



Research Article

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# Essential Oils, an Answer to the Dilemma of Multiply Resistant Bacteria?

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## Abstract

Antibiotic resistance is an increasing worldwide phenomenon. Recently, there has been an interest in using essential oils (EO) both in and out of the hospital. Most current research concentrates on the antimicrobial activity of Tea tree. The purpose of this study is to evaluate the activity of 10 EO, (Tea tree, Thyme, Lavender, German chamomile, Peppermint, Palma rosa, Eucalyptus globulus, Eucalyptus citriodora, Lemongrass and Rose) against multiply resistant organisms. Twenty five strains of Methicillin Resistant *Staphylococcus aureus* (MRSA), ten strains of vancomycin resistant *Enterococcus* sp. (VRE), and 5 strains of extended spectrum beta-lactamase (ESBL) or Amp C resistant gram negative rods were tested. An aromatoqram (Bauer- Kirby [KB]), killing curve, and synergy tests were performed against the isolates. Organisms were classified as multiply resistant using NCCLS approved methods. The bacteria were obtained from the Pinnacle Health microbiology repository. The ten EO were tested in three phases.

Antimicrobial activity occurred between several EO and the multiply resistant bacteria. Lemongrass, Thyme and Palma rosa oils were the most active compounds overall. The Lemongrass and Thyme were the most active compounds versus MRSA. Lemongrass and Palma rosa showed the greatest activity versus VRE and Tea Tree and Thyme showed the most activity against resistant gram negative rods. EO shows antimicrobial activity versus multiply resistant bacteria *in vitro*. More studies are needed to confirm this activity *in vivo*.

**Keywords:** Essential Oils, Antimicrobial Resistance, Multi Drug Resistant Organisms

## Introduction

Antibiotic resistance is an increasing worldwide phenomenon [1-4]. It is at least partially driven by the overuse of antibiotics, particularly broad-spectrum antibiotics and has been occurring for many years without resolution [5-8]. Physicians, hospitals, drug companies, and government agencies have been looking for new and innovative ways to deal with these resistant strains of bacteria. Over the last few decades, a decrease in the number of new antibiotics have been realized [1]. Between 1983 and 1987 16 new antibiotics were approved while only 2 new antibiotics were introduced between 2008 and 2012. In addition, antibiotic resistance is increasing worldwide [1]. Essential oils (EO) have been used since ancient times for healing and the treatment of patients [9], recently, there is an interest in them as an alternative to

antimicrobial therapy as a replacement or as an adjunctive therapy source [10]. There has been an interest in using essential oils (EO) both in and out of the hospital [10].

The purpose of this study is to evaluate the activity of 10 EO, (Tea tree, Thyme, Lavender, German chamomile, Peppermint, *Palmarosa*, *Eucalyptus globulus*, *Eucalyptus citriodora*, Lemongrass and Rose, see table 1 against multiply resistant organisms using standard methods [10].

## Methods

Twenty five strains of Methicillin Resistant *Staphylococcus aureus* (MRSA), ten strains of vancomycin resistant *Enterococcus* sp. (VRE), and 5 strains of extended spectrum beta-lactamase



(ESBL) or Amp C resistant gram negative rods were tested. An aromagram (Bauer- Kirby [KB]), killing curve, and synergy tests were performed against the isolates [10-12].

Organisms were collected from distinct geographical regions (Illinois, Michigan, North Carolina and Pennsylvania) and stored in the Pinnacle Health microbiology repository. This was done in an attempt to test separate clones of organisms and prevent testing multiples of the same organisms. Organisms were classified as multiply resistant using NCCLS approved methods [13,14]. The ten EO (Table 1) were tested in three phases.

1	<i>Melaleuca alternifolia</i> Cterpineol	Tea Tree
2	<i>Lavandula angustifolia</i>	True Lavender
3	<i>Mentha x piperita</i>	Peppermint
4	<i>Eucalyptus globulus</i>	Eucalyptus globulus
5	<i>Eucalyptus citriodora</i>	Eucalyptus citriodora
6	<i>Cymbopogon martinii</i>	Palmarosa
7	<i>Cymbopogon citratus</i>	Lemongrass
8	<i>Matricaria recutita</i>	German Chamomile
9	<i>Rosa damascena</i>	Rose
10	<i>Thymus vulgaris</i> CT linalool	Thyme

The antimicrobial activity of the essential oils was studied using three phases as described below. **Phase I:** 10 EO were tested versus 25 Methicillin Resistant *Staphylococcus aureus* (MRSA), 10 Vancomycin Resistant *Enterococcus sp* (VRE) and 5 *Escherichia coli* and *Klebsiella pneumoniae* (ESBL/AmpC) isolates using an aromagram. Control organisms suggested by NCCLS (obtained from the American Type Culture Collection were also tested [13,14].

Essential oils tested versus the aforementioned organisms were; Tea Tree, Thyme, Lavender, German chamomile, Peppermint, Palmarosa, Eucalyptus globulus, Eucalyptus citriodora, Lemongrass and Rose. Three  $\mu$ l of each of the oils were placed onto separate blank susceptibility disks (Becton Dickinson BBL, Franklin Lakes, New Jersey) and allowed to absorb.

The Phase I test were setup as previously described and suggested as a preliminary screening method [11]. The inoculum of bacteria in saline was calibrated to a 0.5Mc Farland standard ( $10^8$  cfu (colony forming units)/ml). While the disks were soaking, a 0.01 ml inoculum of the test organisms were streaked on to petri

dishes filled with Mueller Hinton agar (Mueller Hinton agar plates with 5% sheep's blood for the VRE cultures) (Becton Dickinson BBL, Franklin Lakes, New Jersey). The disks were placed onto the Mueller Hinton agar plates, 6 disks per plate. All tests were done in duplicate. The plates were then incubated for 24 hour at 35°C in room air. The zone diameters were measured and recorded. The average zone diameters of duplicate tests were used.

**Phase II:** 5 EO were tested against 7 microorganisms (showing variable results) by exposing them to the EO diluted in a fixed oil (suspensions used *in vivo*) in a test tube and measuring a killing curve for each EO and microorganism. Based upon the results of the first experiment, additional tests, "killing curves" were performed. Tea Tree, Lemongrass, Thyme linalool and German chamomile were used in this phase of the study. These oils were chosen because they were the best and the worst inhibitors in Phase I tests. The oils were mixed with a carrier oil, Sweet Almond oil (*Prunus dulcis*) in a sterile tube and then inoculated with a test organism ( $10^8$  cfu/ml) and covered with parafilm. They were then subbed out and streaked onto a plate at one, two, three, six, and 24 hours after they were placed into the oil. All subcultures were performed in duplicate. The plates were incubated as above. Colony counts of the subculture plates were performed. The average colony counts found upon duplicate tests were used in the study.

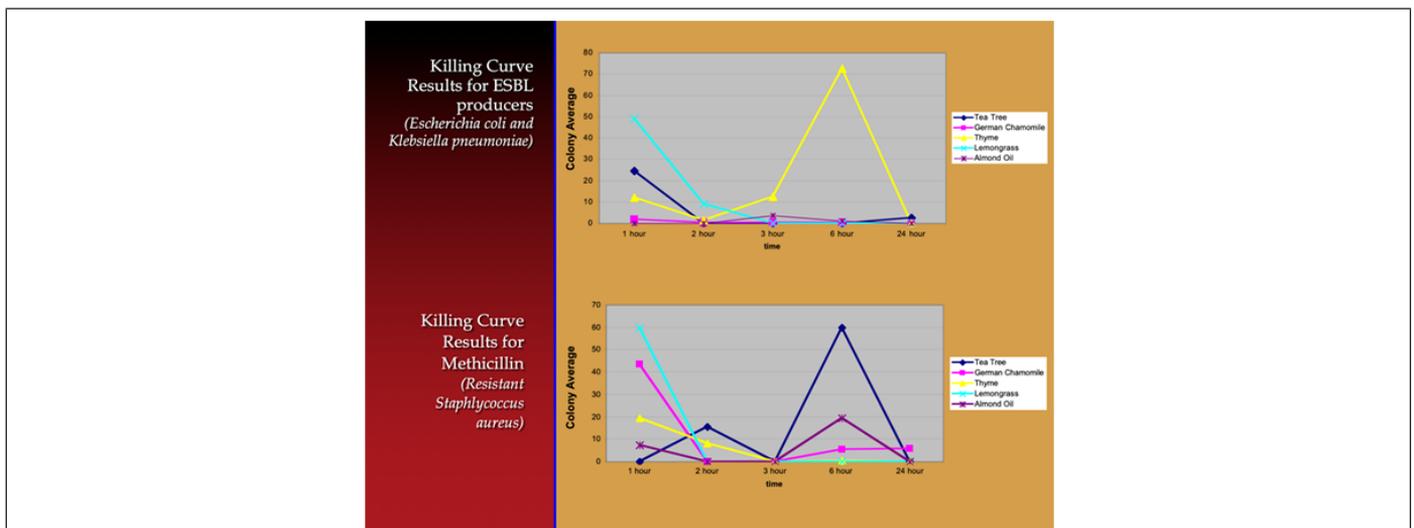
**Phase III:** Synergy/antagonism was tested between the fixed oil with or without vitamin E and 4 EO versus the multiply resistant micro-organisms. Phase III was initiated since the carrier oil showed inhibition. A synergy test was performed similar to testing in Phase I. Disks impregnated with Sweet Almond Oil. Sweet Almond Oil was placed adjacent to a disk containing one of the other test oils from the killing curve onto agar plates at 10mm and 15mm apart. Blunting (antagonism) or enhanced inhibition (synergy) was recorded between Sweet Almond Oil and each other test oil.

## Results

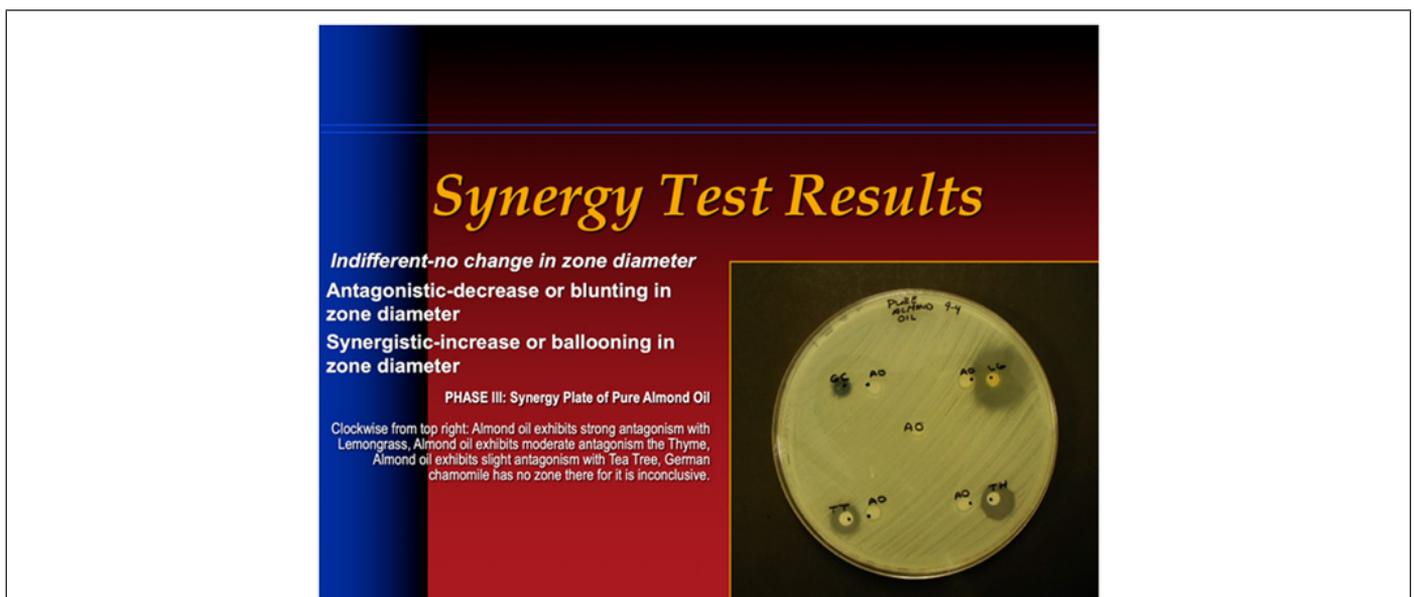
Antimicrobial activity occurred between several EO and the multiply resistant bacteria. Lemongrass, Thyme linalool and Palmarosa oils were the most active compounds overall. The Lemongrass and Thyme were the most active compounds versus MRSA. Lemongrass and Palmarosa showed the greatest activity versus VRE and Tea Tree and Thyme linalool showed the most activity against resistant gram negative rods. EO show antimicrobial activity versus multiply resistant bacteria in vitro. More studies are needed to confirm this activity *in vivo*. (Table 2, Figure 1 & 2).

**Table 2:** Phase 1- Zone Diameter Results of Multi-Resistant Organisms versus Essential Oils.

Essential Oil	VRE	MRSA	ESBL/AMPC
	Average 10 strains *Zone Diameter in MM	Average 25 strains	Average 5 strains
Tea Tree	6*	11.5	14.8
True Lavender	6.1	12.48	10.2
Peppermint	8.5	13	6
Eucalyptus globulus	6	6.52	6
Eucalyptus citriodora	6.6	6.44	6
Palmarosa	12.1	13.64	8.2
Lemongrass	17	24.48	6
German chamomile	6	6	6
Rose	8.2	10.88	6
Thyme linalool	8.7	14.75	12.6



**Figure 1:** PHASE II: Killing curves of Essential oils and effect on Multi-Resistant Organisms over a 24 hour period.



**Figure 2:** PHASE III: Synergy Test Results.Synergy Plate of Pure Sweet Almond Oil; Indifferent-no change in zone diameter; Antagonistic-decrease or blunting in zone diameter; Synergistic-increase or ballooning in zone diameter

\*Clockwise from top right: Sweet Almond oil exhibits strong antagonism with Lemongrass, Sweet Almond oil exhibits moderate antagonism the Thyme linalool, Sweet Almond oil exhibits slight antagonism with Tea Tree, German chamomile has no zone there for it is inconclusive.

## Conclusions

In Phase I the results are consistent with other studies looking at ranges of zone diameters [11]. In the current study the most inhibitory essential oil was Lemongrass against VRE and MRSA. The most inhibitory essential oil against ESBL/AmpC strains was Tea Tree. The least inhibitory essential oil against all the bacteria tested was German chamomile. Consistent results were observed when measuring the zone diameters of inhibition. Some limitations of this testing may be the activity of the solvent as recognized in the current study, the volume of EO on the susceptibility disks and the thickness of the agar layer [11].

In Phase II, of the essential oils tested, Lemongrass and Tea Tree had the most significant killing effect. A control test tube of almond oil (without any essential oil added) also killed the bacteria. Since the almond oil appeared to kill the bacteria faster alone than mixed with essential oil we decided a third phase of the study was necessary.

In Phase III we discovered that the carrier oil often used by aromatherapists in conjunction with essential oils had varying antagonistic effects on the essential oils' antimicrobial activity. In this phase the almond oil alone did not produce zones of inhibition. We believe this happened because the almond oil was too viscous to diffuse through the agar medium.

In the current study, the solvent (Sweet Almond Oil) showed antagonism with several of the EO studied and showed difficulty in diffusion in the test method. Different parameters in this test could affect the result, such as the volume of EO on the paper discs, the thickness of the agar layer and the solvent. As previously seen, this could result in difficulty in reproducibility of screening methods [11,15-17]. An important parameter in phase I is that the zone diameters seen are reproducible in each of the EO tested and are consistent with those seen in other studies [11]. As demonstrated in the current study, a disk test is useful in screening for antibacterial activity of EO, however further studies maybe needed to determine the best EO and solvent to be used for medicinal therapy.

As evidenced in other studies [18], Lemongrass and Tea Tree oil were effective in killing organisms in vitro and in our study did it faster than the other oils. Natural products are interesting alternative or supplemental to antibiotics as a treatment moiety for patients inside and outside the hospital.

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