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Research Article

Homeopathic alternatives to conventional antibiotics

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Abstract. As the number of drug-resistant strains of microorganisms increases, scientists are in search of new ways to treat resistant infections. Essential oils have been used for centuries in homeopathic medicine and many are claimed to have antibacterial and antiviral properties. Several homeopathic substances were tested first using the disc diffusion method to determine activity against selected bacterial and fungal species. Microorganisms used for the study were *Escherichia coli, Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Candida kefyr*, *Rhodotorula rubra* and *Saccharomyces cerevisiae*. Substances with the most consistent action against microorganisms were tested further using a broth microdilution method to determine a minimum inhibitory concentration for each substance. *Rhodotorula rubra* was eliminated from the broth microdilution assay due to the difficulty in culturing the microorganism and its low incidence of infection. Homeopathic substances tested were garlic, honey, tea tree oil, oregano oil, thyme oil, olive leaf extract, wintergreen oil, and lemon oil. The most effective were tea tree oil, oregano oil, thyme oil, wintergreen oil, and lemon oil. Oregano oil was determined to have the greatest antimicrobial activity with a mean minimum inhibitory concentration (MIC) of 0.56 (%v/v) followed by thyme oil with a mean MIC of 2.47 (%v/v).

Introduction

Since penicillin was first introduced in 1940, treatment of infections with antibiotics has increased significantly (Lowy, 2003). Now antibiotic resistance has been detected in bacteria for all of the more than 100 antibiotics and germicidal disinfectants used today, and resistant genes have been identified in several medically important bacterial species. Many of these species are resistant to multiple agents, making choosing an antibiotic to treat such infections difficult (McDermott et. al, 2003). The increase in resistance, both to antibiotics and other disinfectants and germicides, along with the adverse side effects associated with conventional treatments, led researchers to investigate other options, including essential oils, in treating both antibiotic-resistant and –susceptible infections (Mondello et al., 2003).

One essential oil that has been studied extensively is tea tree oil. It is obtained by steam distillation of *Melaleuca alternifolia*, a plant native to Australia (Carson et al., 2002). Tea tree oil is believed to have antibacterial, anti-inflammatory, analgesic, and antiviral properties (Hammer et al., 1999). The antimicrobial properties of tea tree oil may be due to the lipophilic nature of

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some of the components and their ability to penetrate cell membranes (Banes-Marshall et al., 2001).

Many scientists have studied the minimum inhibitory concentration (MIC) of tea tree oil using a broth microdilution method. Mondello et al. (2003) found that tea tree oil inhibited *Candida* species that were both resistant and susceptible to the most commonly used fungicides at concentrations between 0.015% and 0.5% (v/v) depending on the species tested. In addition, there was no evidence that resistance was induced in the microorganisms that survived treatment with tea tree oil.

Oregano oil is another essential oil that has been studied extensively for its antibacterial properties. Studies on the MIC of oregano oil show that it has approximately the same antibacterial activity against many of the most common strains of bacteria. The MIC of oregano oil for *C. albicans* and *S. aureus* was 0.25 mg/mL (Preuss et al., 2005). The minimum bactericidal concentration (MBC) for *S. aureus*, *E. coli*, *Mycobacterium terrae*, and *K. pneumoniae* was 0.5 mg/mL. The MBC of oregano oil for *B. anthracis* and *Helicobacter pylori* was 0.25 mg/mL (Preuss et al., 2005).

Not all homeopathic antibiotics are essential oils. Garlic has long been used as an herbal treatment. In 1969, Johnson and Vaughn investigated its effects, along with the effects of onion, on bacteria. They found that a 1% concentration of onion and a 5% concentration of garlic caused detectible death in microorganisms.

Honey is another natural substance that has been used for centuries as an antibacterial agent. Much of the evidence concerning its use is anecdotal. Cooper et al. (2002) used two naturally occurring honeys and one artificial honey that was a mixture of the predominant sugars in natural honey. The study of honey used strains of bacteria that were both sensitive and resistant to common antibiotics; these included methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA), vancomyocin-resistant enterococci (VRE), and vancomyocin-sensitive enterococci (VSE). The results obtained for each strain were consistent over repeated trials and the results for MSSA strains were similar to MRSA strains (Table 1; Cooper et al., 2002).

The olive tree naturally protects itself from microbial attack using a variety of antimicrobial substances. A study conducted by Markin et al. (2003) using the extract of the leaves of the olive tree found that it also inhibited a variety of microorganisms.

In this study, the antibacterial effects of selected essential oils and natural compounds: tea tree oil, oregano oil, thyme oil, wintergreen oil, lemon oil, garlic, olive leaf extract, and honey; were tested to determine the susceptibility of *Escherichia coli*, *Klebsiella pneumoniae*, *Candida kefyr*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Rhodotorula rubra*, and *Saccharomyces cerevisiae* using the disc diffusion method. The compounds that were determined to have the greatest antibacterial effect were tested further using a broth microdilution method to determine minimum inhibitory concentrations.

Materials and Methods

Disc diffusion

Bacterial and fungal cultures were obtained from Carolina Biological Supply, essential oils were obtained from www.MotherNature.com, and garlic tablets and honey were obtained from mass realtors.

Organisms were seeded onto the appropriate agar using a sterile swab. *Escherichia coli*, *K. pneumoniae*, *P. aeruginosa*, and *P. mirabilis* were grown in tryptic soy broth (TSB) and seeded onto tryptic soy agar (TSA). *Staphylococcus aureus* was grown in TSB and seeded onto brain-heart infusion agar (BHI). *Candida kefyr*, *S. cerevisiae*, and *R. rubra* were grown in Sabouraud dextrose broth (SDB) and seeded

Table 1. MIC (%v/v) values for pasture, manuka, and artificial honey (adapted from Cooper et al., 2002).

Organism	Pasture Honey	Manuka Honey	Artifical Honey
MRSA	3.07 ± 0.26	2.98 ± 0.14	>30
VSE	9.66 ± 0.46	4.92 ± 0.28	29.7 ± 0.47
VRE	8.25 ± 1.03	4.61 ± 0.51	28.9 ± 0.99

onto Sabouraud dextrose agar (SDA). Sterile filter paper discs were soaked in the test compounds at decreasing concentrations and placed onto the surface of the agar with sterile forceps. Essential oils were diluted with mineral oil from 100% to concentrations of 50%, 25% and 12.5%. Garlic, olive leaf extract, and honey were diluted with sterile, distilled water to the same concentrations. The plates were incubated for 48 hours at 37ºC and zones of inhibition were observed and recorded as present or absent. Four trials were performed.

Broth microdilution

The compounds that consistently produced zones of inhibition were further tested by broth microdilution for all the microorganisms except *R. rubra,* due to difficulty with consistent growth.

Serial doubling dilutions from 100% to 0.098 % were performed across a 96-well microplate using 100 µL of 0.5% Tween 80 as an emulsifier for 100 µL of essential oil. One hundred microliters of overnight cultures grown in Methyl Red-Voges Proskauer (MR-VP) broth were added to each well with the wells of the last column being used as positive growth controls. Also included were a Tween 80 growth control, a methyl red growth control, and an oxidase growth control. The plates were incubated for 24 hours at 37ºC for bacteria and 24 hours at 25ºC for fungi. To determine bacterial growth, 20 µL of 4% methyl red indicator was added to each well, except the oxidase control, for *E. coli*, *K. pneumoniae*, *S. aureus*, *P. mirabilis,* and *S. cerevisiae*. One drop of oxidase reagent was added to the oxidase control on all plates and to all the wells on the *P. aeruginosa* plate. The presence of bacterial growth was indicated by the presence or absence a color change in the methyl red test and the development of a deep purple color in the oxidase test. Growth on the *C. kefyr* plates was determined visually by observation of a pellet of growth at the bottom of the well. Wells initially determined to be the MIC and the well of the next lower concentration were plated onto TSA or SDA to determine the lowest concentration that inhibited growth. A volume of 200 µL was taken from the well and plated; the plates were incubated for 24 hours at 37°C or 25°C. Wells were plated until no growth was observed. The well with the lowest concentration that inhibited growth was recorded as the MIC. Six trials were performed and the mean MIC for each essential oil for each microorganism was determined.

Results and Discussion

The disc diffusion assay showed that the essential oils tea tree, oregano, thyme, wintergreen, and lemon showed the greatest amount of antibacterial action. Olive leaf extract showed antibacterial activity only in isolated cases. Garlic and honey gave inconsistent results of low bactericidal activity (data not shown). Based on this, only the essential oils were used for the broth microdilution assay.

For the broth microdilution results, outliers, those falling greater than 2 standard deviations from the mean were removed for graphical analysis. In all cases, this was no more than 1 outlier. Additionally, those samples that had a standard deviation greater than the mean were eliminated.

The broth microdilution showed that oregano oil had the most antibacterial activity demonstrated by consistently low average MIC values, followed by thyme oil (Tables 2 and 3). Ultee et. al (1999) determined that oregano oil did not disrupt the integrity of microorganism membranes at levels consistent with the MIC or MBC. At concentrations greater than or equal to 0.01 mM the membrane potential decreased, which caused increased permeability of the membrane and disruption of the proton gradient. Tea tree oil also had low average MIC values (Tables 2 and 3). The antimicrobial properties of tea tree oil may be due to the lipophilic nature of some of the components and their ability to penetrate cell membranes (Banes-Marshall et. al, 2001). This cell membrane penetration causes ions to leak across the membrane of the cell, disrupting concentration and electrical gradients (Gustafson et. al, 2001). Tea tree oil has also been shown to inhibit respiration, impair the function of membrane ATPase, and increase membrane fluidity. It is hypothesized that the terpenes in tea tree oil

Table 2. Average MIC and standard deviation results for essential oils.

Tea Tree Oil

Organism	N	Mean MIC	Standard Deviation
E. coli	5	0.9626	0
P. mirabilis	5	0.1563	0.1310
P. aeruginosa	5	0.1172	0.0437
C. kefyr	5	0.7031	0.1747
S. cerevisiae	5	0.3125	0.1070

Wintergreen Oil

Organism	N	Mean MIC	Standard Deviation
E. coli	5	0.1758	0.1273
K. pneumoniae	5	3.1250	2.8705
P. mirabilis	5	0.6641	0.2620

Oregano Oil

Organism	N	Mean MIC	Standard Deviation
E. coli	5	0.0977	0
S. aureus	5	0.1563	0.1310
P. aeruginosa	5	0.1172	0.0437
C. kefyr	5	0.0977	0
S. cerevisiae	5	0.0977	0

Lemon Oil

Organism	N	Mean MIC	Standard Deviation
S. aureus	5	35.0	22.3225
K. pneumoniae	5	12.5	11.4820
P. mirabilis	5	37.5	17.6777
C. kefyr	5	0.6250	0.5241
S. cerevisiae	5	0.3516	0.0873

Thyme Oil

Organism	N	Mean MIC	Standard Deviation
E. coli	5	0.1563	0.1310
S. aureus	5	0.9766	0.8023
K. pneumoniae	5	17.5	6.8465
P. mirabilis	5	0.6445	0.5678
P. aeruginosa	5	0.1172	0.0437
C. kefyr	5	0.1367	0
S. cerevisiae	5	0.0977	0

* Standard deviation is 0 when there was no variation in the mean of the samples tested.

Table 3. Overall mean MIC values for each essential oil with outliers removed.

Essential Oil	Overall Mean MIC (%v/v)
Tea Tree Oil	0.4503
Oregano Oil	0.1133
Thyme Oil	2.8041
Wintergreen Oil	1.3216
Lemon Oil	17.2

induced changes in the membrane by inserting between the individual phospholipids, but this action depended on the hydrophobicity of the compounds (Hammer et. al, 2004). A study by Penalver et. al (2005) found that oils with a high percentage of phenolic compounds such as thymol and carvacrol had high inhibitory activities against a wide spectrum of bacteria. These compounds are the main components of thyme oil.

Staphylococcus aureus generally did not respond well to some of the homeopathic antimicrobials, and had average MIC value of 0.15 %v/v, 0.98 %v/v, and 35 %v/v for oregano oil, thyme oil and lemon oil, respectively (Tables 2 and 3). *Staphylococcus aureus* was the only Gram positive bacteria tested and the thick layer of peptidoglycan associated with Gram positive bacteria would inhibit the membrane-disrupting action of many of the essential oils, possibly explaining the increased resistance.

Klebsiella pneumoniae was the most resistant microorganism to all of the essential oils tested, but was the least resistant to oregano oil (Table 2, Figure 1). Because *K. pneumoniae* is resistant to the oils, it is presented separately. Although *K. pneumoniae* is Gram negative, it possesses a capsule as an innate defense, which, like the peptidoglycan of Gram positive bacteria, would block essential oils from accessing the fragile inner membrane.

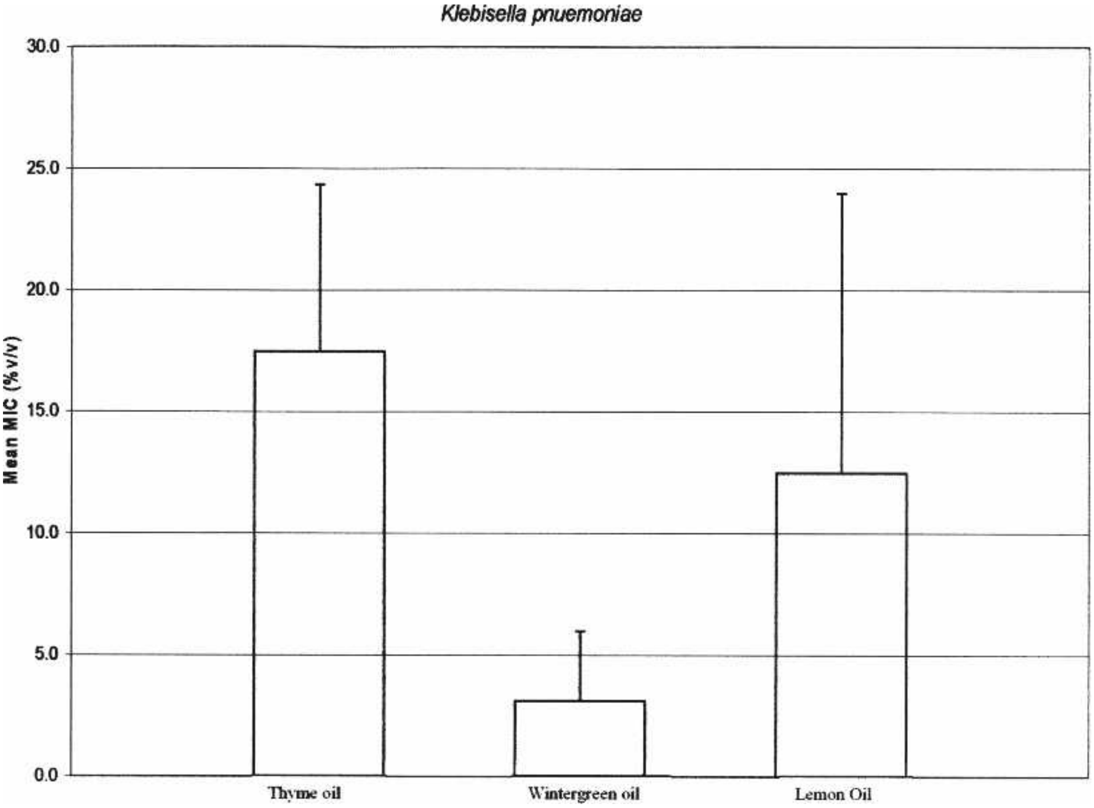

Figure 1. Mean adjusted MICs (%v/v) for *K. pneumoniae*. Error bars represent ± 1 SD.

In some cases there was no variation between the different trials. The same MIC for *E. coli* was obtained in each trial, making the mean of 0.9626% v/v the actual value obtained for the individual trials and the standard deviation zero. The individual MICs for *E. coli*, *C. kefyr* and *S. cerevisiae* with oregano oil also did not show any variation in values and subsequently have a standard deviation of zero, as well the effect of thyme oil on *C. kefyr* and *S. cerevisiae*.

This study did encounter some methodological problems. The lids purchased with the 96-well microplates did not fit on the microplates and evaporation of the broth, particularly in the wells of row A, possibly caused MIC values obtained to be less than the actual antibacterial activity of the oil tested. Additionally, due to lack of availability of Petri dishes, multiple wells were combined onto a single agar plate and an exact MCC could not be found because the samples could not be sufficiently spread across the entire agar surface. The MCC would be the lowest concentration that showed growth of three or fewer colonies. This problem could easily be solved by taking a smaller sample from the wells to be plated. Through the plating method to determine MCC, we found the methyl red and oxidase tests were poor indicators of bacterial growth.

Essential oils do have antibacterial activity in a broth microdilution assay. The oils that performed the best were oregano oil and thyme oil; lemon oil performed the worst out of the five essential oils tested (Table 3).

This work demonstrates that essential oils do have antibacterial properties and these properties need to be utilized, although more work needs to be done to determine more accurate MIC and MCC levels. Although it is not known to what extent the action of essential oils is decreased by debris on the skin surface (Hammer et al., 1999), essential oils could be incorporated into soaps and other bodily cleaning agents at low concentrations in the place of conventional antibiotics (Halcon and Milkus, 2004).

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