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Essential Oils and Future Antibiotics: New Weapons against Emerging 'Superbugs'?

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Abstract

Antibiotic resistance is emerging at an alarming rate, outpacing current research and development efforts to combat this trend. As a result, many infectious diseases have become difficult to treat; in some cases, no treatment options exist. The search for new antibiotics must accelerate to avoid returning to the 'pre-antibiotic' era. Ancient remedies, including essential oils and their components, have been explored on a limited basis as a source of new antimicrobials. Many are known to possess significant antimicrobial activity against a wide range of microorganisms. Elucidation of the mechanism of action of these compounds may lead to identification new antibiotic targets. Such targets, once identified, may represent biosynthetic or regulatory pathways not currently inhibited by available drugs. Novel drugs and targets are vital for continued control of infectious diseases worldwide.

Keywords: Essential oils; Antibiotic resistance; New antibiotics

Introduction

Not long ago, we thought we had conquered infectious disease. The scourge of mankind had been vanquished by the discovery of antibiotics.

"…we can look forward with confidence to a considerable degree of freedom from infectious diseases at a time not too far in the future. Indeed…it seems reasonable to anticipate that within some measurable time…all the major infections will have disappeared…." [1].

The discovery of antibiotics revolutionized medicine; the increasing emergence of antibiotic resistance threatens to return medicine to the pre-antibiotic era. Recently, the US Centers for Disease Control sounded an alarm regarding emerging antibiotic resistance and the threat to public health.

"CRE are nightmare bacteria. They pose a triple threat. First, they're resistant to all or nearly all antibiotics, even some of our last-resort drugs. Second, they have high mortality rates. They kill up to half of people who get serious infections with them. And third, they can spread their resistance to other bacteria." [2].

To combat emerging antibiotic resistance and the rise of 'superbugs', new drugs are needed. However, research and development for new antimicrobial agents is lagging far behind the rate at which bacteria are developing resistance. As a result, many infectious diseases once easily cured have now become increasingly difficult to treat. So where are new antimicrobials to be found? Perhaps by looking to the past, we may discover significant science behind the 'myths' of ancient remedies; science which could lead to the development of new antibiotics and other drugs.

Ancient Remedies: A History

For thousands of years, aromatic oils have been used to relieve a wide variety of human maladies including bronchitis, pneumonia, pharyngitis, diarrhea, periodontal disease, wounds, and numerous other illnesses. Many traditions surrounding the use of these oils are buried in antiquity, passed down orally from master to student until the origin of specific treatments were lost to the ages. In antiquity, medicinal oils were derived from aromatic plants and resins by extraction into other fatty oils such as olive oil and used as a mixture. The earliest recorded use of aromatic oils dates back to 4,500 B.C. in Egypt [3]. The ancient Egyptians recognized that oils could be used in treating illness, including infection and inflammation. So valuable were these oils, that King Tutankhamun was entombed with roughly 350 liters of aromatic oil including cedarwood, frankincense, and myrrh [3]. Myrrh is one of the earliest and well known of the aromatic oils. References to myrrh abound in antiquity. The ancient Hebrews referred to myrrh as 'holy oil' which was more valuable than gold. The ancient Egyptians referred to myrrh as 'the tears of Horus'. Myrrh is derived from the resin of a woody shrub of the genus, *Commiphora*, which grows in hot, arid climates. In ancient Sumer, myrrh was used for treating parasitic infections and periodontal disease. The Greek physician, Dioscorides, used myrrh for bronchial and other infections including the skin [4]. Myrrh was often combined with frankincense, aromatic oil used in antiquity to treat infectious diseases and inflammation. Like myrrh, frankincense is member of a resinous family of plants (*Burseraceae*) commonly found in arid regions of the Middle East and north-east Africa [5,6]. The use of frankincense and myrrh is mentioned numerous times in biblical and other ancient texts [7]. These oils, alone or in combination, were used extensively for the treatment of wounds, inflammation, cystitis, rheumatic joints, skin sores, bleeding, fungal infections, burns, pharyngitis, syphilis, and leprosy [8,9].

Other cultures across the globe have long-standing, medical practices which incorporate the use of aromatic oils and other plant-based therapies, including those found in the Americas, Australia, and the Far East such as the Ayurvedic, Unani, and Chinese traditions. Among the more well-recognized remedies still in use today from North and South America and Australia are purple coneflower (*Echinacea purpurea*), cat's claw (*Uncaria tomentosa*), and eucalyptus (*Eucalyptus globulus*)

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[4]. Ayurvedic traditions include the use of camphor (*Cinnamomum camphora*) and cardamomum (*Elettaria cardamomum*) [4]. China's use of herbal medicine dates as far back as 3000 B.C., when the mythological and legendary ruler Shen Nong Shi (or Shennong) taught humans the use of medicinal plants. His cumulative work, 'Shennong Bencao Jing', is considered one of the earliest medical collections in China [10]. By 500 A.D. the use of aromatic oils had spread throughout most of Asia Minor, and the Mediterranean, spreading along with the Roman and later the Persian Empires [11]. Commonly used aromatic oils included those derived from thyme, clove, rosemary, lavender, and cinnamon.

Today, the term 'essential oils' is used to describe the mixtures derived from aromatic medicinal plants using conventional techniques such as distillation and chromatographic separation. These oils continue to be used for the treatment of infectious disease and inflammation in traditional medicine across the globe. They are administered orally, topically, or via aromatherapy, depending on historical use and chemical composition which for many essential oils has been determined. As a result, a significant amount of toxicity data is available for not only the oils but also the individual components such that many are generally regarded as safe (GRAS) by the FDA. GRAS status has permitted the use of essential oils as flavoring agents in food and as additives to cosmetics, perfumes, and cleaning products.

Ancient Remedies: The Science

Essential oils are derived from a variety of natural sources including plants or components of plants such as flowers, leaves, bark, roots, berries, seeds and/or fruit. These oils are complex mixtures of chemicals, and include various alcohols, aldehydes, terpenes, ethers, ketones, phenols, and oxides. Many essential oils have limited solubility in aqueous solutions and form emulsions with non-ionic surfactants. Previous investigators have reviewed the effect of essential oils, their components and antimicrobial activity [12-17]. However, few studies have determined the antimicrobial-specific mechanism(s) of action of various essential oils or their components [18,19].

Since essential oils are complex mixtures of compounds, it is likely the observed antimicrobial activity is due to inhibition or interaction with multiple targets in the cell [20,21]. However, many essential oils exert non-specific antimicrobial effects due to the hydrophobic properties of the mixtures and components. For instance, the hydrophobic character of many essential oils facilitates entry into cell membranes leading to alteration in architecture, leakage of cell contents, and eventually death [22-26]. In 2009, Fisher and Phillips demonstrated uptake of *Citrus sinensis* and *Citrus bergamia* oils into *Enterococcus faecium* and *E. faecalis* resulting in multiple membrane-related changes: a 2- or 40-fold increase in membrane permeability, a decrease in intracellular pH, the loss of membrane potential, and a reduction in ATP concentration [25]. This is not surprising given that many essential oils contain high concentrations of phenolic compounds including carvacrol, thymol, and eugenol. Phenols are known to disrupt cell membranes resulting in the dissolution of the proton motive force and a subsequent decrease in ATP synthesis [27-29]. Inhibition of ATP synthesis may also result from essential oil-mediated alteration of protein-protein interactions in the cell membrane or direct binding of oil components, especially cyclic hydrocarbons, to lipophilic regions of membrane-bound proteins [28,30]. Diminished ATP levels would necessarily lead to reduction in other energy-dependent cellular processes including synthesis of enzymes and toxins. For example, previous studies have demonstrated a significant decrease in the amount of diarrheal toxin detected in *Bacillus cereus* when exposed to carvacrol. The authors hypothesized that the decrease in toxin detection may be connected to the decrease in ATP production which is required not only for toxin synthesis but also export [31].

Although the spectrum of activity for most essential oils is relatively broad, as would be expected with a mechanism of action related to membrane disruption, evidence is emerging which suggests more specific targets may exist. Such specific targets may vary between organisms, thus explaining the more narrow range of activity of some essential oils and/or components. In such cases, specificity may be related to individual essential oil components. Recently, investigators attempted to determine the mechanism of action of cold-pressed Valencia orange oil against methicillin-resistant *Staphylococcus aureus* (MRSA) [32]. Microarray data showed a 24-fold increase in expression of *cwrA* following exposure to the oil. Interestingly, upregulation of *cwrA* was also demonstrated following exposure to known cell wall-active antibiotics such as penicillin G, oxacillin, phosphomycin, imipenem, and vancomycin suggesting a similar mechanism of action [33-35]. Other specific effects of citrus oil on MRSA include increased expression of penicillin-binding–protein-4 (PBP 4), involved in peptidoglycan synthesis, and genes in the *dlt*ABCD operon. This operon controls alanylation of teichoic acids of the cell wall which may play a role in autolysin activity of *S. aureus* [32]. Autolysin activity was also suggested by Carson and coworkers who noted that tea tree oil resulted in release of membrane-bound, cell wall autolytic enzymes leading to cell lysis and death [21].

Specific targets have also been implicated by the differential activity of essential oils observed against various microorganisms [12]. For instance, multiple studies have shown that essential oils work well against a number of Gram-positive bacteria, with only moderate to little effect on Gram-negative organisms [12]. Some investigators postulated that Gram-negative organisms were intrinsically more resistant to the effects of essential oils due to the presence of the outer membrane which provides an additional permeability barrier [36]. However, susceptibility of Gram-negative bacteria can vary by genus and species. *Aeromonas hydrophila*, a Gram-negative bacteria commonly found in water, was highly susceptible to the effects of essential oils via an unknown mechanism; *Enterobacter aerog*enes was inhibited by cinnamon oil via interaction with various amino acid decarboxylases [37-40]. In these examples, the difference in susceptibility may be due to the presence or absence of the essential oil-specific target versus other Gram-positive or –negative bacteria; alternatively, the specific target may be present but exist in a different isoform resulting in altered susceptibility.

Other specific mechanisms of action have been identified which involve quorum sensing, cellular division, sporulation, stress responses and efflux pumps. Many Gram-positive and –negative bacterial organisms communicate in a complex interplay known as 'quorum sensing' which is used to regulate various cellular functions ranging from biofilm formation and swarming to expression of virulence factors and toxins [12]. It has been suggested that interruption of these bacterial communication networks may inhibit attachment and invasion by some pathogens exploiting an alternative pathway for antimicrobial development as compared with current antibiotics [41, 42]. Interference of quorum sensing has been demonstrated by a number of plant extracts, including garlic, which resulted in significant inhibition of biofilm formation in *P. aeruginosa* [43,44]. This inhibition not only appeared to be concentration dependent, but also illustrated properties of competitive binding as suggested by structure-activity relationship studies [43,44]. Biofilm formation was also inhibited in *S. aureus* and *Salmonella enterica* serovar *typhimurium* following

exposure to carvacrol, a monoterpene found in many essential oils [45]. These findings suggest inhibition of quorum sensing and biofilm formation may provide unique and as yet, unexplored targets for development of new antibiotics. However, other new drug targets may exist, which disrupt cellular division and sporulation as observed with filamentous fungi exposed to various essential oils [46]. In 2006, Pawar and Thaker [45] demonstrated that cinnamon bark oil was highly active against *Aspergillus niger* resulting in reduced production of hyphae and spores and in some cases complete inhibition of growth. The underlying mechanism(s) for these observations were not determined. However, previous investigators identified a correlation between inhibition of sporulation and cellular respiration versus growth [47]. Specifically, essential oils such as citron and lavender significantly inhibited sporulation and cellular respiration, with little effect on growth, whereas oils from cinnamon bark and lemongrass decreased growth, with little to no effect on sporulation or cellular respiration [47]. The effect on cellular respiration has implications for additional drug targets, especially those involving energy-dependent processes such as efflux of various macromolecules as seen with bacterial efflux pumps. Bacterial efflux pumps are responsible for multidrug resistance in a number of bacteria including the AcrAB-TolC efflux system in the *Enterobacteriaceae* and the MexAB-OprM system in *Pseudomonas aeruginosa* [12]. Recent evidence suggests that these efflux mechanisms may in part be responsible for the decreased susceptibility of many Gram-negative organisms to plant-derived phytochemicals and essential oils. However, some oils such as falcarindiol, derived from *Levisticum officinale,* and the geraniol containing *Helicrysum italicum* have demonstrated anti-efflux activity especially in combination with ciprofloxacin and chloramphenicol, respectively, against Gram-negative bacteria [48,49].

Other common components of essential oils with specific antimicrobial activity are alcohols and aldehydes. Alcohols, especially the terpene alcohols, have significant bactericidal activity against a wide range of microorganisms. This bactericidal activity is thought to occur via a number of mechanisms including denaturation of proteins, dehydration of bacterial cells, or solvation of bacterial cell membranes [50,51]. In comparison, aldehydes are thought to interfere with reactions involving electron transfer, especially when conjugated to a carbon-carbon double bond. Such an electronegative molecular arrangement would result in interference with a large number of biological reactions of central metabolism (e.g. respiration and carbon cycling) resulting in rapid cell death [51].

Ancient Remedies: Combining the Old and the New?

Research and development of new antibiotics decreased significantly in the 1970's when the need for new drugs was thought to be negligible since infectious diseases were becoming a concern of the past. As a result, when new antibiotics were needed (e.g. when resistance emerged), pharmaceutical companies merely modified existing antibiotics via slight structural alterations. This approach was more economical than developing a completely new drug, especially at a time when the prevailing perception was that humanity had conquered infectious disease [52]. Today, infections have been documented which are resistant to all known drugs; treatment is often problematic and unsuccessful [53]. Unfortunately, antibiotics of 'last resort' are often used, including drugs previously abandoned due to overt toxicity or serious side effects [54]. Yet even this approach fails to offer long-term solutions for emerging microbial resistance to existing agents and prevention of resistance to new drugs. Perhaps what is needed is a paradigm shift, a fundamental alteration of the way we use antibiotics to treat infectious diseases. In this regard, there are lessons to be learned from plants. For example, plants produce a number of antimicrobial compounds including a large number of essential oils. These essential oils are comprised of numerous compounds which vary in potency and spectrum of activity both individually and as mixtures. Plants need this diversity considering the variability in microbial threats encountered in the environment. Thus, essential oils often inhibit a wide range of microbes due to the synergy afforded by individual components against multiple bacterial targets. Likewise, synergy has been documented between existing antibiotics with specific combinations utilized heavily in current medical practice (e.g. trimethoprim/sulfamethoxazole; amoxicillin/clavulanate; piperacillin/tazobactam) [55]. However, synergy between existing antibiotics and essential oils and/or components has not been thoroughly investigated; although to date, limited studies have been conducted [56]. For example, β-lactam antibiotics inhibit cell wall synthesis through interaction with penicillin-binding proteins (PBP's) [57]. PBP2a, is a specific PBP in *S. aureus* with reduced affinity for β-lactam antibiotics resulting in resistance to these drugs [58]. Interestingly, when β-lactam antibiotics were combined *in vitro* with corilagin, a polyphenol derived from *Arctostaphylos uva-ursi*, the PBP2a-mediated resistance in MRSA was overcome with a concomitant reduction in MIC [59]. The authors postulated that corilagin may interfere with binding of β-lactams to the PBP2a enzyme resulting in reversion of resistance [60]. Other plant derived compounds from green tea demonstrated a similar effect in a dose-dependent manner suggesting the presence of a specific target [61]. Synergy has also been documented with linalool and α-terpineol from *Melaleuca leucodendron* when combined with ampicillin and kanamycin [62]. In addition, synergy was seen with totarol, ferulenol, and plumbagin in combination with isoniazid (INH) and rifampin (RIF) against *Mycobacterium tuberculosis* (MTB). These combinations increased the potency of INH 4-fold against MTB [62]. Another compound isolated from the roots of *Euclea natalensis* decreased the MIC 4- to 6-fold for INH and RIF, respectively [63]. Taken together, these are important findings due to the rapid emergence of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB). MDR-TB is defined as resistance to INH and RIF; XDR-TB is defined as resistance to INH, RIF, and any of the fluoroquinolones and one of the injectable second-line drugs (e.g. capreomycin, amikacin, or kanamycin) [64,65]. Unfortunately, these drug-resistant patterns in MTB may become "obsolete" in the near future, as MTB strains with alarming and more extensive resistance patterns have been isolated from multiple locations on the globe. These strains exhibited resistance to nearly all drugs ever used for treatment of tuberculosis and other mycobacterial infections including: INH, RIF, ethambutol, pyrazinamide, ofloxacin, moxifloxacin, capreomycin, kanamycin, amikacin, para-aminosalicylic acid, ethionamide, cycloserine, rifabutin, clofazimine, dapsone, clarithromycin, and thiacetazone [64]. Although consensus is lacking for a specific acronym for describing these strains (extremely- versus totally-drug-resistant TB; XXDR and TDR, respectively), the fact that they have been isolated is cause for great concern. In the absence of new antibiotics becoming quickly available for treatment, an alternative approach may be to combine existing drugs with essential oils. Yet, viable combinations will require a significant investment to better understand the mechanism of action of essential oils and components, determine individual and combined toxicity, characterize metabolism *in vivo*, as well as define their selectivity and bioavailability.

Summary

Since antiquity, essential oils and their constituents have been

used to treat a large number of human illnesses. Today, essential oils are used in alternative and holistic medicine for similar purposes and administered orally, topically or via aromatherapy. A growing number of scientific investigators have begun the process of elucidating the specific mechanism(s) of action of essential oils and components. Emerging evidence has shown that many essential oils have both non-specific and specific mechanisms of action which varies based on the relative abundance and chemical composition of the components. Elucidation of the mechanism of action of these compounds may enable identification of new antibiotic targets and exploitation of novel biochemical pathways; pathways not currently targeted by existing antibiotics. Additionally, combination of existing drugs with essential oils and/or components may provide an alternative approach to combat emerging drug resistance. Since antibiotic resistance is currently outpacing research and development to find new drugs, humanity is facing a return to the 'pre-antibiotic era'. Perhaps the remedies of the past combined with scientific study may provide the antibiotics of tomorrow.

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