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# Antimicrobial activity of multipurpose essential oil blends

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## ABSTRACT

**Two types of multipurpose essential oil blends, blend[11] containing eleven different essential oils and blend[12] containing twelve, were tested against bacterial strains of *Pseudomonas aeruginosa* ATCC 9027, *Serratia marcescens* ATCC 13880 and *Staphylococcus aureus* ATCC 6538 and against the fungi, *Candida albicans* ATCC 10231, *Aspergillus fumigatus* ATCC 10894 and *Fusarium solani* ATCC 36031 to determine the spectrum of *in vitro* antimicrobial activity using aromatograms (paper disc diffusion assays). Microbial growth was decreased by multipurpose blend[11] and blend[12] in a similar manner. The saline control disc did not inhibit anti-microbial growth while the two blends exhibited significant zones of inhibition for all 3 bacteria and for the 3 fungi. The greatest antibacterial activity of blend[11] and blend[12] was exhibited with *P. aeruginosa* and *S. marcescens* followed by *S. aureus*. A high level of activity was associated with *C. albicans* and a lower level with *F. solani* followed by *A. fumigatus*. It is clearly evident from previous published studies that no single essential oil will effectively inhibit the growth of all of the organisms in our study. However, our results demonstrate that blend[11] and blend[12] have a broad range of inhibitory activity affecting all of the microorganisms tested.**

**Keywords:** Aromatic Essential Oils; Antimicrobial Activity

## 1. INTRODUCTION

The use of aromatic essential oils for human preventative medical purposes began in ancient Greece and is currently being studied for control of microbial agents. Individual essential oil blends contain combinations of volatile compounds extracted from single plant types and within each plant type, the components of the essential oils function as antimicrobial and/or antifungal agents [1,2].

Previous studies of microbiological inhibition by individual essential oils derived from a single plant species reveal that only a few oils are capable of inhibiting molds, yeasts and bacterial pathogens and only at very high concentrations (**Tables 1** and **2**). Independent studies of the effects of aromatic compounds suggest that their use in combinations in liquid mixtures could broaden their capabilities to prevent antimicrobial growth. To address this question, the present study assessed the ability of two types of multipurpose essential oil blends as antibacterial and antifungal agents using the *in vitro* disc diffusion method. Blend[11] and blend[12] contain mixtures of eleven and twelve different essential oils, respectively, extracted from aromatic plants species known to have antimicrobial activity.

The present study was designed to determine the antimicrobial activity of two types of essential oil blends, blend[11] and blend[12], against *Aspergillus fumigatus* ATCC 10894, *Fusarium solani* ATCC 36031, *Candida albicans* ATCC 10231, *Pseudomonas aeruginosa* ATCC 9027, *Serratia marcescens* ATCC 13880 and *Staphylococcus aureus* ATCC 6538. The purpose was to compare the antimicrobial killing of two multipurpose essential oils to data from previous reports of single antimicrobial plant extracts using a paper disc diffusion assay.

## 2. MATERIALS AND METHODS

The six microorganisms, *A. fumigatus*, *F. solani*, *C. albicans*, *P. aeruginosa*, *S. marcescens* and *S. aureus* were obtained from the American Type Culture Collection (Rockville, MD). Molds and yeast species were cultured on Sabouraud's Dextrose agar (Difco, Sparks, MD) for 10 days at 23 C or for two days at 37 C, respectively. Blend[11] (GermBullet[TM]) is the original multipurpose, Inhalable blend and seven of it's 12 essential oils are

**Table 1.** Comparison of the antibacterial activity of essential tree and herb oils using agar disc diffusion.

| | *Pseudomonas aeruginosa* | *Serratia marcescens* | *Staphylococcus aureus* |
|---|---|---|---|
| | Radius of zone of inhibition (mm) | | |
| Trees | | | |
| *Eucalyptus sideroxylon* | 8 [3] | NA | 12 [3] |
| *Melaleuca alternifolia* | 0 [4] | 32 [5] | 10 [6] |
| *Pinus densiflora* | 14 [7] | NA | 14 [7] |
| Herbs | | | |
| *Lavandula angustifolia* | 12 [8] | NA | 10 [6] |
| *Mentha piperita* | 13 [8] | NA | 8 [8] |
| *Origanum vulgare* | 7 [9] | 10 [9] | 56 [9] |
| *Rosmarinus officinalis* | 23 [8] | NA | 12 [8] |
| *Thymus vulgaris* | NA | NA | 19 [10] |

Reference numbers are in brackets, NA is not analyzed.

**Table 2.** Comparison of the antifungal activity of tree and herb essential oils using agar disc diffusion.

| | *Candida albicans* | *Aspergillus fumigatus* | *Fusarium solani* |
|---|---|---|---|
| | Radius of zone of inhibition (mm) | | |
| Trees | | | |
| *Eucalyptus sideroxylon* | 10 [11] | NA | 21 [12] |
| *Melaleuca alternifolia* | 11 [6] | NA | NA |
| *Pinus densiflora* | 11 [13] | 12 [14] | NA |
| Herbs | | | |
| *Lavandula angustifolia* | 10 [6] | NA | NA |
| *Mentha piperita* | 18 [6] | 10 [15] | NA |
| *Origanum vulgare* | 19 [16] | NA | 28 [16] |
| *Rosmarinus officinalis* | 6 [17] | NA | NA |
| *Thymus vulgaris* | 36 [6] | NA | NA |

Reference numbers are in brackets, NA is not analyzed.

Lavender A. Vera (*Lavendula vera*), Bergamot (*Citrus aur bergamia*), Eucalyptus Globulus (*Eucalyptus globulus*), Pine Sylvestre (*Pinus negra*), Eucalyptus Radiata (*Eucalyptus radiata*), Rosemary Cineol (*Rosemarinus officinalis*), and Lavender Spike (*Lavendula officinalis*) listed in descending order of concentration and comprise over 80% of the ingredients (FCD, Boca Raton, FL). Blend[12] (Germbullet[TM14]) is a slight variation of Blend[11] and is also a multipurpose blend used in this study. Specific concentrations of ingredients are not listed, Streptomycin, miconazole and fluconazole (Becton Dickinson Diagnostic Systems, Sparks, MD) discs were used as positive controls and a normal saline treated Whatman disc as a negative control.

Plate cultures of bacteria and *C. albicans* were grown for two days and plate cultures of the molds for seven days, harvested in normal saline to a concentration of $1 \times 10^6$ cells per ml, plated on nutrient and Sabouraud's agar plates, respectively, and dried. Sterile six mm Whatman discs (Fisher Scientific, Suwanee, GA) were dipped into each of the two undiluted blends, dried and placed into the center of each of the inoculated agar dishes. A standardized inoculum of $1 \times 10^6$ CFU/ml was prepared for each microorganism. Agar plates were inoculated with $1 \times 10^6$ microorganisms in 1 ml suspensions and allowed to dry. Paper discs containing each of the mixtures,

blend[11] and blend[12], were applied to inoculated plates followed by incubation of the molds at 23 C for 72 hours and the bacteria and yeasts at 37 C for 24 hours. Following incubation, the zone of inhibition for each blend was recorded in mm (including the disc). Plates were done in triplicate and an average + SD was recorded.

## 3. RESULTS

*In vitro* paper disc diffusion assays clearly showed antimicrobial activity for both multipurpose essential oil preparations (**Table 3**) and blend[11] and blend[12] both inhibited microbial growth in a similar fashion. Zone radii (in mm) differed between bacteria and fungi but were consistent between the two essential oil mixtures, from 19.5 + 1 to 42.3 + 3 for blend[11] and 22.8 + 1 to 42.9 + 3 for blend[12].

Antibacterial activity associated with both blend[11] and blend[12] differed between species (*P. aeruginosa* > *S. marcescens* > *S. aureus*) with killing activity observed in all three bacteria (**Table 3**). Antifungal activity was displayed in the three fungi tested but with variations (*C. albicans* > *F. solani* > *A. fumigatus*).

## 4. DISCUSSION

Previous studies of the antifungal activity of essential oils using broth dilution and agar dilution methods report similar findings to those of this study [18-20]. Obviously, other methods of measuring antimicrobial activity (minimum inhibiting concentration) have been reported but were not included in the comparison of data obtained from the present study due to inherent differences in test methodology concerning the use of solubilizing agents, choice of plant extract and/or test microorganism [8,21, 22].

The two ubiquitous molds, *A. fumigatus* and *F. solani* were affected by blend[11] and blend[12] demonstrating that the growth of common airborne molds and their potential pathogenic activity in the nasal cavity can be controlled. Of interest is the fact that the spores of these molds are resistant to harsh environmental changes but not to blend[11] and blend[12], which is relatively harmless and safe for humans. The results of these findings support the contention that mixing individual aromatic essential oils results in blends that are capable of broad spectrum antimicrobial activity. The observed antimicrobial and antifungal activity of both blends reflects the previously reported properties attributed to the activity of the individual essential oil preparations.

Both *S. marcescens* and *P. aeruginosa* are bacteria which are normally found abundantly in the environment and that are capable of causing serious infections in humans. Obviously, *P. aeruginosa* is most studied due to its known resistance to antimicrobial drug therapy. An interesting finding was the antibacterial action of the two blends on both *P. aeruginosa* and *S. marcescens* using

**Table 3.** Comparison of the antimicrobial activity of multipurpose blend[11] and blend[12] on selected bacteria and fungi using paper disc diffusion assays.

| | Radius of zone of inhibition (mm) + 1SD | | |
|---|---|---|---|
| | Bacteria | | |
| | *Pseudomonas aeruginosa* | *Serratia marcescens* | *Staphylococcus aureus* |
| Treatment | | | |
| Blend[11] | 30.9 + 1.2 | 32.5 + 2.1 | 19.5 + 0.8 |
| Blend[12] | 33.3 + 2.3 | 26.2 + 1.9 | 24.2 + 1.1 |
| Normal saline | 0.0 | 0.0 | 0.0 |
| Streptomycin | 14.3 + 0.2 | 17.1 + 0.09 | 21.2 + 0.6 |
| | | Fungi | |
| | *Candida albicans* | *Aspergillus fumigatus* | *Fusarium solani* |
| Treatment | | | |
| Blend[11] | 42.3 + 3.1 | 21.8 + 1.0 | 25.1 + 1.3 |
| Blend[12] | 42.9 + 3.3 | 22.9 + 1.3 | 23.4 + 0.9 |
| Normal saline | 0.0 | 0.0 | 0.0 |
| Fluconazole | 4.1 + 0.8 | - | - |
| Miconazole | - | 29 + 1.1 | 27 + 1.8 |

 

the paper disc diffusion method.

Multipurpose essential oil blend[11] and blend[12] have the potential of providing protection against microorganisms causing respiratory problems that originate in the nose. The duration of their usage before exposure is short, if not immediate, they are convenient for the consumer, are stable and do not irritate sensitive or damaged tissues. These properties, along with the current finding of a broad spectrum of antimicrobial activity, demonstrate that either blend[11] or blend[12] can be effective if used as an inhalant.

From the data presented here, it seems plausible that introduction of blend[11] or blend[12] into the nasal cavities of humans may modify the growth of the resident microbial populations and result in control of harmful microorganisms in the respiratory tract. The inhibitory effect of both blends on *P. aeruginosa*, *S. aureus* and *C. albicans*, which are found as normal human flora, supports the concept that they may help prevent infections caused by bacteria and yeast that, under certain circumstances, are pathogens. Possibly the reduction in the growth of symbiotic/pathogenic organisms may lower the numbers of the microorganisms below a critical threshold level necessary to cause infections.

Regular use of either blend[11] or blend[12] before, during or after exposure provides protection against a variety of potential pathogens that are encountered when exposed to people who carry respiratory infections. Future studies on the effect of multipurpose blends on the antimicrobial flora of the human respiratory tract will be necessary to determine the health benefits acquired from nasal inhalation.

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