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MAJOR ARTICLE

In Vitro Activity of Essential Oils Against Gram-Positive and Gram-Negative Clinical Isolates, Including Carbapenem-Resistant *Enterobacteriaceae*

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Background. There is increasing demand for compounds to treat antimicrobial-resistant pathogens, and essential oils have gained interest. Moreover, previous studies have demonstrated antimicrobial activity of these nonpharmaceutical products. We investigated the activity of essential oils against multiresistant bacteria and other clinical isolates to evaluate the potential of their use topically and/or internally for treatment of bacterial infections.

Methods. We studied the in vitro activity of 10 essential oils and 1 essential oil blend against clinical isolates including extended-spectrum beta-lactamase-producing *Enterobacteriaceae*, carbapenem-resistant *Enterobacteriaceae*, multidrug-resistant *Pseudomonas aeruginosa*, and methicillin-resistant *Staphylococcus aureus.*

Results. Essential oils of oregano, thyme, cinnamon bark, and lemongrass had the largest zones of inhibition against Gram-positive organisms, whereas cinnamon bark had the largest zone of inhibition against *P aeruginosa*. Oregano, thyme, and cinnamon bark had the largest zones of inhibition against *Enterobacteriaceae*.

Conclusions. Essential oils have promising in vitro activity that warrants further study of their activity and use in the clinical setting.

Keywords. carbapenem-resistant *Enterobacteriaceae*; essential oils; in vitro susceptibility.

Antimicrobial resistance is a major public health problem around the world. Resistance in *Klebsiella pneumoniae*, *Escherichia coli*, including carbapenem-resistant *Enterobacteriaceae* (CRE), and multidrug-resistant *Pseudomonas aeruginosa* continues to increase, and methicillin-resistant *Staphylococcus aureus* is widespread [1]. The WHO Global Action Plan to combat antimicrobial resistance encourages public-private partnerships for research on new antimicrobial agents (Objective 5) [2]. The National Action Plan for Combating Antibiotic-Resistant Bacteria in the United States calls for developing nontraditional therapeutics, including natural compounds such as essential oils (Goal 4.4) [3]. Previous studies have suggested the potential of essential oils for antimicrobial activity [4–7]. The global essential oils market demand has increased from 61.8 kilotons in 2014 to 226.9 kilotons in 2018 and is still increasing [8], combined with a growing inclination by consumers toward natural and holistic therapies. These factors, in addition to rising antimicrobial resistance, warrant further study of antimicrobial effects, particularly against more recent multidrug-resistant pathogens. A previous pilot study by our group showed in vitro activity of essential oils (EOs) against CRE and selected American Type Culture Collection (ATCC) strains [9] and led us to investigate activity against a larger sample of pathogens. To that end, our objective was to explore the activity of essential oils against multiresistant bacteria and other clinical isolates to evaluate the potential of their use topically and/or internally for treatment of bacterial infections.

MATERIALS AND METHODS

Essential Oils and Bacterial Isolates

Essential oils of cinnamon bark (*Cinnamomun zeylanicum*), clove (*Szygium aromaticum*), lemongrass (*Cymopogon flexuosus*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), thyme (*Thymus vulgaris*), tea tree (*Melaleuca alternifolia*), manuka (*Leptospermum scoparium*), and the oil blend, Thieves (a proprietary blend of cinnamon, clove, lemon, eucalyptus, and rosemary), were provided by Young Living Essential Oils (Lehi, UT) who also performed gas chromatography/flame ionization detector analysis. Major constituents from the essential oils are shown in Table 1. Thirty Gram-positive, including methicillin-susceptible *S aureus* (MSSA) and methicillin-resistant *S aureus*

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Table 1. Content of Each Major Constituent Was Determined From a Peak Area Relative to the Total Peak Area in Gas Chromatography/Flame Ionization Detector Analysis: The Top Three Constituents Are Listed

Essential Oils Used and Their Major Constituents	
Cinnamon bark oil	trans-cinnamaldehyde 68.4%, eugenol 8.5%, cinnamyl acetate 4.1%
Clove oil	eugenol 78.9%, eugenyl acetate 13.0%, trans-beta-caryophyllene 6.5%
Lemongrass oil	geranial 39.2%, neral 32.0%, geraniol 6.9%
Manuka oil	leptospermone 16.4%, calamenene 12.8%, isoleptospermone 6.1%
Oregano oil	carvacrol methyl ether 67.4%, para-cymene 7.7%, gamma-terpinene 4.8%
Tea tree oil	terpinene-4-ol 37.1%, gamma-terpinene 20.8%, alpha-terpinene 11.3%
Thieves oil	eugenol 35.5%, limonene 16.9%, trans-cinnamaldehyde 12.7%
Thyme oil	thymol 43.3%, para-cymene 16.0%, gamma-terpinene 7.7%

(MRSA), and 70 Gram-negative bacterial clinical isolates, including extended-spectrum beta-lactamase and CRE strains, were tested using Clinical and Laboratory Standards Institute methods [10]. Selected ATCC quality control isolates (ATCC 29213 methicillin-susceptible *S aureus*, ATCC 49619 *Streptococcus pneumoniae*, ATCC 25922 *E coli*, and ATCC 27853 *P aeruginosa*) were also included. All isolates were maintained as frozen stocks in the Microbial Pathology Research Laboratory at UT Health San Antonio and were subcultured twice before testing.

Susceptibility Testing

Isolates were grown overnight on tryptic soy agar, 0.5 McFarland suspensions were prepared in sterile saline, and Mueller-Hinton agar plates were inoculated by streaking for susceptibility testing using the Kirby-Bauer method. Twenty microliters of full-strength oils were pipetted onto blank paper disks in a sterile dish. Twenty microliters corresponded to approximately half of a drop of essential oil, a conservative estimate of an amount that would be feasible to apply topically when diluted in a carrier oil, and an amount that could be ingested when diluted in a carrier oil. Disks were placed aseptically onto the plates immediately after inoculating disks. In the previous pilot study, lemongrass lacked substantial Gram-negative in vitro activity, and in this study it was only tested against Gram-positive isolates. For positive control comparators and as quality controls, vancomycin was tested against Gram-positive isolates and meropenem was tested against Gram-negative isolates. Replicates were not performed. All plates were read using a benchtop desk lamp, and a ruler was used to measure zone sizes. No pop-up colonies were noted within the zone recorded.

RESULTS

Mean and median zone diameters of essential oils are shown in Tables 2–4. Representative sample disk diffusion agar plates are shown in Figure 1. Essential oils oregano, thyme, cinnamon bark, and lemongrass had the largest zones of inhibition against Gram-positive clinical isolates. Manuka and Thieves also showed significant Gram-positive activity. Manuka had greater activity against Gram positives than Gram negatives, with the exception of *E coli*. Oregano, thyme, and cinnamon bark had the largest zones of inhibition against *Enterobacteriaceae*. Cinnamon bark had the largest zone of inhibition against *P aeruginosa*. Zone sizes for *P aeruginosa* showed the most variability. This was due to 1 carbapenem-susceptible *P aeruginosa* isolate and 1 carbapenem-resistant *P aeruginosa* isolate that displayed much larger zone sizes with the essential oils than the other *P aeruginosa* isolates. Zone sizes for the positive control comparator vancomycin showed that the Gram-positive isolates were susceptible as expected. Zone sizes for meropenem for the Gram-negative isolates confirmed susceptibility for the non-CRE isolates and resistance for the CRE isolates, as expected. Vancomycin and meropenem zone sizes for the respective Gram-positive and Gram-negative selected ATCC strains confirmed susceptibility, as expected.

DISCUSSION

The ancient and traditional use of plants as medicine, the emergence of multidrug-resistant pathogens, and the increasing use of essential oils make the study of their antimicrobial activity timely and relevant. Essential oils have been shown to have antimicrobial activity, in particular, oregano, thyme, and tea tree oil [5–7, 11, 12]. Commonly used essential oils have not been studied against recently isolated multidrug-resistant pathogens, which prompted our study. Oregano, thyme, cinnamon bark, lemongrass, and manuka had notable activity against Gram-positive pathogens. In our pilot study, lemongrass had more Gram-positive than Gram-negative activity. In this study, manuka had more Gram-positive activity than Gram-negative activity, with the exception of *E coli*.

The least activity was demonstrated against *P aeruginosa*, but cinnamon bark oil had the largest zone of inhibition against *P aeruginosa*. Of note, the Thieves essential oil blend (containing clove, cinnamon, lemon, eucalyptus, and rosemary) had the next largest zone of inhibition against *P aeruginosa*. Oregano, thyme, and cinnamon bark had the largest zones of inhibition against *Enterobacteriaceae*. Cinnamon bark demonstrated the broadest spectrum of activity across Gram-positive and Gram-negative isolates, including *P aeruginosa*. Excluding *P aeruginosa*, oregano, thyme, close, tea tree oil, and Thieves had the broadest spectrum. It is of interest that 1 carbapenem-susceptible and 1 carbapenem-resistant *P aeruginosa* isolate showed more susceptibility to essential oils oregano, thyme, cinnamon bark, and Thieves than the other isolates. This suggests that these oils could be useful for some *P aeruginosa* isolates, based on susceptibility testing.

Major constituents of essential oils with antimicrobial activity include terpenoids such as the phenols thymol, carvacrol, and geraniol, phenylpropenes such as eugenol, as well as para-cymene and cinnamaldehyde [6, 7, 12]. These compounds were demonstrated as components in several of the essential

Table 2. Mean, Standard Deviation, Median, and Range of Zone Diameters (mm) for Essential Oils Against Gram-Positive Clinical Isolates

Species (n)	Oregano	Thyme	Cinnamon Bark	Lemongrass	Manuka	Clove	Tea Tree	Thieves	Vanco
MRSA (10)									
Mean (SD)	23.6 (0.84)	26.8 (1.69)	29.7 (0.82)	29.2 (2.04)	19.7 (1.34)	12.8 (0.63)	9.4 (0.52)	18.8 (1.81)	17.8 (0.42)
Median, range	23, 2	26, 5	30, 3	29, 5	20, 4	13, 2	9, 1	18, 1	18, 1
MSSA (10)									
Mean (SD)	26.6 (2.21)	29.7 (1.77)	30.2 (2.78)	31.4 (6.40)	18.8 (1.62)	13.4 (1.35)	8.9 (2.42)	19.5 (2.72)	18.2 (3.05)
Median, range	26, 7	30, 6	29, 7	30, 20	18, 5	13, 5	8.5, 7	19, 8	19, 11
GAS									
Mean (SD)	18.3 (0.48)	19.7 (1.57)	13.1 (0.32)	23.2 (3.33)	14.0 (1.94)	13.0 (0.47)	6.9 (0.99)	19.1 (1.60)	21.0 (1.16)
Median, range	18. 1	19.5, 4	13, 1	22, 11	13, 2	13, 2	6.5, 2	18.5, 5	20.5, 3

Abbreviations: GAS, group A streptococcus; *Streptococcus pyogenes*; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S aureus*; SD, standard deviation.

Table 3. Mean, Standard Deviation, Median, and Range of Zone Diameters (mm) for Essential Oils Against Gram-Negative, Carbapenem-Susceptible Clinical Isolates

Species (n)	Oregano	Thyme	Cinnamon Bark	Manuka	Clove	Tea Tree	Thieves	Meropenem
ESBL *Escherichia coli* (19)								
Mean (SD)	23.6 (0.84)	26.8 (1.69)	29.7 (0.82)	19.7 (1.34)	12.8 (0.63)	9.4 (0.52)	18.8 (1.81)	30.0 (1.81)
Median, range	23, 2	26, 5	30, 3	20, 4	13, 2	9, 1	18, 1	30, 7
ESBL *Klebsiella pneumoniae* (7)								
Mean (SD)	21.3 (3.50)	15.4 (1.27)	22.3 (1.60)	6.0 (0)	10.7 (1.38)	14.4 (0.79)	11.6 (1.41)	30.3 (0.95)
Median, range	23, 10	15, 19	22, 5	6, 0	10, 4	15, 2	12, 3	30, 3
Enterobacter spp (10)								
Mean (SD)	18.5 (5.15)	14.8 (5.60)	22.3 (4.08)	6.3 (0.48)	9.8 (2.44)	13.6 (3.27)	11.5 (3.10)	26.4 (4.86)
Median, range	20.5, 14	20.5, 14	21, 14	6, 1	10, 8	14.5, 12	11, 10	27, 17
Pseudomonas aeruginosa (4)								
Mean (SD)	13.0 (11.27)	11.0 (7.81)	28.5 (13.08)	10.0 (6.16)	11.0 (5.83)	8.5 (3.0)	13.8 (6.24)	12.8 (2.22)
Median, range	20, 7	7, 14	27.5, 27	7.5, 13	10.5, 11	8, 6	14, 13	13, 5

Abbreviations: ESBL, extended-spectrum beta-lactamase-producing; SD, standard deviation.

oils tested. In general, essential oils are composed of 20 to 60 different compounds [7, 12]. Many of these compounds have antimicrobial activity, and the presence of the compounds together can be more powerful than the action of 1 compound alone. This combined approach is contrary to most currently used antibiotics, which are typically an expansion of 1 functional molecular structure, with activity extended by synthetic rearrangements. The presence of multiple compounds in essential oils may strengthen and prolong antimicrobial activity against microbes. Some studies have noted synergy between essential oil components and antibiotics, which could be another useful role of these agents [13].

The mechanisms of activity of essential oils against bacteria is suggested to be (1) increased cell permeability due to the

Table 4. Mean, Standard Deviation, Median, and Range of Zone Diameters (mm) for Essential Oils Against Gram-Negative, Carbapenem-Resistant Clinical Isolates

Species (n)	Oregano	Thyme	Cinnamon Bark	Manuka	Clove	Tea Tree	Thieves	Meropenem
Klebsiella pneumoniae (13)								
Mean (SD)	19.7 (3.57)	16.2 (3.16)	21.2 (1.54)	6.0 (0)	12.8 (0.63)	14.7 (1.93)	12.1 (1.04)	10.8 (2.35)
Median, range	20, 12	15, 12	21, 5	20, 4	6, 0	15, 8	12, 5	11, 8
Enterobacter spp (4)								
Mean (SD)	20.0 (0)	16.8 (2.36)	21.3 (1.50)	6.0 (0)	9.0 (2.58)	15.3 (1.26)	10.5 (1.92)	11.3 (2.36)
Median, range	20, 0	16, 5	21, 3	6, 0	9, 6	15, 3	11, 4	16, 5
Pseudomonas aeruginosa (11)								
Mean (SD)	9.2 (10.23)	9.2 (10.23)	18.0 (8.12)	6.7 (2.41)	7.5 (4.82)	6.7 (2.10)	8.7 (5.06)	6.7 (1.10)
Median, range	6, 34	6, 34	16, 29	6, 8	6, 16	6, 7	6, 17	6, 3

Abbreviations: SD, standard deviation.

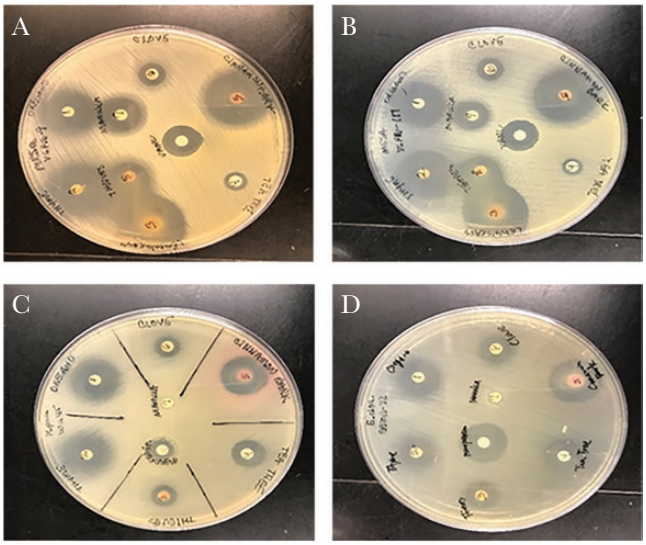

Figure 1. Agar disk diffusion results: (A) methicillin-resistant *Staphylococcus aureus*; (B) methicillin-susceptible *S aureus*; (C) *Klebsiella pneumoniae*; (D) *Enterobacter cloacae*.

hydrophobicity of the oils and (2) toxic effects on membrane structure and function [6, 7]. Similar to our study, some previous studies have observed that essential oils have more activity against Gram-positive than Gram-negative isolates [6]. This is thought to be due to the more complex, rigid outer membrane of Gram-negative bacteria with lipopolysaccharide that limits the diffusion of hydrophobic compounds. The complex outer membrane is not present in Gram-positive bacteria, and the peptidoglycan cell wall provides less resistance against the hydrophobic compounds. Some essential oils have been noted to eradicate biofilms of *Pseudomonas* spp and *S aureus* [14], which could provide additional mechanisms of antimicrobial action.

Methods used to assess antimicrobial activity of essential oils varies among publications, but agar disk diffusion is frequently used for initial studies [4] and was chosen for this study. This may be considered a limitation of the study, and it may explain some of the variability seen in results. Although broth minimum inhibitory concentration detection is more precise for testing antimicrobial effects, the hydrophobicity of essential oils necessitates the use of a solvent to allow uniform distribution for accurate broth microdilution results.

Essential oils may be able to be used topically or by ingestion for antimicrobial activity, either alone or in combination with traditional antibiotics. Although essential oil antimicrobial activity looks promising, it must be remembered that safety and toxicity studies for the ingestion of essential oils are limited. In addition, these essential oils must be significantly diluted when used topically [15]. Based on current knowledge, the safest way to use essential oils is by inhalation. Essential oil inhalation has been noted to affect the autonomic nervous system, suggesting some significant systemic absorption [16], but data on systemic levels achieved via inhalation are limited. So, although these activities are promising, clinical studies are needed to determine the practicality and applicability of use.

CONCLUSIONS

Essential oils can inhibit growth of a broad range of pathogens correlating to their presence in aromatic plants. We showed significant in vitro activity against clinical isolates, including multidrug-resistant pathogens and CRE. Further study of the clinical activity of essential oils is warranted.

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