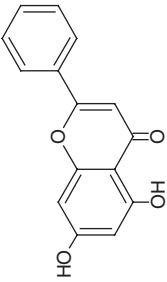
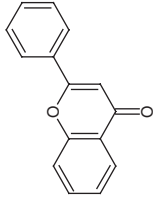
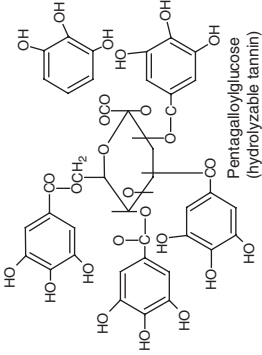
Class and description	Subclass	Example	Mechanism	Structure
<p>Phenolics</p> <ul style="list-style-type: none"> • Simple phenolic ring structures that may exist in their highest oxidation state. For example, cinnamic and caffeic acid, which are phenylpropene-derived compounds. • Number and site of hydroxyl group(s) present on the compound may indicate the relative toxicity to microorganisms. 	Simple phenol and phenolic acid	Catechol, epicatechin and caffeic acid	Substrate deprivation and membrane disruption	
<ul style="list-style-type: none"> • Highly oxidised phenols or those with increased hydroxylation have increased microbial inhibitory effects. • Active against various types of bacteria, viruses and fungi. • May cause iron deprivation, reaction with sulphhydryl groups, protein-related polyamide polymers via non-specific interaction and hydrogen bonding to inactivate proteins or enzymes in the cell. 	Quinone	Hypericin	Binds to adhesins, complex with cell wall and inactivate enzyme	
	Flavonoids	Chrysin	Binds to adhesins	
	Flavones	Abyssinone	Complexes with cell wall and inactivates enzymes	
	Tannins	Ellagitannin	Binds to proteins, adhesins, inhibits enzymes, causes substrate deprivation, complexes with cell wall, membrane disruption and metal ion complexation	

Table 1 Continued

Class and description	Subclass	Example	Mechanism	Structure
Coumarins Warfarin	Coumarins Warfarin	Warfarin	Interactions with eukaryotic DNA and has antiviral properties	
Terpenoids • Actively disrupts various membranes of bacteria, fungi, protozoa and enveloped viruses via its lipophilic properties.	Monoterpenes Terpinen-4-ol	Terpinen-4-ol	Membrane disruption	
Alkaloids • Secondary plant metabolites that have antimicrobial activity.	N/A Berberine and piperine	Berberine and piperine	Intercalate into cell wall or DNA, differentially inhibit sterol and chitin biosynthesis	
Polyacetylenes • Has antibacterial, antimycobacterial, anti-inflammatory, anti-platelet-aggregatory effects.	N/A Falcarinol	Falcarinol	Mechanism unknown	
Lectins and polypeptides • Inhibition of membrane function and integrity via interaction with proteins, ion channels and displacement of lipids. • Presence of cholesterol and increasing ionic strength in the target membrane reduces the antimicrobial activity due to stabilisation of the lipid bilayer as well as the weakened electrostatic interaction to encourage binding to target membrane. Note: interaction with membrane peptides or weakening of electrostatic charge interactions required for antimicrobial activity.	N/A Mannose-specific agglutinin, fabatin, defensins and thionins	Mannose-specific agglutinin, fabatin, defensins and thionins	Inhibit adhesion and fusion interactions between virus and host cell. May form ion channels in microbial membranes or reduce adhesion of microbial proteins to host receptors via competitive inhibition	

The amount of active compound present in botanical extracts varies depending on the main adaptation of the plant to its environment, harvesting period, the extraction process, dehydration procedures, purification and storage methods (25). The extraction of secondary metabolites from plants is usually by distillation (water or steam) to produce a volatile essential oil. Essential oils are aromatic compounds synthesised by plants as secondary metabolites and are well known for their antibacterial, antifungal and antiviral properties (6,29), in addition to anti-cancer, anti-diabetic, anti-inflammatory and antioxidant activities (29,32). Essential oils are multi-component compounds, usually with terpenes and their derivatives (terpenoids) as the major components. After extraction, they present as clear, almost colourless volatile liquids, soluble in organic solvents and lipids in addition to some hydrophilic components (29). Due to the versatility and wide-ranging properties of essential oils, they are used not only in the pharmaceutical industry but also incorporated into the cosmetics, agriculture, sanitation, disinfection, food preservation and manufacturing industries (25,29). Examples of the various uses of essential oils are summarised in Table 2.

Tea tree essential oil as an antimicrobial

History, background of use and production of TTO

Bundjalung Aborigines of northern New South Wales traditionally used crushed tea tree leaves as a treatment for coughs and colds (inhalation), a herbal infusion for sore throats and also sprinkled them directly onto skin wounds to promote healing (33). The use of TTO as an antimicrobial agent became a common practice after Penfold published reports on its antimicrobial properties in the 1920s and 1930s (33). In modern society, the useful properties of TTO are made commercially available, either as the essential oil per se or alternatively formulated into various products, including antiseptic creams, soaps, shampoo, anti-acne creams, toothpaste and household cleaning agents.

TTO is recognised as having broad spectrum antibacterial, antifungal, antiviral, antimycoplasmal and antiprotozoal activity as well as anti-inflammatory and anti-cancer properties (33,34). There are over one hundred different components in whole TTO, of which its major component, terpinen-4-ol, is primarily responsible for its active antimicrobial properties (33). TTO is extracted from the leaves and terminal branches of the *Melaleuca alternifolia* (*M. alternifolia*) plant via steam distillation and condensed to yield a pale yellow oil (33). The potential variation in the composition of TTO has led to the classification of six chemotypes of *M. alternifolia*, based on the amount of terpinen-4-ol (one type), terpinolene (one type) and 1,8-cineole (four types) (33). Commercially acceptable grade of TTO are classed in the terpinen-4-ol chemotype and have to comply with ISO4730:2004 standards for 'Oil of Melaleuca, terpinen-4-ol type', which defines the maximum and minimum level for the 14 major components of the essential oil, including terpinen-4-ol (33).

Mode of action

The physicochemical properties of the oil include those from hydrophilic hydrocarbon compounds with sufficient

lipophilicity and allow the oil to partition preferentially into biological membranes, causing bilayer expansion (33). Thus, TTO components diffuse easily through the hydrophobic lipid bilayer of the microbial cell membrane, causing disruption to integrity and function, increased fluidity, loss of permeability and inhibition of embedded membrane enzymes. Consequently, the cell loses essential metabolites and repair enzymes, ultimately resulting in cell death (33,35). The microbicidal properties of active monoterpenes in particular can mainly be attributed to disruption of the cell membrane's barrier function; cells are thus unable to establish control over membrane-coupled energy-transducing processes, solute transport, regulation of metabolism and maintenance of turgor pressure (35). However, when a compound is highly lipophilic, its low solubility in aqueous media hinders its ability to contact with and permeabilise cell membranes (36).

Cox *et al.* (2000) examined membrane disruption of *E. coli*, *S. aureus* and *Candida albicans* by TTO via the leakage of potassium ions, materials that absorb at 280 nm (proteins) and uptake of fluorescent nucleic acid stain, propidium iodide (35). All three microorganisms showed decreased microbial viability (inhibition of respiration), increased uptake of propidium iodide and increased leakage of 280 nm absorbing material. Leakage of potassium ions was prominent in *E. coli* and *S. aureus* but less so in *C. albicans* (35,37). Carson *et al.* (2002) assessed the release of 260 nm absorbing materials (nucleic acids) from *S. aureus* after treatment with whole TTO, terpinen-4-ol, 1,8-cineole and α -terpineol (38). These results showed significant leakage of nucleic acids, suggesting extensive damage to the cytoplasmic membrane.

Applications

Preparations containing TTO are commonly used as antiseptic agents with antimicrobial, cleansing, healing and itch relieving properties (39). For example, creams containing 5% TTO have been used to treat acne and toenail onychomycosis. A 6% gel formulation was shown to have antiherpetic effects; and TTO has been used as an antiseptic agent in handwash soaps and mouthwashes as well as for the treatment of microbial infections such as folliculitis and vaginitis (39–43). Whole TTO essential oil applied over 12 days successfully and permanently removed skin warts, whereas salicylic acid (12% w/w) and lactic acid (4% w/w) only resulted in temporary removal of the warts (44). Treatment with 4% TTO nasal ointment together with a 5% TTO body wash performed better at eradicating MRSA from patients than 2% mupirocin nasal ointment together with 2% triclosan body wash (45). Similarly, a 10% TTO cream and 5% TTO body wash was more effective at clearing MRSA on skin compared with 4% chlorhexidine gluconate soap and 1% silver sulfadiazine cream (46). In contrast, treatment of MRSA in the nasal cavity alone, even with 10% TTO cream, showed only 47% eradication (46).

TTO has been widely used in the management of wounds because of its antimicrobial and therapeutic properties. Burnaid® (Mundipharma Pty Limited, Sydney, Australia) is a commercial hydrogel dressing impregnated with TTO for the treatment of burns (47). Other studies have reported the enhancement of antimicrobial activity when using TTO

Table 2 Common essential oils extracted from plants and their medicinal and/or antimicrobial activity

Common name	Scientific name	Active compound	Activity or therapeutic properties	Application/product	References
Aloe	<i>Aloe barbadensis</i> , <i>Aloe vera</i>	Anthraquinones (aloin, emodin and resistanol), β -sitosterol and gibberellins	<ul style="list-style-type: none"> • Antimicrobial • Increases wound healing • Induces hypoglycaemic effect • Lowers blood cholesterol 	<ul style="list-style-type: none"> • Used on burns and wounds • Treatment of psoriasis and genital herpes • Mediate insulin production 	(42, 103–107)
Arnica	<i>Arnica montana</i>	Sesquiterpene and lactones	<ul style="list-style-type: none"> • Anti-inflammatory 	<ul style="list-style-type: none"> • Applied to bruises and swelling • Osteoarthritis treatment 	(108)
Basil	<i>Ocimum basilicum</i>	Linalool, methylchavicol, epi- α -cadinol, α -bergamotene, γ -cadinene and germacrene D	<ul style="list-style-type: none"> • Antimicrobial • Antioxidant 	<ul style="list-style-type: none"> • Antimicrobial activity in food preservation and packaging industry 	(109, 110)
Calendula (Marigold)	<i>Calendula officinalis</i>	Triterpenoid	<ul style="list-style-type: none"> • Antimicrobial • Anti-inflammatory • Anti-tumorigenic 	<ul style="list-style-type: none"> • Herbal antimicrobial mouthwash • Treatment against athletes foot, ringworm and candidal infections 	(111–113)
Cinnamon	<i>Cinnamomum zeylanicum</i>	Cinnamaldehyde	<ul style="list-style-type: none"> • Analgesic • Antiseptic 	<ul style="list-style-type: none"> • Inhibits biofilm formation by clinical strains of <i>Staphylococcus epidermidis</i> • Active against common food spoilage microbes 	(114, 115)
Clove	<i>Syzygium aromaticum</i>	Eugenol	<ul style="list-style-type: none"> • Antibacterial • Antifungal 	<ul style="list-style-type: none"> • Used to treat toothache and skin sores 	(104, 116, 117)
Eucalyptus	<i>Eucalyptus globulus</i>	1,8-cineole, Tannin	<ul style="list-style-type: none"> • Antimicrobial • Insect repellent • Skin penetration enhancer for drugs 	<ul style="list-style-type: none"> • Herbal antimicrobial mouthwash • Inhibits growth of food pathogen • Fragrant in pharmaceuticals, soaps, detergents and food • Natural pesticide/insecticide 	(118–121)
Garlic	<i>Allium sativum</i>	Allicin	<ul style="list-style-type: none"> • Antimicrobial • Potential anti-cancer agent • Lowers cholesterol and triglycerides 	<ul style="list-style-type: none"> • <i>In vitro</i> antimicrobial activity against <i>Helicobacter pylori</i> • Food preservative • Reduce risk of hypertension 	(32, 104, 122, 123)
Green tea extract	<i>Camellia sinensis</i>	Catechins, tannins, caffeine	<ul style="list-style-type: none"> • Anti-inflammatory • Antimicrobial • Anti-cancer • Antioxidant 	<ul style="list-style-type: none"> • Reduces dental caries • Lowers risk of <i>Helicobacter</i> infection 	(111, 124–126)

Table 2 Continued

Common name	Scientific name	Active compound	Activity or therapeutic properties	Application/product	References
Lemon verbena	<i>Aloysia triphylla</i> or <i>Lippia citriodora</i>	Verbacoside	<ul style="list-style-type: none"> • Antimicrobial, • Anti-cancer agent • Antioxidant • Anti-inflammatory • Antispasmodic • Antipyretic 	<ul style="list-style-type: none"> • <i>In vitro</i> antimicrobial activity against <i>H. pylori</i>, <i>Escherichia coli</i>, <i>Mycobacterium tuberculosis</i>, <i>Staphylococcus aureus</i> and <i>Candida albicans</i> • Treatment of asthma, cold, fever and indigestion 	(127–130)
Manuka	<i>Leptospermum scoparium</i>	α -terpineol, cineole, β -triketone	<ul style="list-style-type: none"> • Antimicrobial • Anti-inflammatory 	<ul style="list-style-type: none"> • Aids wound healing • Relieve fevers, inflammation and pain • Respiratory ailments 	(23,111)
Oregano	<i>Origanum vulgare</i>	Thymol, carvacol, p -cymene, γ -terpinene, Thymoquinone	<ul style="list-style-type: none"> • Antimicrobial • Antioxidative 	<ul style="list-style-type: none"> • Used in food preservation 	(131,132)
Rooibos	<i>Aspalathus linearis</i>	Flavonoids, aspalathin, iso-orientin, orientin and rutin	<ul style="list-style-type: none"> • Antimicrobial • Antioxidant activity 	<ul style="list-style-type: none"> • <i>In vitro</i> antimicrobial and antifungal activity against <i>Bacillus cereus</i>, <i>Micrococcus luteus</i> and <i>C. albicans</i>, • <i>In vitro</i> anti-HIV activity 	(104,133–135)
Rosemary	<i>Rosmarinus officinalis</i>	α -pinene, bornyl acetate, eucalyptol, camphor, 1,8-cineole	<ul style="list-style-type: none"> • Antimicrobial • Antioxidant • Anti-cellulitis 	<ul style="list-style-type: none"> • <i>In vitro</i> antifungal activity, particularly against <i>Malassezia (Pityrosporum)</i> spp. • Food preservation 	(131,136–139)
Tea Tree Oil	<i>Melaleuca alternifolia</i>	Terpinen-4-ol, α -terpineol, 1,8-cineole	<ul style="list-style-type: none"> • Antimicrobial • Anti-cancer • Anti-inflammatory 	<ul style="list-style-type: none"> • Treatment of wounds • Pharmaceutical cosmetics and cleaning product applications 	(32,33,111)

in combination with other antimicrobial agents such as chlorhexidine (8,23), tobramycin (48) and silver ions (34).

Wound-healing benefits

In addition to antimicrobial activity, TTO also plays a role in wound healing and modulation of the inflammatory response (6,49,50). Water soluble components of TTO, especially terpinen-4-ol, contribute to inflammatory regulation by suppressing monocyte production of superoxide ions (50) as well as inflammatory-inducing mediators, for example, TNF α , IL-1 β , IL-8, IL-10 and PGE $_2$ (49). This in turn limits further production of other inflammatory cytokines (49) and reduces

oxidative damage to cells (6), thereby enhancing wound healing.

The aromatic vapours and analgesic properties of TTO may promote wound healing by providing temporary relief to patients (47,51). Burnaid[®] reduces skin temperature at the burn site by approximately 2°C within 20 min, providing localised soothing and cooling effects (47), and may improve the patients' ability to cope with the treatment. Using TTO to treat patients with malodorous skin ulcers showed a significant reduction of the malodour as well as of infection and pus secretion (51,52). The TTO compounds therefore improved the patient's well-being by reducing social isolation associated with the malodour.

Resistance

Despite the popularity of TTO in many applications, concerns of microorganisms developing resistance have not been totally neglected. In vitro exposure of *S. aureus* to stepwise increasing concentrations of TTO resulted in a selection of TTO-resistant sub-populations (53).

Although there is little evidence of cross-resistance to conventional antibiotic-resistant strains with TTO (33), mutant strains of *S. aureus* with reduced susceptibility to household cleaning agents containing plant extracts were less susceptible to TTO (54). Based on the available data, the activity of TTO may not favour spontaneous development of resistance; however, it is still important to minimise exposure to sublethal concentrations to limit the possibility of resistance development (33,53).

TTO resistance is also noted in gram-negative bacteria because of the nature of the outer membrane, which is composed of lipopolysaccharide, proteins and phospholipids. This membrane provides a hydrophilic permeability barrier, which is an essential factor in the tolerance of *P. aeruginosa* to membrane-damaging agents, such as TTO (36).

Toxicity concerns

As with most drugs, overdose or extended exposure induces toxic side effects. Evidence from several reports has shown that toxicity of TTO when ingested is rarely, if ever, fatal (33). In general, the symptoms of oral toxicity of TTO vary according to age and dose ingested. Reported symptoms arise from effects on the central nervous system, resulting in changes in respiration rate, oxygen saturation levels, pupil reactivity, electrolyte and blood glucose concentration, development of systemic hypersensitivity, ataxia, muscle weakness, unconsciousness and hallucinations (55,56). Similar toxic symptoms including lack of coordination, muscle tremors, dehydration, hypothermic, ataxic effects and, in more severe cases, death have been observed in animals (57).

Dermal toxicity of TTO has been reported to cause irritation and allergic reactions (33). The localised cooling effect on treated burn wound sites may lead to triggering hypothermia when using TTO-based dressings on large areas of the skin (47).

Heavy metals as antimicrobial agents

Heavy metals have a density of at least 5 g/cm³ and are located centrally in the periodic table, along with all the other transition elements, because of their ability to form complexes via their partially filled d-orbitals (58). These d-orbitals allow the heavy metals to accept electrons to form complexes, either by redox or other biochemical reactions in the cell. At low concentrations, some heavy metals may serve as essential trace elements to maintain normal cell function. However, at high concentrations, cell toxicity may result from the formation of unspecified complexes within the cell (58,59). Three classes of heavy metals have been proposed by Nies, 1999 (58):

- i. low toxicity and play an important role as trace elements, for example, iron (Fe), molybdate (Mo), manganese (Mn),

- ii. toxic but high to moderate importance as a trace element, for example, zinc (Zn), nickel (Ni), copper (Cu), vanadium (V), cobalt (Co), tungsten (W), chromium (Cr); and
- iii. toxic with little or no beneficial action, for example, arsenic (As), silver (Ag), antimony (Sb), cadmium (Cd), mercury (Hg), lead (Pb) and uranium (U).

Amongst the various biological functions of heavy metals, their antimicrobial activity will be discussed in further detail. The sequence of antimicrobial toxicity is reported as follows: Ag > Hg > Cu > Cd > Cr > Pb > Co > Au > Zn > Fe > Mn > Mo > Sn (60). The mechanism of antimicrobial action of heavy metal cations varies because of differences in chemical characteristics and their effect on different biochemical pathways in the cell (Table 3). These effects result in disruption of the microorganisms' normal cell function, leading to irreversible damage and cell death (58,61).

Metal cations interact with ionisable intracellular groups such as carboxylates and phosphates in the lipopolysaccharide layer of gram-negative bacterial cells, peptidoglycan and teichoic acids of gram-positive bacterial cells (62). Metal cations may get incorporated into the cell membrane, causing loss of fluidity, followed by further transport into the cell cytoplasm to inhibit vital biochemical processes, hence disturbing the normal growth (58,63). This transport is usually achieved via an unspecified system using a chemiosmotic gradient between the cell and its environment or, alternatively, an ATP-dependant specific transport system (58,61).

Within the cytoplasm, metal cations may affect various cell processes (62); for example, they may bind to sulfhydryl or thiol groups, leading to inhibition of various enzymes (58). Some examples of toxic activities of metal cations towards microorganisms are detailed in Table 3.

Despite their usefulness as antimicrobial compounds, the intensity of exposure (duration and concentration applied) should be carefully considered. Cobalt, cadmium and mercury are too toxic to be used clinically as antimicrobial agents. Copper, zinc and silver are less toxic to human cells. These are often incorporated into antiseptic creams or cleaning agents as well as surfaces of medical devices surfaces, including hospital taps and door handles (64,65). Examples of applications using heavy metal ions and their mechanism of action are detailed in Table 4.

Silver as an antimicrobial

History and background of the uses of silver

The antimicrobial properties of silver, including its use as an active water disinfectant, have been documented since 1000 B.C. (66). The use of silver as an antimicrobial has been reported since ancient Greek and Roman times, in which silverware was used to store perishable food and drinks. By the 19th century, the use of silver ions, Ag⁺ (as silver nitrate), in medical applications became more widespread, with records of it being used to treat venereal diseases, salivary gland fistulae, bone and perianal abscesses and removal of granulation tissue prior to epithelialisation (66,67). After the discovery of penicillin and the rapid expansion in the number and use of

Table 3 Examples of heavy metals with antimicrobial properties

Metal cations	Activity towards microorganism	References
Cadmium, Cd ²⁺	Reacts with sulphhydryl groups on various intracellular proteins	(140)
Copper, Cu ²⁺	Membrane-bound copper ions may undergo Cu(I) to Cu(II) redox cycle, catalysing formation of highly toxic hydroperoxide radicals (R-OOH)	(58,141,142)
Cobalt, Co ²⁺	Competes with zinc for the active site of urease, thus inhibiting growth of <i>Helicobacter pylori</i>	(143)
Silver, Ag ⁺	Interacts with intracellular components to suppress expression of enzymes and proteins essential for ATP production and condenses DNA, thus impairing replication	(73)
Zinc, Zn ²⁺	Inhibits nutrient uptake, acid production and glycolysis in oral pathogens and rhinoviral replication. Interferes with proton transfer	(65,144,145)

Table 4 Applications of heavy metals with lower toxicity to human cells

Heavy metal ions	Antimicrobial mechanism and uses	References
Copper, Cu ²⁺	<ul style="list-style-type: none"> • Inhibition of bacterial growth or bactericidal activity at 50–250 ppm. • Substitutes essential ions, blocks protein functional groups, inactivates enzymes, weakens membrane integrity and produces hydrogen peroxide-free radicals when membrane bound. • For example, antimicrobial hand rub, coated contact surfaces (copper toilet seats, brass taps, door handles, door push plates) and cleaning materials (ultra-microfibre cloths and mops). 	(64,146–149)
Silver, Ag ⁺	<ul style="list-style-type: none"> • Microbiocidal activity at 0.05 ppm in phosphate-buffered saline or at >50–60.5 ppm in complex biological fluids. • Interact strongly with thiol groups, inhibits bacterial enzymes, interferes with electron transport, binds to DNA, thus inhibiting normal cell replication. • Formulated as creams and incorporated into dressings for the treatment of burns and wound infections. Also incorporated into polyalkenoate dental cements, textiles, water filters, kitchen appliances and medical devices (catheters). 	(5,28,65,150,151)
Zinc, Zn ²⁺	<ul style="list-style-type: none"> • Inhibits replication of rhinovirus at <0.1 mmol/l (equivalent to <6.539 ppm). At 0.01–0.1 mM (equivalent to 0.6539–6.539 ppm), inhibits acid production by oral plaque bacteria, effective against plaque and gingivitis when combined with trichlosan. Inhibits nutrient uptake, proton transfer and sugar transport. • Incorporated into polyalkenoate dental cements, stainless steel surface coatings, air conditioning ventilation, intake and exhaust ducts. 	(65,144,145,150,152,153)

antibiotics (54,66,68), the antimicrobial use of silver declined. Recent widespread emergence of antibiotic-resistant microorganisms has resulted in Ag⁺-based agents regaining popularity as their multi-target action in microbial cells is less likely to lead to silver resistance (60,69).

Silver in its non-ionised form is an inert metal that does not react with human cells (70). Compared with other heavy metals, Ag⁺ has relatively low toxicity towards human cells at concentrations that are still highly effective against microbial pathogens (71–73).

The reactivity of Ag⁺, even at concentrations as low as 10⁻⁹ to 10⁻⁶ mol/l (equivalent to 0.000108–0.108 ppm), has shown broad spectrum antibacterial, antiviral, antiprotozoal and antifungal activity (74). In addition, the antimicrobial activity and low toxicity of silver towards human cells is also accompanied by wound-healing properties (67). Silver is able to treat infections while enhancing wound healing, which could be especially useful in the management of severe wounds such as burns and slow/non-healing ulcers.

Mode of action

Ag⁺ is classified as highly reactive moieties, which readily bind anions formed by electron donor groups containing sulphur (thiols), oxygen and nitrogen (74). Ag⁺ demonstrates broad spectrum antimicrobial activity at concentrations as low as 0.05 ppm in phosphate-buffered saline or between >50 and 60.5 ppm in complex organic biological fluids (5,75–77). Ag⁺ expresses its antimicrobial activity initially by binding to cell surface proteins and enzymes, resulting in morphological cellular changes, inhibition of cell replication (75,78,79) and impairment of solute and electron transport systems, leading to reduced production of vital cell components, such as ATP (80). Subsequent uptake of Ag⁺ into the cell cytoplasm, either via non-specific or substrate-specific transport systems, allows Ag⁺ to bind and interfere with the activity of essential intracellular enzymes and DNA (74,78,79).

In the presence of oxygen, Ag⁺ also promotes the generation of reactive oxygen species through the inhibition of respiratory enzymes, such as NADH dehydrogenase II (81), or

by impairing superoxide-radical-scavenging enzymes, such as superoxide dismutase (82). Evidence also suggests that the antimicrobial activity of Ag^+ may be a consequential result of its ability to bind to essential enzyme sulphhydryl groups (thiols), thereby breaking these protein bonds (83). The binding of Ag^+ to anionic groups, most notably disulfide, amino, imidazole, carbonyl and phosphate residues, results in intracellular and nuclear membrane (in eukaryotes) permeability changes as well as structural modifications of the cell wall (79,84,85).

Ag^+ may also bind to DNA bases, causing condensation and degenerative changes of DNA strands, leading to inhibition of cell replication and cell death (72,79). In general, the antimicrobial mechanism of action of Ag^+ is described as a cascade of four steps (80):

- The Ag^+ binds to receptors (especially in protein residues including sulphhydryl, amino, imidazole, phosphate and carboxyl groups) on the microbial cell membrane, resulting in membrane damage.
- After entering the cytoplasm, Ag^+ binds to other essential enzymes, restricting their activity and the production of vital metabolites.
- Binding to microbial DNA follows, thereby impairing cell replication.
- With weakened membrane structure and inhibition of cellular processes, vital components leak out; the bacterial cell cannot maintain normal function, resulting in cell death.

Applications

Formulations containing silver are commonly used to treat a variety of Gram-positive and Gram-negative bacteria as well as common highly antibiotic-resistant microorganisms such as *P. aeruginosa* (79,86). Silver-based pharmaceutical preparation, for example, silver sulfadiazine (Flamazine[®], Smith and Nephew Healthcare Limited, Hull, UK), has been used for the treatment of burn wounds. It has been found that the antimicrobial activity of Ag^+ depends not only on the amount of bioactive ions present but also on bioavailability, which is influenced by the physical and chemical form of silver, the affinity for moisture, rate of release and distribution (74,79,84,87).

In the skin, silver may form a temporary reservoir by binding to proteins with an estimated half-life of 10–12 hours (26). Being a highly reactive species, Ag^+ can readily bind to components present in the wound bed, for example, negatively charged proteins, RNA, DNA and chloride ions (5), thereby limiting the bioavailability of the ions. This ‘quenching’ effect of the host tissues may lead to the need for the application of higher and potentially damaging doses of Ag^+ for effective treatment.

Recent advances in controlled delivery systems, which incorporate antimicrobial agents such as Ag^+ into delivery devices or dressings, may play a role in overcoming the potential problems caused by the increased exposure and concentration of active antimicrobial agents. Some examples of silver-containing wound dressings available for current wound management applications include DuoDERM[®] (hydrocolloids, ConvaTec, Skillman, NJ, USA), Aquacel[®]

Ag (hydrofibre dressings containing antimicrobial silver ions, ConvaTec, Skillman, NJ, USA), Tegaderm[™] (films, 3M United Kingdom PLC, Bracknell, UK), Vaseline[™] (gauze, Unilever, London, UK), Sorbsan[®] (alginates, Bertek Pharmaceuticals, Research Triangle Park, NC, USA), Lyofoam[®] C (foam dressing, ConvaTec, Skillman, NJ, USA) and Nu-Gel[™] (hydrogels, Johnson and Johnson Wound Management, Somerville, NJ, USA) (16,17).

Wound-healing benefits

As discussed above, Ag^+ helps to promote wound healing by reducing the bacterial load at wound site. Ag^+ can also enhance wound healing directly by modulating the inflammatory response (88). At wound sites, Ag^+ is taken into epithelial cells responsible for the regulation of tissue metal homeostasis, heavy metal detoxification and wound healing (5). This uptake induces the synthesis of low molecular weight, cysteine-rich, metal-binding proteins called metallothioneins (metallothioneins I and II). This activity of metallothioneins, which protect the healthy cells from the toxic effects of metals, is induced by several other xenobiotic metals such as cadmium, gold and mercury (88). Metallothioneins also play an important role in promoting the uptake of key trace elements, such as zinc and copper, promoting RNA and DNA synthesis, cell proliferation, epithelialisation and tissue repair (5,88). Rising zinc levels induced by the accumulation of Ag^+ in wounds increased the activity of the zinc metalloenzymes, thus promoting cell proliferation and re-epithelialisation in rats (88).

During wound repair and the inflammatory response, matrix metalloproteinases (MMPs) are present within the wound. MMPs function to cause breakdown of the extracellular matrix, autolytic debridement, dissolution of basement membranes, growth promotion of capillary beds, re-epithelialisation and tissue remodelling (72). Hence, MMPs are essential in wound healing, but excessive levels degrade fibronectin and peptide growth factors needed for optimal re-epithelialisation (5,72). Ag^+ may form stable complexes with MMPs, thus down-regulating excessive localised inflammatory responses to promote wound healing (72,88). Comparative studies revealed that patients had improved epithelialisation on the skin grafts when treated with nanocrystalline silver dressings in comparison to topical antibiotics (85). Results indicated that Acticoat[™], a nanocrystalline silver-containing product, was effective as a dressing that reduced the wound bio-burden and altered the process of inflammation, thus facilitating the healing process (85,89). The ability of Acticoat[®] to provide prolonged sustained release of silver onto the wound site helps to avoid the development of resistance microorganisms while reducing potentially toxic side effects (85,89).

Resistance

While this multi-locus action of silver makes development of resistant microorganisms less likely, such resistance has been observed (90), and with the increasing use of silver, it may be a cause for concern (68,91). The occurrence of resistance genes has been reported and may be chromosomal- or plasmid-born (92,93). The identified silver resistance plasmid,

pMG101, codes for periplasmic binding proteins (SilE and SilF) and a chemiosmotic efflux pump (SilCBA), which exports Ag⁺ donated from an ATPase efflux pump (SilP) via SilF (90,92–94).

The initial mechanism of resistance may be because of periplasmic Ag⁺-binding proteins, SilE, each of which binds five Ag⁺ in the periplasmic space. Synthesis of SilE is stimulated by the presence of Ag⁺ during growth (90). Although SilE has high affinity for Ag⁺, the actual release mechanism of the bound Ag⁺ has yet to be determined. It is possible that the Ag⁺ may be released to the cell exterior via the SilCBA protein trimer. The SilCBA assembly functions as a transmembrane cation/proton antiporter, moving Ag⁺ from the cytoplasm directly to the cell exterior. It is classed as a member of the resistance, nodulation and cell division (RND) superfamily, sharing homologous sequences with similar resistance mechanisms in metal-resistant *Alcaligenes* and multi-drug resistance mechanisms in *E. coli* (90,93,94).

Silver resistance has been found in clinical isolates of *E. coli*, *Enterobacter cloacae*, *Proteus mirabilis* and *Klebsiella pneumoniae* in a burns unit. In addition, silver nitrate and SSD treatment has also been associated with resistance in *Proteus* spp., *E. cloacae* and miscellaneous Enterobacteriaceae (95). Similarly, resistance to multiple antibiotics and Ag⁺ has been shown in *Salmonella* species (96). Laboratory exposure of clinical strains of *E. coli* to stepwise increases of silver nitrate or SSD has been shown to induce cross-resistance against both compounds (97).

A *sil E* gene has been detected in MRSA strains, isolated from dogs, and is ≥95% homologous with the *sil E* from plasmid, pMG101 (98). However, these isolates were still sensitive to treatment with silver-impregnated hydrofibres. The results from this study are compatible with those of Silver, 2003 and suggest that there is low prevalence of silver resistance in MRSA (90). The restricted occurrence of a single *sil E* gene encoding for resistance is not sufficient to induce significant resistance against silver (97).

Toxicity concerns

In common with many drug treatments, over exposure to the agents causes unwelcome side effects. Long-term topical exposure to high concentrations of Ag⁺ leads to a build up of Ag⁰ in the dermis, causing an irreversible blue-grey discolouration of the skin (argyria). This is particularly pronounced in areas exposed to sunlight, which accelerates the photoreduction and deposition of Ag⁰ (27,70). Some patients treated with silver-containing dressings reported the occurrence of skin rashes, stinging and burning sensations when treated with silver-impregnated dressings (26). Other more serious problems associated with topical application can include disturbances in electrolyte concentration, resulting in hyponatraemia or hypochloraemia (80).

Despite the beneficial properties of various silver-based treatments, the potential toxicity and safety issues of silver use have to be carefully considered. With the increased availability of formulations, administration of silver can be tailored to the patient's condition, thus limiting the potential risk of side effects.

Conclusion

There has been increasing interest in the use of alternative, broad spectrum, pre-antibiotic antimicrobial agents, such as essential oils and metal ions, to address issues relating to increased antibiotic-resistant hospital infections. The versatility of alternative agents such as TTO and Ag⁺ against a wide range of different microorganisms because of their multiple target sites impedes the development of resistance and might be useful in improving the current wound treatment strategies. Despite the effectiveness of the agents, the potential development of side effects or toxicity to healthy host cells because of prolonged exposure at higher concentrations should be carefully monitored. Efforts to combine the use of these alternative antimicrobial agents with advances in targeted delivery techniques may help to address the issue of localised overloading and toxicity. Based on the current findings, which showed the efficacy and beneficial therapeutic properties of both Ag⁺ and TTO, the potential advantages of using these agents in wound treatment regimes should be explored further.

References

1. Glasser JS, Guymon CH, Mende K, Wolf SE, Hospenthal DR, Murray CK. Activity of topical antimicrobial agents against multidrug-resistant bacteria recovered from burn patients. *Burns* 2010;**36**:1172–84.
2. Toy LW, Macera L. Evidence-based review of silver dressing use on chronic wounds. *J Am Acad Nurse Pract* 2011;**23**:183–92.
3. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S, Krishnan P, Kumar AV, Maharjan S, Mushtaq S, Noorie T, Paterson DL, Pearson A, Perry C, Pike R, Rao B, Ray U, Sarma JB, Sharma M, Sheridan E, Thirunaryan MA, Turton J, Upadhyay S, Warner M, Welfare W, Livermore DM, Woodford N. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis* 2010;**10**:597–602.
4. Tacconelli E. Antimicrobial use: risk driver of multidrug resistant microorganisms in healthcare settings. *Curr Opin Infect Dis* 2009;**22**:352–8.
5. Atiyeh BS, Costagliola M, Hayek SN, Dibo SA. Effect of silver on burn wound infection control and healing: review of the literature. *Burns* 2007;**33**:139–48.
6. Warnke PH, Becker ST, Podschun R, Sivananthan S, Springer IN, Russo PAJ, Wiltfang J, Fickenscher H, Sherry E. The battle against multi-resistant strains: renaissance of antimicrobial essential oils as a promising force to fight hospital-acquired infections. *J Craniomaxillofac Surg* 2009;**37**:392–7.
7. Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev* 2001;**14**:244–69.
8. Karpanen TJ, Worthington T, Hendry ER, Conway BR, Lambert PA. Antimicrobial efficacy of chlorhexidine digluconate alone and in combination with eucalyptus oil, tea tree oil and thymol against planktonic and biofilm cultures of *Staphylococcus epidermidis*. *J Antimicrob Chemother* 2008;**62**:1031–6.
9. Low WL, Martin C, Hill DJ, Kenward MA. Antimicrobial efficacy of liposome encapsulated silver ions and tea tree oil against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. *Lett Appl Microbiol* 2013;**57**:33–9.
10. Martin C, Low WL, Amin MCIM, Radecka I, Raj P, Kenward MA. Current trends in the development of wound dressings, biomaterials and devices. *Pharm Pat Anal* 2013;**2**:341–59.
11. Reddy PD, Swarnalatha D. Recent advances in novel drug delivery systems. *Int J Res Ayurveda Pharm* 2010;**2**:2025–7.

12. Martin C, Low WL, Gupta A, Amin MCIM, Radecka I, Britland ST, Raj P, Kenward MA. Strategies for antimicrobial drug delivery to the biofilm. *Curr Pharm Des* 2015;**21**:43–66.
13. Vowden P, Cooper RA. An integrated approach to managing wound infection. *European wound management association*. Position document: management of wound infection. London: MEP Ltd., 2006: 2–6.
14. Robinson DA, Griffith RW, Shechtman D, Evans RB, Conzemius MG. In vitro antibacterial properties of magnesium metal against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Acta Biomater* 2010;**6**:1869–77.
15. Mears A, White A, Cookson B, Devine M, Sedgwick J, Phillips E, Jenkinson H, Bardsley M. Healthcare-associated infection in acute hospitals: which interventions are effective? *J Hosp Infect* 2009;**71**:307–13.
16. Fonder MA, Lazarus GS, Cowan DA, Aronson-Cook B, Kohli AR, Mamelak AJ. Treating the chronic wound: a practical approach to the care of nonhealing wounds and wound care dressings. *J Am Acad Dermatol* 2008;**58**:185–206.
17. Ovington LG. Advances in wound dressings. *Clin Dermatol* 2007;**25**:33–8.
18. Shaw TJ, Martin P. Wound repair at a glance. *J Cell Sci* 2009;**122**:3209–13.
19. Wild T, Rahbarnia A, Kellner M, Sobotka L, Eberlein T. Basics in nutrition and wound healing. *Nutrition* 2010;**26**:862–6.
20. Stojadinovic A, Carlson JW, Schultz GS, Davis TA, Elster EA. Topical advances in wound care. *Gynecol Oncol* 2008;**111**:S70–80.
21. Schultz GS, Sibbald RG, Falanga V, Ayello EA, Dowsett C, Harding K, Romanelli M, Stacey MC, Teot L, Vanscheidt W. Wound bed preparation: a systematic approach to wound management. *Wound Repair Regen* 2003;**11**:S1–28.
22. Martin KW, Ernst E. Herbal medicines for treatment of bacterial infections: a review of controlled clinical trials. *J Antimicrob Chemother* 2003;**51**:241–6.
23. Filoche SK, Soma K, Sissons CH. Antimicrobial effects of essential oils in combination with chlorhexidine digluconate. *Oral Microbiol Immunol* 2005;**20**:221–5.
24. Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev* 1999;**12**:564–82.
25. Pandey RR, Dubey RC, Saini S. Phytochemical and antimicrobial studies on essential oils of some aromatic plants. *Afr J Biotechnol* 2010;**9**:4364–8.
26. Lansdown AB, Williams A. How safe is silver in wound care? *J Wound Care* 2004;**13**:131–6.
27. Drake PL, Hazelwood KJ. Exposure-related health effects of silver and silver compounds: a review. *Ann Occup Hyg* 2005;**49**:575–85.
28. Castellano JJ, Shafii SM, Ko F, Donate G, Wright TE, Mannari RJ, Payne WG, Smith DJ, Robson MC. Comparative evaluation of silver-containing antimicrobial dressings and drugs. *Int Wound J* 2007;**4**:114–22.
29. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils – a review. *Food Chem Toxicol* 2008;**46**:446–75.
30. Gershenzon J, Dudareva N. The function of terpene natural products in the natural world. *Nat Chem Biol* 2007;**3**:408–14.
31. Greay S, Ireland D, Kissick H, Levy A, Beilharz M, Riley T, Carson C. Induction of necrosis and cell cycle arrest in murine cancer cell lines by *Melaleuca alternifolia* (tea tree) oil and terpinen-4-ol. *Cancer Chemother Pharmacol* 2010;**65**:877–88.
32. Edris AE. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. *Phytother Res* 2007;**21**:308–23.
33. Carson CF, Hammer KA, Riley TV. *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. *Clin Microbiol Rev* 2006;**19**:50–62.
34. Low WL, Martin C, Hill DJ, Kenward MA. Antimicrobial efficacy of silver ions in combination with tea tree oil against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. *Int J Antimicrob Agents* 2011;**37**:162–5.
35. Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warming-ton JR, Wyllie SG. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *J Appl Microbiol* 2000;**88**:170–5.
36. Mann CM, Cox SD, Markham JL. The outer membrane of *Pseudomonas aeruginosa* NCTC 6749 contributes to its tolerance to the essential oil of *Melaleuca alternifolia* (tea tree oil). *Lett Appl Microbiol* 2000;**30**:294–7.
37. Cox S, Mann C, Markham J, Gustafson J, Warming-ton J, Wyllie S. Determining the antimicrobial actions of tea tree oil. *Molecules* 2001;**6**:87–91.
38. Carson CF, Mee BJ, Riley TV. Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. *Antimicrob Agents Chemother* 2002;**46**:1914–20.
39. Reichling J, Landvatter U, Wagner H, Kostka KH, Schaefer UF. In vitro studies on release and human skin permeation of Australian tea tree oil (TTO) from topical formulations. *Eur J Pharm Biopharm* 2006;**64**:222–8.
40. Hammer KA, Carson CF, Riley TV. In-vitro activity of essential oils, in particular *Melaleuca alternifolia* (tea tree) oil and tea tree oil products, against *Candida* spp. *J Antimicrob Chemother* 1998;**42**:591–5.
41. Messenger S, Hammer KA, Carson CF, Riley TV. Effectiveness of hand-cleansing formulations containing tea tree oil assessed ex vivo on human skin and in vivo with volunteers using European standard EN 1499. *J Hosp Infect* 2005;**59**:220–8.
42. Syed TA, Ahmad SA, Holt AH, Ahmad SA, Ahmad SH, Afzal M. Management of psoriasis with *Aloe vera* extract in a hydrophilic cream: a placebo-controlled, double-blind study. *Trop Med Int Health* 1996;**1**:505–9.
43. Thornfeldt C. Cosmeceuticals containing herbs: fact, fiction, and future. *Dermatol Surg* 2005;**31**:873–81.
44. Millar BC, Moore JE. Successful topical treatment of hand warts in a paediatric patient with tea tree oil (*Melaleuca alternifolia*). *Complement Ther Clin Pract* 2008;**14**:225–7.
45. Caelli M, Porteous J, Carson CF, Heller R, Riley TV. Tea tree oil as an alternative topical decolonization agent for methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect* 2000;**46**:236–7.
46. Dryden MS, Dailly S, Crouch M. A randomized, controlled trial of tea tree topical preparations versus a standard topical regimen for the clearance of MRSA colonization. *J Hosp Infect* 2004;**56**:283–6.
47. Cuttle L, Kempf M, Kravchuk O, George N, Liu PY, Chang HE, Mill J, Wang XQ, Kimble RM. The efficacy of *Aloe vera*, tea tree oil and saliva as first aid treatment for partial thickness burn injuries. *Burns* 2008;**34**:1176–82.
48. D'Arrigo M, Ginestra G, Mandalari G, Furneri PM, Bisignano G. Synergism and postantibiotic effect of tobramycin and *Melaleuca alternifolia* (tea tree) oil against *Staphylococcus aureus* and *Escherichia coli*. *Phytomedicine* 2010;**17**:317–22.
49. Hart PH, Brand C, Carson CF, Riley TV, Prager RH, Finlay-Jones JJ. Terpinen-4-ol, the main component of the essential oil of *Melaleuca alternifolia* (tea tree oil), suppresses inflammatory mediator production by activated human monocytes. *Inflamm Res* 2000;**49**:619–26.
50. Koh KJ, Pearce AL, Marshman G, Finlay-Jones JJ, Hart PH. Tea tree oil reduces histamine-induced skin inflammation. *Br J Dermatol* 2002;**147**:1212–7.
51. Warnke PH, Sherry E, Russo PAJ, Açil Y, Wiltfang J, Sivananthan S, Sprengel M, Roldàn JC, Schubert S, Bredee JP, Springer ING. Antibacterial essential oils in malodorous cancer patients: clinical observations in 30 patients. *Phytomedicine* 2006;**13**:463–7.
52. Warnke PH, Terheyden H, Açil Y, Springer IN, Sherry E, Reynolds M, Russo PAJ, Bredee JP, Podschun R. Tumor smell reduction with antibacterial essential oils. *Cancer* 2004;**100**:879–80.
53. Nelson RRS. Selection of resistance to the essential oil of *Melaleuca alternifolia* in *Staphylococcus aureus*. *J Antimicrob Chemother* 2000;**45**:549–50.

54. Davies J. Microbes have the last word. A drastic re-evaluation of antimicrobial treatment is needed to overcome the threat of antibiotic-resistant bacteria. *EMBO Rep* 2007;**8**:616–21.
55. Hammer KA, Carson CF, Riley TV, Nielsen JB. A review of the toxicity of *Melaleuca alternifolia* (tea tree) oil. *Food Chem Toxicol* 2006;**44**:616–25.
56. Mozelsio NB, Harris KE, McGrath KG, Grammer LC. Immediate systemic hypersensitivity reaction associated with topical application of Australian tea tree oil. *Allergy Asthma Proc* 2003;**24**:73–5.
57. Bischoff K, Guale F. Australian tea tree (*Melaleuca alternifolia*) oil poisoning in three purebred cats. *J Vet Diagn Invest* 1998;**10**:208–10.
58. Nies DH. Microbial heavy-metal resistance. *Appl Microbiol Biotechnol* 1999;**51**:730–50.
59. Adarsh VK, Madhusmita M, Sanhita C, Sudarshan M, Thakur AR, Chaudhuri SR. Studies on metal microbe interaction of three bacterial isolates from East Calcutta Wetland. *J Biol Sci* 2007;**7**:80–8.
60. Ansari MA, Khan HM, Khan AA, Malik A, Sultan A, Shahid M, Shujatullah F, Azam A. Evaluation of antibacterial activity of silver nanoparticles against MSSA and MSRA on isolates from skin infections. *Biol Med* 2011;**3**:141–6.
61. Gadd GM. Metals, minerals and microbes: geomicrobiology and bioremediation. *Microbiology* 2010;**156**:609–43.
62. Karthikeyan S, Beveridge TJ. *Pseudomonas aeruginosa* biofilms react with and precipitate toxic soluble gold. *Environ Microbiol* 2002;**4**:667–75.
63. Gilbert P, Moore LE. Cationic antiseptics: diversity of action under a common epithet. *J Appl Microbiol* 2005;**99**:703–15.
64. Casey AL, Lambert PA, Elliott TSJ. Staphylococci. *Int J Antimicrob Agents* 2007;**29**:S23–32.
65. Rusin P, Bright K, Gerba C. Rapid reduction of *Legionella pneumophila* on stainless steel with zeolite coatings containing silver and zinc ions. *Lett Appl Microbiol* 2003;**36**:69–72.
66. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol Adv* 2009;**27**:76–83.
67. Edwards-Jones V. The benefits of silver in hygiene, personal care and healthcare. *Lett Appl Microbiol* 2009;**49**:147–52.
68. Chopra I. The increasing use of silver-based products as antimicrobial agents: a useful development or a cause for concern? *J Antimicrob Chemother* 2007;**59**:587–90.
69. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH, Cho MH. Antimicrobial effects of silver nanoparticles. *Nanomed Nanotech Biol Med* 2007;**3**:95–101.
70. Leak K, Johnson J. Silver therapy in practice: clinical considerations. *Prim Health Care* 2007;**17**:43–7.
71. Mooney EK, Lippitt C, Friedman J. Plastic Surgery Educational Foundation DC. Silver dressings. *Plast Reconstr Surg* 2006;**117**:666–9.
72. Warriner R, Burrell R. Infection and the chronic wound: a focus on silver. *Adv Skin Wound Care* 2005;**18**:2–12.
73. Yamanaka M, Hara K, Kudo J. Bactericidal actions of a silver ion solution on *Escherichia coli*, studied by energy-filtering transmission electron microscopy and proteomic analysis. *Appl Environ Microbiol* 2005;**71**:7589–93.
74. Maillard JY, Denyer SP. Demystifying silver. *European wound management association*. Position document: management of wound infection. London: MEP Ltd., 2006: 7–10.
75. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J Biomed Mater Res* 2000;**52**:662–8.
76. Inoue Y, Hoshino M, Takahashi H, Noguchi T, Murata T, Kanzaki Y, Hamashima H, Sasatsu M. Bactericidal activity of Ag-zeolite mediated by reactive oxygen species under aerated conditions. *J Inorg Biochem* 2002;**92**:37–42.
77. Kawahara K, Tsuruda K, Morishita M, Uchida M. Antibacterial effect of silver-zeolite on oral bacteria under anaerobic conditions. *Dent Mater* 2000;**16**:452–5.
78. Graham C. The role of silver in wound healing. *Br J Nurs* 2005;**14**:S22.
79. Lansdown AB. Silver. I: its antibacterial properties and mechanism of action. *J Wound Care* 2002a;**11**:125–30.
80. Hermans MH. Silver-containing dressings and the need for evidence. *Am J Nurs* 2006;**106**:60–8.
81. Matsumura Y, Yoshikata K, Kunisaki S, Tsuchido T. Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate. *Appl Environ Microbiol* 2003;**69**:4278–81.
82. Park HJ, Kim JY, Kim J, Lee JH, Hahn JS, Gu MB, Yoon J. Silver-ion-mediated reactive oxygen species generation affecting bactericidal activity. *Water Res* 2009;**43**:1027–32.
83. Jung WK, Koo HC, Kim KW, Shin S, Kim SH, Park YH. Antibacterial activity and mechanism of action of the silver ion in *Staphylococcus aureus* and *Escherichia coli*. *Appl Environ Microbiol* 2008;**74**:2171–8.
84. Lansdown AB. Silver in health care: antimicrobial effects and safety in use. *Curr Probl Dermatol* 2006;**33**:17–34.
85. Leaper DJ. Silver dressings: their role in wound management. *Int Wound J* 2006;**3**:282–94.
86. Matsuura T, Abe Y, Sato Y, Okamoto K, Ueshige M, Akagawa Y. Prolonged antimicrobial effect of tissue conditioners containing silver-zeolite. *J Dent* 1997;**25**:373–7.
87. Burd A, Kwok CH, Hung SC, Chan HS, Gu H, Lam WK, Huang L. A comparative study of the cytotoxicity of silver-based dressings in monolayer cell, tissue explant, and animal models. *Wound Repair Regen* 2007;**15**:94–104.
88. Lansdown AB. Silver. 2: toxicity in mammals and how its products aid wound repair. *J Wound Care* 2002b;**11**:173–7.
89. Fong J, Wood F. Nanocrystalline silver dressings in wound management: a review. *Int J Nanomedicine* 2006;**4**:441–9.
90. Silver S. Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. *FEMS Microbiol Rev* 2003;**27**:341–53.
91. Chen X, Schluesener HJ. Nanosilver: a nanoparticle in medical application. *Toxicol Lett* 2008;**176**:1–12.
92. Cutting K, White R, Edmonds M. The safety and efficacy of dressings with silver – addressing clinical concerns. *Int Wound J* 2007;**4**:177–84.
93. Silver S, Phung le T, Silver G. Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds. *J Ind Microbiol Biotechnol* 2006;**33**:627–34.
94. Gupta A, Matsui K, Lo JF, Silver S. Molecular basis for resistance to silver cations in *Salmonella*. *Nat Med* 1999;**5**:183–8.
95. Lansdown AB, Williams A. Bacterial resistance to silver in wound care and medical devices. *J Wound Care* 2007;**16**:15–9.
96. Percival SL, Bowler PG, Russell D. Bacterial resistance to silver in wound care. *J Hosp Infect* 2005;**60**:1–7.
97. Li XZ, Nikaido H, Williams KE. Silver-resistant mutants of *Escherichia coli* display active efflux of Ag⁺ and are deficient in porins. *J Bacteriol* 1997;**179**:6127–32.
98. Loh JV, Percival SL, Woods EJ, Williams NJ, Cochrane CA. Silver resistance in MRSA isolated from wound and nasal sources in humans and animals. *Int Wound J* 2009;**6**:32–8.
99. Christensen LP, Brandt K. Bioactive polyacetylenes in food plants of the Apiaceae family: occurrence, bioactivity and analysis. *J Pharm Biomed Anal* 2006;**41**:683–93.
100. Slobodníková L, Košťálová D, Labudová D, Kotulová D, Kettmann V. Antimicrobial activity of *Mahonia aquifolium* crude extract and its major isolated alkaloids. *Phytother Res* 2004;**18**:674–6.
101. Zasloff M. Antimicrobial peptides of multicellular organisms. *Nature* 2002;**415**:389–95.
102. Okoro IO, Osagie A, Asibor EO. Antioxidant and antimicrobial activities of polyphenols from ethnomedicinal plants of Nigeria. *Afr J Biotechnol* 2010;**9**:2989–93.
103. Maenthaisong R, Chaiyakunapruk N, Niruntraporn S, Kongkaew C. The efficacy of *Aloe vera* used for burn wound healing: a systematic review. *Burns* 2007;**33**:713–8.

104. Sandasi M, Leonard CM, Viljoen AM. The in vitro antibiofilm activity of selected culinary herbs and medicinal plants against *Listeria monocytogenes*. *Lett Appl Microbiol* 2009;**50**:30–5.
105. Barcroft A, Myskja A. *Aloe vera: nature's silent healer*. London: BAAM, 2003:8–11.
106. Vogler BK, Ernst E. *Aloe vera*: a systematic review of its clinical effectiveness. *Br J Gen Pract* 1999;**49**:823–8.
107. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. *J Ethnopharmacol* 2002;**81**:81–100.
108. Wagner S, Merfort I. Skin penetration behaviour of sesquiterpene lactones from different Arnica preparations using a validated GC-MSD method. *J Pharm Biomed Anal* 2007;**43**:32–8.
109. Hussain AI, Anwar F, Hussain-Sherazi ST, Przybylski R. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chem* 2008;**108**:986–95.
110. Suppakul P, Sonneveld K, Bigger SW, Miltz J. Efficacy of polyethylene-based antimicrobial films containing principal constituents of basil. *LWT-Food Sci Technol* 2008;**41**:779–88.
111. Lauten JD, Boyd L, Hanson MB, Lillie D, Gullion C, Madden TE. A clinical study: Melaleuca, Manuka, Calendula and green tea mouth rinse. *Phytother Res* 2005;**19**:951–7.
112. Okoh OO, Sadimenko AP, Asekun OT, Afolayan AJ. The effects of drying on the chemical components of essential oils of *Calendula officinalis* L. *Afr J Biotechnol* 2008;**7**:1500–2.
113. Roveroni-Favaretto L, Lodi K, Almeida J. Topical *Calendula officinalis* L successfully treated exfoliative cheilitis: a case report. *Cases J* 2009;**2**:9077.
114. Nuryastuti T, van der Mei HC, Busscher HJ, Irvati S, Aman AT, Krom BP. Effect of cinnamon oil on icaA expression and biofilm formation by *Staphylococcus epidermidis*. *Appl Environ Microbiol* 2009;**75**:6850–5.
115. Trajano VN, Lima EO, Travassos AE, Souza EL. Inhibitory effect of the essential oil from *Cinnamomum zeylanicum* Blume leaves on some food-related bacteria. *Food Sci Technol* 2010;**30**:771–5.
116. Angienda PO, Onyango DM, Hill DJ. Potential application of plant essential oils at sub-lethal concentrations under extrinsic conditions that enhance their antimicrobial effectiveness against pathogenic bacteria. *Afr J Microbiol Res* 2010;**4**:1678–84.
117. Pinto E, Vale-Silva L, Cavaleiro C, Salgueiro L. Antifungal activity of the clove essential oil from *Syzygium aromaticum* (*Eugenia caryophyllus*) on *Candida*, *Aspergillus* and dermatophyte species. *J Med Microbiol* 2009;**58**:1454–62.
118. Batish DR, Singh HP, Kohli RK, Kaur S. Eucalyptus essential oil as a natural pesticide. *Forest Ecol Manag* 2008;**256**:2166–74.
119. Karpanen T, Conway B, Worthington T, Hilton A, Elliott T, Lambert P. Enhanced chlorhexidine skin penetration with eucalyptus oil. *BMC Infect Dis* 2010;**10**:278.
120. Gupta C, Garg AP, Uniyal RC, Kumari A. Antimicrobial activity of some herbal oils against common food-borne pathogens. *Afr J Microbiol Res* 2008;**2**:258–61.
121. Souza EL, Lima EO, Freire KRL, Sousa CP. Inhibitory action of some essential oils and phytochemicals on the growth of various moulds isolated from foods. *Braz Arch Biol Technol* 2005;**48**:245–50.
122. O'Gara EA, Hill DJ, Maslin DJ. Activities of garlic oil, garlic powder, and their diallyl constituents against *Helicobacter pylori*. *Appl Environ Microbiol* 2000;**66**:2269–73.
123. Sallam KI, Ishioroshi M, Samejima K. Antioxidant and antimicrobial effects of garlic in chicken sausage. *LWT-Food Sci Technol* 2004;**37**:849–55.
124. Lee JH, Shim JS, Chung MS, Lim ST, Kim KH. In vitro anti-adhesive activity of green tea extract against pathogen adhesion. *Phytother Res* 2009;**23**:460–6.
125. Cabrera C, Artacho R, Gimenez R. Beneficial effects of green tea – a review. *J Am Coll Nutr* 2006;**25**:79–99.
126. Yee YK, Koo MWL, Szeto ML. Chinese tea consumption and lower risk of *Helicobacter* infection. *J Gastroenterol Hepatol* 2002;**17**:552–5.
127. Bilia AR, Giomi M, Innocenti M, Gallori S, Vincieri FF. HPLC-DAD-ESI-MS analysis of the constituents of aqueous preparations of verbena and lemon verbena and evaluation of the antioxidant activity. *J Pharm Biomed Anal* 2008;**46**:463–70.
128. Funes L, Fernández-Arroyo S, Laporta O, Pons A, Roche E, Segura-Carretero A, Fernández-Gutiérrez A, Micol V. Correlation between plasma antioxidant capacity and verbascoside levels in rats after oral administration of lemon verbena extract. *Food Chem* 2009;**117**:589–98.
129. Ohno T, Kita M, Yamaoka Y, Imamura S, Yamamoto T, Mitsufuji S, Kodama T, Kashima K, Imanishi J. Antimicrobial activity of essential oils against *Helicobacter pylori*. *Helicobacter* 2003;**8**:207–15.
130. Pereira CG, Meireles MAA. Evaluation of global yield, composition, antioxidant activity and cost of manufacturing of extracts from lemon verbena (*Aloysia triphylla* [L'herit.] Britton) and mango (*Mangifera indica* L.) leaves. *J Food Process Eng* 2007;**30**:150–73.
131. Burt S. Essential oils: their antibacterial properties and potential applications in foods – a review. *Int J Food Microbiol* 2004;**94**:223–53.
132. Chouliara E, Karatapanis A, Savvaidis IN, Kontominas MG. Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4°C. *Food Microbiol* 2007;**24**:607–17.
133. Marnewick JL. Rooibos and honeybush: recent advances in chemistry, biological activity and pharmacognosy. In: *African natural plant products: new discoveries and challenges in chemistry and quality*. American Chemical Society, 2009:277–94.
134. Almajano MP, Carbó R, Jiménez JAL, Gordon MH. Antioxidant and antimicrobial activities of tea infusions. *Food Chem* 2008;**108**:55–63.
135. Nakano M, Nakashima H, Itoh Y. Anti-human immunodeficiency virus activity of oligosaccharides from rooibos tea (*Aspalathus linearis*) extracts in vitro. *Leukemia* 1997;**11**:128–30.
136. Gutierrez J, Barry-Ryan C, Bourke P. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *Int J Food Microbiol* 2008;**124**:91–7.
137. Fu Y, Zu Y, Chen L, Shi X, Wang Z, Sun S, Efferth T. Antimicrobial activity of clove and rosemary essential oils alone and in combination. *Phytother Res* 2007;**21**:989–94.
138. Moreno S, Scheyer T, Romano CS, Vojnov AA. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radic Res* 2006;**40**:223–31.
139. Prabhmanju M, Shankar GS, Babu K. Herbal vs. chemical actives as antidandruff ingredients – which are more effective in the management of dandruff? An overview. *Ethnobot Leaflets* 2009;**13**:1373–81.
140. Chopra I. Mechanism of plasmid-mediated resistance to cadmium in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1975;**7**:8–14.
141. Noyce JO, Michels H, Keevil CW. Potential use of copper surfaces to reduce survival of epidemic methicillin-resistant *Staphylococcus aureus* in the healthcare environment. *J Hosp Infect* 2006;**63**:289–97.
142. Magnani D, Solioz M. How bacteria handle copper. In: Nies D, Silver S, editors. *Molecular microbiology of heavy metals*. Springer: Berlin/Heidelberg, 2007:259–85.
143. Bruggraber SFA, French G, Thompson RPH, Powell JJ. Selective and effective bactericidal activity of the cobalt (II) cation against *Helicobacter pylori*. *Helicobacter* 2004;**9**:422–8.
144. Phan TN, Buckner T, Sheng J, Baldeck JD, Marquis RE. Physiologic actions of zinc related to inhibition of acid and alkali production by oral streptococci in suspensions and biofilms. *Oral Microbiol Immunol* 2004;**19**:31–8.
145. Singh M, Das RR. Zinc for the common cold. *Cochrane Database Syst Rev* 2011;**2**:CD001364.
146. Hall TJ, Wren MWD, Jeanes A, Gant VA. A comparison of the antibacterial efficacy and cytotoxicity to cultured human skin cells of 7 commercial hand rubs and Xgel, a new copper-based biocidal hand rub. *Am J Infect Control* 2009;**37**:322–6.
147. Luna VA, Hall TJ, King DS, Cannons AC. Susceptibility of 169 USA300 methicillin-resistant *Staphylococcus aureus* isolates to

- two copper-based biocides, CuAL42 and CuWB50. *J Antimicrob Chemother* 2010;**65**:939–41.
148. Faundez G, Troncoso M, Navarrete P, Figueroa G. Antimicrobial activity of copper surfaces against suspensions of *Salmonella enterica* and *Campylobacter jejuni*. *BMC Microbiol* 2004;**4**:19.
149. Ibrahim SA, Yang H, Seo CW. Antimicrobial activity of lactic acid and copper on growth of *Salmonella* and *Escherichia coli* O157:H7 in laboratory medium and carrot juice. *Food Chem* 2008;**109**:137–43.
150. Coughlan A, Scanlon K, Mahon BP, Towler MR. Zinc and silver glass polyalkenoate cements: an evaluation of their antibacterial nature. *Biomed Mater Eng* 2010;**20**:99–106.
151. Monteiro DR, Gorup LF, Takamiya AS, Ruvollo-Filho AC, Camargo ER, Barbosa DB. The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. *Int J Antimicrob Agents* 2009;**34**: 103–10.
152. Keegan G, Smart J, Ingram M, Barnes L, Rees G, Burnett G. An in vitro assessment of bioadhesive zinc/carbomer complexes for antimicrobial therapy within the oral cavity. *Int J Pharm* 2007;**340**: 92–6.
153. Dardenne M. Zinc and immune function. *Eur J Clin Nutr* 2002;**56**: S20–3.