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

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Abstract:	The increasing occurrence of hospital infections and the emerging problems posed by antibiotic resistant strains contribute to escalating treatment costs. Infection on the wound site can potentially stall the healing process at the inflammatory stage, leading to the development of acute wounds. Traditional wound treatment regime 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. In recent research, 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 been identified to have potent antimicrobial, antifungal, antiviral, anti-inflammatory, anti-oxidant and other beneficial therapeutic properties. Similarly, heavy metal ions have also been used as disinfecting agents due to their broad spectrum activities. Such activities is contributed by reactive properties of the metal cations, which allows the ions to interact with many different intracellular compounds, thereby resulting in the disruption of vital cell function leading to cell death. This review will discuss the potential properties of essential oils and heavy metal ions, in particular tea tree oil and silver ions as alternative antimicrobial agents for the treatment of wounds.

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, anti-oxidant and other beneficial therapeutic properties. Similarly, heavy metal ions have also been used as disinfecting agents due to 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, in particular tea tree oil and silver ions, as alternative antimicrobial agents for the treatment of chronic, infected wounds.

Keywords: -Antimicrobial, Silver, Tea Tree Oil, Wound Infection

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 chronic wound with increased potential to develop into 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 coli*, multidrug-resistant *Acinetobacter baumannii*, ciprofloxacin-resistant *P. aeruginosa*) [3, 4] have renewed interest in pre-antibiotic antibacterial agents such as metal ions and essential oil (TTO) [5, 6]. Alternative, non-antibiotic based treatments are attractive due to their decreased side effects compared to 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, 9, 10, 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 patients 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 pathogenic invasion by these microorganisms [7]. Colonization 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, 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 colonizing microorganisms, thus triggering a series of systemic responses which delay the healing process [7, 13].

The increasing occurrence of hospital infections and widespread emergence of resistant microorganisms contribute to escalating treatment costs. Additionally, hypersensitivity reactions to antibiotics and the lack of access to new treatments within the health care industry, makes provision of sufficient support and care for patients difficult. Modern lifestyles which frequently lack physical activity increase the possibility of developing various life-long (interconnected) health conditions, *e.g.* 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 due to their decreased side effects compared to 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 due to 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, 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 colonizing microorganisms, thus triggering a series of systemic responses which 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 *e.g.* visitors [15].

Cutaneous wounds offer a favourable (moist, warm, nutritious) environment to support bacterial growth and proliferation. Heavy microbial infection (above critical colonization) 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 colonization 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 due to 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 synthesize aromatic secondary metabolites to protect against predation and prevent colonization 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 tea tree oil, ylang ylang, betel pepper, manuka, eucalyptus, arnica, lemon verbena, rosemary, green tea extract and cadendula [24, 23]. 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 concentration 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 due to 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. When faced with microbial invasion or attack leading to infection, plants have their own defence mechanisms which rely on the production of compounds which interfere with the cellular and intra-cellular structure of microorganisms [29]. These antimicrobial plant compounds which 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 irritant to other organisms, and may flow out from injured plants to prevent colonization by microorganisms [30, 31]. These secondary metabolites are mainly present as volatile compounds such as phenols or their oxygen-substituted derivatives, which are categorized 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 synthesize these compounds.

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 synthesized by plants as secondary metabolites and are well known for their antibacterial, antifungal and antiviral properties [6, 29], in addition to anticancer, anti-diabetic, anti-inflammatory and anti-oxidant activities [29, 32]. Essential oils are

multi-component compounds, usually with terpenes and their derivatives (terpeniods) 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 summarized in Table 2.

Tea tree essential oil as an antimicrobial

History, background of use and production of tea tree oil

Bundjalung Aborigines of northern New South Wales traditionally used crushed tea tree leaves as a treatment for coughs and colds (inhalation), as a herbal infusion for sore throats and also sprinkled them directly onto skin wounds to promote healing [33]. The use of tea tree oil (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.~~ 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* plant *via* steam distillation, and condensed to yield a

pale yellow oil [33]. ~~Due to t~~The potential variation in the composition of ~~the~~ TTO ~~components,~~ has lead to the classification of six chemotypes of *Melaleuca alternifolia*, ~~has been described~~ 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 haves ~~s~~ to comply with ISO4730:2004 standards for "Oil of Melaleuca, terpinen-4-ol type", which defines the maximum and minimum level for the fourteen 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 microbiocidal 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 permeabilize cell membranes [36].

Cox *et al.* (2000) examined membrane disruption of *E. coli*, *S. aureus* and *C. albicans* by TTO *via* the leakage of potassium ions, materials that absorb at 280nm (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 280nm 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 tea tree oil, terpinen-4-ol, 1,8-cineole and α-terpineol [32]. 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, mouthwashes, as well as for the treatment of microbial infections such as folliculitis and vaginitis [39, 40, 41, 42, 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 methicillin resistant *S. aureus* (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 to 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 due to its antimicrobial and therapeutic properties. Burnaid® 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 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 *e.g.* TNFα, IL-1β, 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 to 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 minutes, providing localized 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 wellbeing 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 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 due to 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]. Generally, 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 hallucination [55, 56]. Similar toxic symptoms including lack of co-ordination, 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 localized 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/cm^3 and are located centrally in the periodic table, along with all the other transition elements, due to 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, *e.g.* iron (Fe), molybdate (Mo), manganese (Mn),
ii. toxic but high to moderate importance as a trace element, *e.g.* zinc (Zn), nickel (Ni), copper (Cu), vanadium (V), cobalt (Co), tungsten (W), chromium (Cr); and
iii. toxic with little or no beneficial action, *e.g.* 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 due to 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 utilising 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 has been have been documented since 1000 B.C. [66]. The use of silver as an antimicrobial tool has been reported since ancient Greek and Roman periodstimes, 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 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 since 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 to other heavy metals, Ag^+ have relatively low toxicity towards human cells at concentrations that are still highly effective against microbial pathogens [71, 72, 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 characteristics towards human cells of silver is also accompanied by wound healing properties [67]. Silver is able to treat infections whilst 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^+ are classified as highly reactive moieties, which readily bind anions formed by electron donor groups containing sulphur (thiols), oxygen and nitrogen [74]. Ag^+ demonstrate broad spectrum antimicrobial activity at concentrations as low as 0.05 ppm in phosphate buffered saline, or between >50 ppm to 60.5 ppm in complex organic biological fluids [5, 75, 76, 77]. Ag^+ express their 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, allow Ag^+ to bind and interfere with the activity of essential intracellular enzymes and DNA [74, 78, 79].

In the presence of oxygen, Ag^+ also promote the generation of reactive oxygen species through 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 their ability to bind to essential enzyme sulphydrals 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 binds to DNA bases, causing condensation and degenerative changes of DNA strands, leading to inhibition of cell replication and cell death [72, 79]. Generally 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 sulphydryl, amino, imidazole, phosphate and carboxyl groups) on the microbial cell membrane, resulting in membrane damage.
- After entering the cytoplasm, Ag^+ bind to other essential enzymes, restricting their activity and the production 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®) has been used for the treatment of burn wounds. It has been found that the antimicrobial activity of Ag^+ depend not only on the amount of bioactive ions present, but also 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 agent. Some examples of silver containing wound dressings available for current wound management applications include DuoDERM® (hydrocolloids), Aquacel® Ag (hydrofibre dressings containing antimicrobial silver ions), TegadermTM (films), VaselineTM (gauze), Sorbsan® (alginates), Lyofoam® C (foam dressing) and Nu-GelTM (hydrogels) [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^+ are 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 reepithelialisation in rats [88].

During wound repair and the inflammatory response, matrix metalloproteinases (MMPs) are present in within the wound. MMPs function to cause breakdown of the extracellular matrix, autolytic debridement, dissolution of basement membranes, growth promotion of capillary beds, reepithelialisation 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 localized inflammatory response 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]. ~~Research data have shown~~Results indicated that Acticoat™, a nanocrystalline silver containing product, was effective as a dressing which reduced the wound bioburden ~~whilst~~ and alter~~eding the~~ the process of inflammat~~ory~~ ion,~~events to~~ thus facilitat~~e~~ing 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 whilst reducing potential 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 [~~89~~90] and with the increasing use of silver it may be a cause for concern [68, ~~90~~91]. The occurrence of resistance genes have been reported and may be chromosomal or plasmid born [~~91~~92, ~~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 [~~89~~90, ~~91~~92, ~~92~~93, ~~93~~94].

The initial mechanism of resistance may be due to 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 [~~89~~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* [8990, 9293, 9394].

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., *Enterobacter cloacae* and miscellaneous Enterobacteriaceae [9495]. Similarly, resistance to multiple antibiotics and Ag^+ has been shown in *Salmonella* species [9596]. 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 [9697].

A *sil* E gene has been detected in MRSA strains, isolated from dogs, and is ≥95% homologous with the *sil* E from plasmid, pMG101 [9798]. However these isolates were still sensitive to treatment with silver impregnated hydrofibers. The results from this study are compatible with those of Silver, 2003 and suggest that there is low prevalence of silver resistance in MRSA [8990]. The restricted occurance 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 Ag^0 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 Ag^0 [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 hyponatremia or hypochloremia [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 due to 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 due to 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 localized 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 regime should be explored further.

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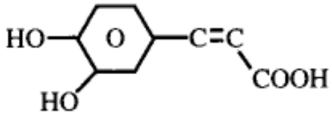
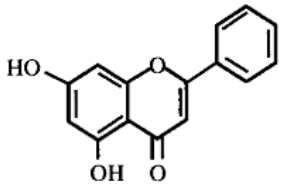
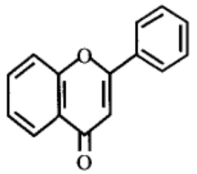
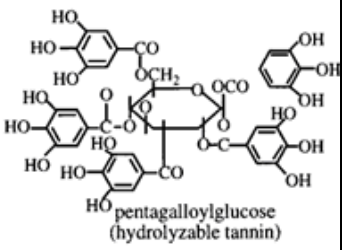
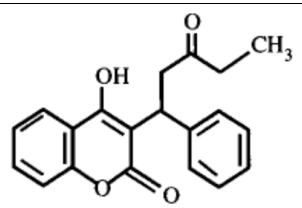
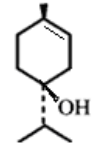
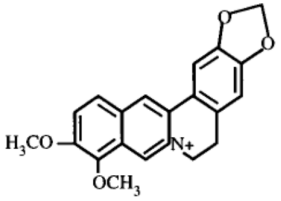
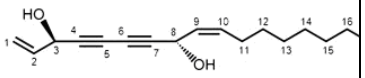
Key Message

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 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 tea tree oil indicate 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.

Table 1: Summary of the major classes of active antimicrobial compounds from plants (summarized from references 24, 29, 39, 98, 99, 100, 101)

Class and description	Subclass	Example	Mechanism	Structure
Phenolics ▪ Simple phenolic ring structures which may exist in their highest oxidation state. *E.g.* 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. ▪ Highly oxidised phenols or those with increased hydroxylation have increased microbial inhibitory effects. ▪ Active against various types of bacteria,	Simple phenol and phenolic acid	Catechol, epicatechin and caffeic acid	Substrate deprivation,and membrane disruption	 caffeic acid
	Quinone	Hypericin	Binds to adhesins, complex with cell wall and inactivate enzyme	 quinone
	Flavonoids	Chrysin	Binds to adhesins	 chrysin

viruses and fungi. ▪ May cause iron deprivation, reaction with sulphydryl groups, protein related polyamide polymers via nonspecific interaction and hydrogen bonding to inactivate proteins or enzymes in the cell.	Flavones	Abyssinone	Complexes with cell wall and inavtivates enzymes	 flavone
	Tannins	Ellagitannin	Binds to proteins, adhesins, inhibits enzymes, causes substrate deprivation, complexes with cell wall, membrane disruption and metal ion complexation	 pentagalloylglucose (hydrolyzable tannin) **Tannins**

	Coumarins	Warfarin	Interactions with eukaryotic DNA, and has antiviral properties	 warfarin
Terpenoids ▪ Actively disrupts various membranes of bacteria, fungi, protozoa and enveloped viruses *via* its lipophilic properties.	Monoterpenes	Terpinen-4-ol	Membrane disruption	 Terpinen-4-ol
Alkaloids ▪ Secondary plant metabolites that have antimicrobial activity.	N/A	Berberine and piperine	Intercalate into cell wall or DNA, differentially inhibit sterol and chitin biosynthesis	 berberine
Polyacetylenes ▪ Has antibacterial, antimycobacterial, anti-inflammatory, anti-platelet-	N/A	Falcarinol	Mechanism unknown	 Falcarinol

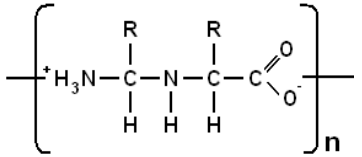
aggregatory effects.				
Lectins and polypeptides ▪ Inhibition of membrane function and integrity *via* interaction with proteins, ion channels and displacement of lipids. ▪ Presence of cholesterol and increasing ionic strength in the target membrane reduces the antimicrobial activity due to stabilization 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	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	 polypeptide

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	*Aloe barbadensis, Aloe vera*	Anthraquinones (aloin, emodin and resistanol), β-sitosterol and gibberellins	• Antimicrobial • Increases wound healing • Induces hypoglycemic effect • Lowers blood cholesterol	• Used on burns and wounds. • Treatment of psoriasis and genital herpes • Mediate insulin production	42, 102, 103, 104, 105, 106
Arnica	*Arnica montana*	Sesquiterpene and lactones	• Anti-inflammatory	• Applied to bruises and swelling • Osteoarthritis treatment	107
Basil	*Ocimum basilicum*	Linalool, methylchavicol, epi-α-cadinol, α-bergamotene, γ-cadinene and germacrene D	• Antimicrobial • Antioxidant	• Antimicrobial activity in food preservation and packaging industry	108, 109

Calendula (Marigold)	*Calendula officinalis*	Triterpenoid	• Antimicrobial • Anti-inflammatory • Anti-tumorogenic	• Herbal antimicrobial mouthwash • Treatment against athletes foot, ringworm and Candidal infections	110, 111, 112
Cinnamon	*Cinnamomum zeylanicum*	cinnamaldehyde	• Analgesic • Antiseptic	• Inhibits biofilm formation by clinical strains of *Staphylococcus epidermidis* • Active against common food spoilage microbes	113, 114
Clove	*Syzygium aromaticum*	Eugenol	• Antibacterial • Antifungal	• Used to treat toothache and skin sores	103, 115, 116
Eucalyptus	*Eucalyptus globulus*	1,8-cineole, Tannin	• Antimicrobial • Insect repellent, • Skin penetration enhancer for drugs	• Herbal antimicrobial mouthwash • Inhibits growth of food pathogen • Fragrant in pharmaceuticals, soaps, detergents and food	117, 118, 119, 120

				• Natural pesticide/insecticide	
Garlic	*Allium sativum*	Allicin	• Antimicrobial • Potential anti-cancer agent • lowers cholesterol and triglycerides	• *In vitro* antimicrobial activity against *H. pylori* • Food preservative • Reduce risk of hypertension	32, 103, 121, 122
Green tea extract	*Camellia sinensis*	Catechins, tannins, caffeine	• Anti-inflammatory • Antimicrobial • Anti-cancer • Antioxidant	• Reduces dental caries • Lowers risk of *Helicobacter* infection	110, 123, 124, 125
Lemon verbena	*Aloysia triphylla or Lippia citriodora*	Verbacoside	• Antimicrobial, • Anti cancer agent • Antioxidant • Anti-inflammatory • Antispasmodic	• *In vitro* antimicrobial activity against *H. pylori*, *E. coli, Mycobacterium tuberculosis, S. aureus* and *C. albicans* • Treatment of asthma, cold, fever	126, 127, 128, 129

			• Antipyretic	and indigestion	
Manuka	*Leptospermum scoparium*	α-terpineol, cineole, β-triketone	• Antimicrobial • Anti-inflammatory	• Aids wound healing • Relieve fevers, inflammation and pain • Respiratory ailments	23, 110
Oregano	*Origanum vulgare*	Thymol, carvacol, ρ-cymene, γ-terpinene, Thymoquinone	• Antimicrobial • Antioxidative	• Used in food preservation	130, 131
Rooibos	*Aspalathus linearis*	Flavonoids, aspalathin, isoorientin, orientin and rutin	• Antimicrobial • Antioxidant activity	• *In vitro* antimicrobial and antifungal activity against *Bacillus cereus*, *Micrococcus luteus* and *Candida albicans*, • *In vitro* anti-HIV activity	103, 132, 133, 134
Rosemary	*Rosmarinus officinalis*	α-pinene, bornyl acetate, eucalyptol,	• Antimicrobial • Antioxidant	• *In vitro* antifungal activity particularly against *Malassezia*	130, 135, 136, 137, 138

		camphor, 1,8-cineole	• Anti-cellulitis	(*Pityrosporum*) spp., • Food preservation	
Tea Tree Oil	*Melaleuca alternifolia*	Terpinen-4-ol, α-terpineol, 1,8-cineole	• Antimicrobial • Anti-cancer • Anti-inflammatory	• Treatment of wounds • Pharmaceutical cosmetics and cleaning product applications	32, 33, 110

Table 3: Examples of heavy metals with antimicrobial properties

Metal cations	Activity towards microorganism	References
Cadmium, Cd^{2+}	Reacts with sulphdryl groups on various intracellular proteins.	139
Copper, Cu^{2+}	Membrane-bound copper ions may undergo Cu(I) to Cu(II) redox cycle catalyzing formation of highly toxic hydroperoxide radicals (R-OOH).	58, 140, 141
Cobalt, Co^{2+}	Competes with zinc for the active site of urease, thus inhibiting growth of *Helicobacter pylori*.	142
Silver, Ag^{+}	Interacts with intracellular components to suppress expression of enzymes and proteins essential for ATP production, 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, 143, 144

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

Heavy metal ions	Antimicrobial mechanism and uses	References
Copper, Cu^{2+}	• 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. • *E.g.* 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, 145, 146, 147, 148
Silver, Ag^{+}	• 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, 149, 150
Zinc, Zn^{2+}	• Inhibits replication of rhinovirus at <0.1mmol/L	65, 143, 144, 149,

	(equivalent to <6.539 ppm). At 0.01-0.1mM (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.	151, 152