

Inhibition of methicillin-resistant *Staphylococcus aureus* (MRSA) by essential oils

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ABSTRACT: Ninety-one essential oils, each distilled from a single plant source, and 64 blended essential oils obtained from a commercial source were screened using the disc diffusion assay for inhibitory activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Of the 91 single essential oils, 78 exhibited zones of inhibition against MRSA, with lemongrass, lemon myrtle, mountain savory, cinnamon and melissa essential oils having the highest levels of inhibition. Of 64 blended essential oils, 52 exhibited inhibitory activity against MRSA, with R.C. (a combination of myrtle, *Eucalyptus globulus*, *Eucalyptus australiana*, *Eucalyptus radiata*, marjoram, pine, cypress, lavender, spruce, peppermint and *Eucalyptus citriodora* oils), Motivation (a combination of Roman chamomile, ylang ylang, spruce and lavender oils) and Longevity (a combination of frankincense, clove, orange and thyme oils) blended essential oils having the highest inhibitory activity. These results indicate that essential oils alone and in combination can inhibit MRSA *in vitro*. Application of these results may include the potential use of essential oils as an alternative therapy for various diseases sustained by *S. aureus* MRSA. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS: essential oils; MRSA; inhibition

Introduction

Staphylococcus aureus is a normal inhabitant in the anterior nares of 25–30% of people and can also reside transiently on the skin. Following the introduction of β -lactamase-stable cephalosporins and semi-synthetic penicillins, such as methicillin, during the late 1950s and early 1960s, methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were soon noted. Since then, the Centers for Disease Control and Prevention (CDC) have reported that the proportion of overall staphylococcal infections due to MRSA has risen steadily from 2% in 1974, to 22% in 1995, to 63% in 2004¹ in the USA. Although initially most MRSA infections were acquired in the hospital (HA-MRSA), the first appearance of community-acquired MRSA (CA-MRSA) occurred in 1982 among intravenous drug users in Detroit.² Since then CA-MRSA has grown annually into an ever-increasingly important source of infections among individuals previously labelled as belonging to low risk groups.

Because only 10 new antibiotics have been placed on the market since 1998 and there are few prospects for new antibiotic agents, the future health of our society may depend on researching alternative therapies. Since infections caused by CA-MRSA and HA-MRSA are increasing, as are rates of antibiotic therapy failures,

many have called for new measures to treat and prevent these infectious diseases.^{3–6} Research has revealed some promising novel antimicrobial candidates including essential oils, particularly interesting since some oils have been used by native groups for curative purposes in the past.^{3–6}

In vitro data indicate that many essential oils have antimicrobial activity.⁷ For instance, tea tree oil obtained from the Australian tree *Melaleuca alternifolia* has been shown to be active against a wide range of microorganisms, including bacteria, fungi and viruses.⁶ The activities of other oils have also been investigated and their actions against various pathogens, including MRSA, have been demonstrated.^{8–14}

There are also several clinical studies^{15,16} and case reports^{17,18} noting the successful use of essential oils in treating MRSA nasal carriage or MRSA infections. Specifically, Dryden *et al.*¹⁵ and Caelli *et al.*¹⁶ reported that topical tea tree oil was as effective as standard therapy for reducing MRSA nasal colonization; Sherry *et al.*^{17,18} reported successful treatment of patients with staphylococcal wound infections. However, several review articles^{19–21} have reported that, while use of essential oils as antimicrobial agents is promising, additional *in vivo* studies and more clinical studies involving larger numbers of subjects are needed.

Previous studies in our laboratory have shown inhibition of *Streptococcus pneumoniae* and other pathogens by various essential oils, but these oils were not tested specifically against MRSA.^{7,22,23} Thus, this study was

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initiated to screen essential oils for inhibitory activity against this medically important bacterium to determine their potential as candidates for use as disinfectants, antiseptics or even as topical treatments against MRSA.

Materials and Methods

Bacterial Culture

Staphylococcus aureus ssp. *aureus* ATCC 700699 (American Type Culture Collection, Manassas, VA, USA) is a methicillin-resistant strain which also shows reduced sensitivity to vancomycin. A loop of the ATCC culture was placed into 5 ml sterile trypticase soy broth (TSB; Difco, Becton Dickinson and Co., Sparks, MD, USA) containing vancomycin to a final concentration of 4 µg/ml, according to instructions by ATCC. This culture was distributed to five sterile culture tubes, so that each tube contained 800 µl culture and 200 µl sterile glycerol. These tubes were frozen at -70 °C until use. *Staphylococcus aureus* ATCC 700699 was shown to be a pure isolate by streaking it onto trypticase soy agar (TSA) plates (Difco, Becton Dickinson). For the disc diffusion assay, a vial of bacteria was thawed and a loop of the culture was plated on TSA and incubated for 24 h at 35 °C. A few colonies were selected and transferred to a tube of sterile TSB and incubated at 35 °C in a water bath until the turbidity was comparable to the 0.5 McFarland standard, prepared according to standard procedure.²⁴

Essential Oils

Ninety-one individual essential oils listed in Table 1 and 64 essential oil blends listed in Table 2 were obtained from Young Living Essential Oils (Orem, UT, USA). The commercial essential oils were water-distilled extracts of various portions of the source plants. The chemical composition of the oils was quantitatively analysed by GC/FID (Hewlett-Packard 6890; Table 3a, b). An HP-1 column (50 m × 0.32 mm i.d., 0.5 µm film thickness) was used, with helium as the carrier gas at 1.3 ml constant flow. The GC oven temperature was started at 70 °C and programmed to 250 °C at a rate of 3 °C/min. Pure oil (0.5 ml) was injected at 1:100 split ratio. The injector and detector temperatures were 250 °C.

Disc Diffusion Assay

The disc diffusion assay was performed in accordance with the *Manual of Clinical Microbiology of the American Society for Microbiology*²⁵ and has been recommended for the screening of products for antimicrobial activity.^{4,26} Ninety-one essential oils (Table 1) from a single plant

source and 64 blended essential oils (Table 2) were screened for their inhibitory activity against MRSA.

The disc diffusion assay was performed by dipping a sterile cotton swab into the diluted culture, prepared as described above. Discs containing 5 µg methicillin were used as controls; 30 µl of an individual essential oil or blended essential oil was placed on a sterile 13 mm disc (BBL, Becton Dickinson) placed in the centre of the MHA plate. The plates were then incubated as placed at 35 °C for 24 h and the diameter of the zone of inhibition measured. Each oil was tested in triplicate and the diameters of the zone of inhibition were averaged. Each oils' inhibitory level was ranked based on the diameter of the zone of inhibition, measured in mm as is standard for this procedure.²⁵

Results

In Table 1, the 91 essential oils, each from a single plant source, that were screened by the disc diffusion assay, are listed alphabetically with their zones of inhibition against MRSA; 78 of these essential oils had measurable inhibitory activity, while 13 exhibited no detectable inhibitory activity against MRSA. Control plates with methicillin showed MRSA growth as expected. Of the essential oils tested, lemongrass, lemon myrtle, mountain savory, cinnamon bark and melissa essential oils showed the highest levels of inhibition with zones ≈ 60 mm. Remarkably, lemongrass essential oil completely inhibited all MRSA growth on the plate. Significant zones of inhibition (45–57 mm) were found for thyme, cumin, *Eucalyptus citriodora*, tsuga, oregano, *Melaleuca alternifolia*, and limette essential oils. Intermediate inhibition zones (35–42 mm) were detected for essential oils derived from ledum, *Eucalyptus dives*, niaouli, manuka, peppermint, elemi and rosewood.

The antimicrobial activity of the 64 blended essential oils tested is shown in Table 2. Of these blended oils, 52 showed zones of inhibition, while 12 had no detectable inhibitory activity against MRSA. Of these, R.C., which is a combination of myrtle, *Eucalyptus globulus*, *Eucalyptus australiana*, *Eucalyptus radiata*, marjoram, pine, cypress, lavender, spruce, peppermint and *Eucalyptus citriodora* oils, totally inhibited MRSA. The blended oil Motivation, a blend of Roman chamomile, ylang ylang, spruce and lavender oils, was the next most inhibitory. Longevity, a combination of frankincense, clove, orange and thyme oils, was the third most inhibitory blend against MRSA. The major components of these three blended oils are listed in Table 3a, b.

Discussion

Utilizing the disc diffusion assay as a screening method, the majority (78/91) of commercial essential oils from a

Table 1. Zones of inhibition of MRSA by essential oils (30:1)

Oils	Botanical name	Diameter (mm)	Oils	Botanical Name	Diameter (mm)
Angelica	<i>Angelica archangelica</i>	14	Lemon myrtle	<i>Backhousia citriodora</i>	65
Anise	<i>Pimpinella anisum</i>	15	Lemongrass	<i>Cymbopogon flexuosus</i>	>83
Basil	<i>Ocimum basilicum</i>	18	Lime	<i>Citrus aurantifolia</i>	16
Bay laurel	<i>Laurus nobilis</i>	17	Limette	<i>Citrus hystrix</i>	45
Bergamot	<i>Citrus bergamia</i>	0	Manuka	<i>Leptospermum scoparium</i>	35
Blue cypress	<i>Callitris intratropica</i>	17	Marjoram	<i>Origanum majorana</i>	28
Cajuput	<i>Melaleuca leucadendra</i>	19	Melaleuca (alt.)	<i>Melaleuca alternifolia</i>	45
Cardamom	<i>Elettaria cardamomum</i>	17	Melaleuca (eric.)	<i>Melaleuca ericifolia</i>	30
Carrot seed	<i>Daucus carota</i>	17	Melissa	<i>Melissa officinalis</i>	60
Cedarwood	<i>Cedrus atlantica</i>	15	Mountain savory	<i>Satureja Montana</i>	62.5
Celery seed	<i>Apium graveolens</i>	15	Myrrh	<i>Commiphora myrrha</i>	16
Chamomile, German	<i>Matricaria recutita</i>	0	Myrtle	<i>Myrtus communis</i>	0
Chamomile, Roman	<i>Chamaemelum nobile</i>	19	Neroli	<i>Citrus aurantium</i>	25
Cinnamon bark	<i>Cinnamomum verum</i>	60	Niaouli	<i>Melaleuca quinquenervia</i>	38
Cistus	<i>Cistus ladanifer</i>	17	Nutmeg	<i>Myristica fragrans</i>	14.5
Citronella	<i>Cymbopogon nardus</i>	19	Orange	<i>Citrus sinensis</i>	0
Clary sage	<i>Salvia sclarea</i>	17	Oregano	<i>Origanum compactum</i>	48
Clove	<i>Syzygium aromaticum</i>	20	Palmarosa	<i>Cymbopogon martinii</i>	22
Coriander	<i>Coriandrum sativum</i>	28	Palo Santo	<i>Bursera graveolens</i>	30
Cumin	<i>Cuminum cyminum</i>	50	Patchouli	<i>Pogostemon cablin</i>	26
Cypress	<i>Cupressus sempervirens</i>	15	Pepper, black	<i>Piper nigrum</i>	14
Dill	<i>Anethum graveolens</i>	16	Peppermint	<i>Mentha piperta</i>	37
Elemi	<i>Canarium luzonicum</i>	36	Petitgrain	<i>Citrus aurantium</i>	21
Eucalyptus (dives)	<i>Eucalyptus dives</i>	40	Pine, Scots	<i>Pinus sylvestris</i>	17
Eucalyptus (globulus)	<i>Eucalyptus globulus</i>	0	Ravensara	<i>Ravensara aromatica</i>	17
Eucalyptus (polybrac.)	<i>Eucalyptus polybractea</i>	17	Rose	<i>Rosa damascena</i>	22
Eucalyptus (radiata)	<i>Eucalyptus radiata</i>	25	Rosemary	<i>Rosmarinus officinalis</i>	26
Eucalyptus Citriodora	<i>Eucalyptus citriodora</i>	50	Rosewood	<i>Aniba rosaedora</i>	35
Fennel	<i>Foeniculum vulgare</i>	14	Sage	<i>Salvia officinalis</i>	14
Fir, Douglas	<i>Pseudotsuga menziesii</i>	17	Sandalwood	<i>Santalum album</i>	16
Fir, Idaho balsam	<i>Abies balsamea</i>	0	Spearmint	<i>Mentha spicata</i>	19
Fir, White	<i>Abies alba</i>	17	Spikenard	<i>Nardostachys jatamansi</i>	18
Fleabane (Conyza)	<i>Conyza canadensis</i>	15	Spruce, black	<i>Picea mariana</i>	19
Frankincense	<i>Boswellia carteri</i>	0	Tangerine	<i>Citrus nobilis</i>	0
Galbanum	<i>Ferula gummosa</i>	27	Tansy, blue	<i>Tanacetum annuum</i>	0
Geranium	<i>Pelargonium graveolens</i>	26	Tansy, Idaho	<i>Tanacetum vulgare</i>	19
Ginger	<i>Zingiber officinale</i>	15	Tarragon	<i>Artemisia dracunculid</i>	15
Goldenrod	<i>Solidago canadensis</i>	15	Thyme	<i>Thymus vulgaris</i>	57
Grapefruit	<i>Citrus paradisi</i>	0	Tsuga	<i>Tsuga canadensis</i>	50
Helichrysum	<i>Helichrysum italicum</i>	15	Valerian	<i>Valeriana officinalis</i>	25
Hyssop	<i>Hyssopus officinalis</i>	17	Vetiver	<i>Vetiveria zizanioides</i>	0
Jasmine	<i>Jasminum officinale</i>	15	Western red cedar	<i>Thuja plicata</i>	16
Juniper, Utah	<i>Juniperus osteosperma</i>	30	Wintergreen	<i>Gaultheria procumbens</i>	0
Lavender	<i>Lavandula angustifolia</i>	26	Yarrow	<i>Achillea millefolium</i>	18
Ledum	<i>Ledum groenlandicum</i>	42	Ylang ylang	<i>Cananga odorata</i>	0
Lemon	<i>Citrus limon</i>	20			

single plant source (Table 1) and 52/64 blended essential oils (Table 2) showed inhibitory activity against MRSA. While most of the oils tested in this study had some inhibitory activity, a few (bergamot, German chamomile, *Eucalyptus globulus*, Idaho balsam fir, frankincense, grapefruit, myrtle, orange, tangerine, blue tansy, vetiver, wintergreen and ylang ylang, and 12 combination oils) showed no observable zones of inhibition against MRSA (Tables 1, 2). Since the disc diffusion assay is done on a semi-solid surface and requires that the essential oil is spread across a surface to contact MRSA, a lack of inhibition may be due to the inability of the oils' chemical components to diffuse readily under these conditions. Since essential oils are hydrophobic, they may not diffuse

optimally in an aqueous environment, such as is present in the disc diffusion assay. Likewise, according to Carson *et al.*,⁶ the volatility as well as miscibility of the essential oils may cause problems when assaying their activity. For these reasons, oils which lacked activity in the disk diffusion assay should be re-evaluated by an assay in which their potential antimicrobial activity is optimized.

This observation, that low or moderately inhibitory oils when combined can produce significant zones of inhibition, is suggestive of synergism occurring between oils (Tables 1, 2). For instance, combinations of lavender, spruce, Roman chamomile and ylang ylang in Motivation oil showed anti-MRSA activity greater than individual oils tested alone (55 mm diameter vs. 26, 19, 19 and 0 mm,

Table 2. Inhibition of MRSA by combined essential oils (30:1)

Combined essential oils	Diameter of zone of inhibition (mm)	Combined essential oils	Diameter of zone inhibition (mm)
Abundance	23	Joy	20
Acceptance	15	JuvaCleanse	20
Aroma Life	0	JuvaFlex	15
Aroma Siez	24	Lady Scareol	19
Awaken	0	Legacy	22
Australian Blue	17	Live With Passion	15
Believe	19	Longevity	46
Brain Power	25	Magnify Your Purpose	29
Chivalry	0	Melrose	21
Christmas Spirit	30	M-Grain	26
Citrus Fresh	0	Mister	0
Clarity	27	Motivation	55
Di-Gize	27	PanAway	21
Dragon Time	21	Peace & Calming	0
Dream Catcher	17	Present Time	0
Egyptian Gold	31	Purification	38
En-R-Gee	29	Raven	17
Envision	20	R.C.	>83
Evergreen Essence	17	Release	0
Exodus II	40	Relieve It	22
Forgiveness	17	Sacred Mountain	14
Gathering	25	Sara	0
Gentle Baby	24	SclarEssence	26
Gratitude	29	Sensation	23
Grounding	18	Surrender	27
Harmony	27	Thieves	35
Highest Potential	17	3 Wise Men	0
Hope	16	Transformation	33
Humility	15	Trauma Life	21
ImmuPower	27	Valor	0
Inner Child	20	White Angelica	15
Inspiration	16		
Into the Future	0		

respectively. The diameter of the zone of inhibition is greater than would be expected if the effect was only due to the oils acting independently. Likewise, data for the other two most potent blends, R.C. and Longevity, imply synergism of the combined oils (Tables 1, 2). However, definitive proof of synergism would require further testing.

The major anti-staphylococcal components of many essential oils and other natural compounds have been reviewed.²⁷ While the major chemical components of essential oils have been shown to have antimicrobial activity, the combination of the chemical components, as they arise in plants, may actually be advantageous for antimicrobial activity, since a microorganism would usually have difficulty developing resistance to all the active components present in the inhibitory substance. For instance, tree tea oil contains over 100 components, many of which are antimicrobial in nature.⁶ Another possible advantage of this complexity is suggested by a recent study of the interaction of two components of tree tea oil, 1,8-cineole and terpinene. While 1,8-cineole exhibits little antimicrobial activity inherently, it has been shown to enhance the lethal action of terpinene. It is hypothesized that 1,8-cineole helps permeabilize bacterial membranes, allowing the more active terpinene to enter and kill the

bacterial cell.⁶ These data may have implications particularly for oil blends, in which each oil has different major chemical components that may act synergistically against the target organism. Our data for blended oils suggest that this may be occurring, since none of the individual oils was the most inhibitory for MRSA; however (at lower concentrations than the individual essential oils) the zones of inhibition were as large as the most inhibitory individual essential oils (Tables 1, 2).

While it is known that many essential oils kill bacteria by damaging the cell membrane's structure, inhibiting membrane function,^{28,29} one area that deserves more study is to determine whether synergistic killing occurs when essential oils are used in combination with compounds which act by other mechanisms. Currently only limited evidence of this relationship has been observed.³⁰⁻³² One example is a study by Nascimento *et al.*, who noted the synergism of essential oils (clove, jambolan, pomegranate and thyme) and antibiotics (ampicillin, tetracycline or chloramphenicol) against a *Pseudomonas aeruginosa* strain resistant to these antibiotics. Combining essential oils with conventional antibiotics deserves further studies, using mainly oils from the present study that have effectively inhibited bacteria.

Table 3a. Major components of the single essential oils with high anti-MRSA activity (30 µl)

Single essential oil	Diameter of zone of inhibition (mm)	Major components (area percentage)
Lemongrass	>83	Geranial: 39.5–46.0 Neral: 29.5–34.9 Geranyl acetate: 2.5–6.4 Geraniol: 4.9–8.4
Lemon myrtle	65	Geranial: 51.86 Neral: 39.11
Mountain savory	62.5	Carvacrol: 31.6–34.0 Thymol: 17.2–18.2 γ-Terpinene: 10.4–13.7 p-Cymene: 8.2–10.2
Cinnamon	60	Carvacrol methyl ether: 5.3–5.8 trans-Cinnamaldehyde: 40.2–64.6 Eugenol: 5.4–39.7 trans-β-Caryophyllene: 3.2–6.5 Linalol: 3.1–6.2
Melissa	60	Geranial: 29.6–32.8 Neral: 21.1–22.1 trans-β-Caryophyllene: 15.0–17.1
Thyme	57	Thymol: 41.5–57.1 p-Cymene: 17.9–27.5 γ-Terpinene: 3.9–9.6 Linalol: 3.6–5.3
Cumin	50	Cumin aldehyde: 22.4–26.7 γ-Terpinene: 18.4–21.0 β-Pinene: 15.9–17.4 p-Menth-1,3-dien-7-al: 11.9–17.0 p-Menth-1,4-dien-7-al: 8.5–11.6 p-Cymene: 6.1–8.2
Tsuga	50	Bornyl acetate: 36.6–37.7 α-Pinene: 18.1–19.5 Camphene: 14.4–15.6 Tricyclene: 5.3–6.2
Oregano	48	Carvacrol: 69.4–74.3 p-Cymene: 5.4–6.5 Linalol: 4.4–5.8 γ-Terpinene: 3.0–5.9
<i>Citrus hystrix</i>	45	Citronellal: 66.0–67.6
<i>Melaleuca alternifolia</i>	45	Terpinen-4-ol: 32.9–43.4 γ-Terpinene: 17.3–24.8 1,8-Cineole: 3.3–16.6 α-Terpinene: 7.1–11.8

Table 3b. Major components of the blended essential oils with high anti-MRSA activity (30 µl)

Blended essential oil	Diameter of zone of inhibition (mm)	Major components (area percentage)
R.C.	>83	Eucalyptol: 30.6–35.5 α-Pinene: 22.8–29.2 Citronellal: 4.5–6.0
Motivation	55	Isobutyl angelate + isoamyl methacrylate: 18.1 Bornyl acetate: 9.5 Isoamyl angelate: 7.0 Camphene: 6.5 α-Pinene: 6.2
Longevity	46	Limonene: 30.0 Thymol: 27.3 p-Cymene: 11.7 Eugenol: 7.9 α-Pinene: 5.1

While research, including this study, has noted the potential value of essential oils as therapeutic antimicrobial agents, future studies should focus on testing the clinical safety and efficacy of the essential oils. Although few studies have been done, one determined the therapeutic indices (TIs) of hundreds of chemical components of essential oils, administered either orally or dermally. The data indicate that several components of essential oils could be considered for safe use in humans, based on their TIs; however, more data are needed, as none of these oils currently has FDA approval for human use to treat a disease.¹⁹

Based on these data, the potential use of these essential oils (Tables 1, 2) or their active components (Table 3a, b) is promising, since demonstration of *in vitro* antimicrobial activity is the first step in the regulatory process: This study shows that many essential oils and a number of essential oil blends can inhibit the growth of MRSA, a very significant public health concern. If further studies definitely show safety and efficiency of these essential oils, this may represent a valuable weapon against MRSA.

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