Original Article

# Evaluation of effects of essential oil vapors on the bacterial count in bioaerosols

Vishwaprakash Shetty, K Sri Varalakshmi, A Jacob Prakash, M Vijaya Lakshmi, Harsha M

Department of Oral Pathology, Lenora Institute of Dental Sciences, Rajanagaram, Andhra Pradesh, India

## Abstract

**Background:** The aerosols generated during dental treatments contain bacteria and other microorganisms that penetrate the body through the respiratory system of dental surgeons and cause infectious diseases. Several studies have been done to reduce these hazards. The aim of the present study is to evaluate the effects of the plant extract essential oil (EO) vapors of Neem, Clove, Cinnamon bark, Thyme, Lemon Grass, and *Eucalyptus* on the bacterial count in bioaerosols near dental units.

**Materials and Methods:** Sampling was taken on nutrient blood agar plates by placing them open near dental units using passive air sampling method, before commencement of treatment for 1 h, during treatments for 2 h, and after introducing EO vapors for 2 h. The collected samples were taken for incubation at 37°C for 48 h. The colonies formed were counted in colony-forming units per cubic meter and taken for statistical analysis.

**Results:** After comparing the obtained results, it was found that there was a significant reduction ($P < 0.05$) in the bacterial count for about 43% near the dental units after the introduction of the EO vapours.

**Conclusion:** It is concluded that natural extracts like EOs can reduce bacterial contamination near dental units in the vapourized state, thereby reducing the health hazards in Dental Health Professionals.

**Keywords:** Bioaerosols, dental units, essential oil vapors, index of bacterial air contamination, passive air sampling

**Address for correspondence:** Dr. Vishwaprakash Shetty, Department of Oral and Maxillofacial Pathology, Lenora Institute of Dental Sciences, Rajahmundry, Andhra Pradesh, India.
E-mail: vpshetty@yahoo.com

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## INTRODUCTION

The humidity and temperature of the oral cavity creates a wide range of habitats with different environmental conditions and provides an ideal media for growth and colonization of microorganisms, which include pathogenic (Viridans *Streptococcus, Streptococcus haemolyticus* etc.) and commensal microorganisms (*Staphylococcus* sp., *Micrococcus* sp. etc.). These microorganisms can be easily transmitted by blood or saliva through direct or indirect contact, droplets, aerosols, which are emitted during cavity preparations and ultrasonic scalings or through contaminated instruments and equipment.[1-3] Hence, in the dental clinical environment, the presence of aerosols, droplets and splatter containing microbes liberated during dental operative procedures is a biological hazard promoting an increased risk of cross-infection to dental health professionals and patients. To ensure an effective and safe infection control program, dentists and dental

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laboratories should discuss their infection control programs with each other.[4] The equipment used for infection control are ought to be expensive or hazardous.

In recent years, there has been an increasing interest in the usage of herbal extracts in various fields of medicine (oncology, microbiology) and dentistry. Eugenol, the most widely used plant extract of clove, has been used in treating dental caries because of its anticariogenic activity (through antiadhesive property against the bacteria).[5] Similarly, plant extracts, mainly essential oils (EOs) are having a significant role in treating various diseases. Hence, studies on plant extracts in medicine have to be done to enrich the use of herbal products and avoid the hazards caused due to chemical products. Plant-derived EOs are complex mixtures consisting of mono and sesquiterpenes and volatile phenolics. Apart from being eco-friendly, they are also economical. Not much study has been undertaken using EO vapors directly near dental units to reduce the microbial counts in bioaerosols. The present study evaluates the effects of the vaporised EO blend of six various plants (Plants such as Neem, *Eucalyptus*, Clove, Cinnamon, Thyme and Lemongrass) on the bacterial count in bioaerosols emitted during treatments near dental units.

### Aim

To evaluate the effects of EO vapors on the bacterial count in bioaerosols.

### Objectives

1. To evaluate the bacterial count in bioaerosols near the dental units before the commencement of treatments
2. To evaluate the bacterial count in bioaerosols near the dental units during dental treatments
3. To evaluate the bacterial count in bioaerosols near the dental units after introducing EO vapors during the dental treatments
4. To compare and correlate the bacterial counts in bioaerosols near dental units before and after introducing EO vapours.

## MATERIALS AND METHODS

This study involved 30 different clinical establishments (4 clinical departments, Lenora Institute of Dental Sciences, Rajamahendravaram, and 26 Private Dental Clinics).

### Methodology

Prior consent was taken from the departments and the clinicians from the private dental practice. Each dental unit water tubing system was flushed out with 0.5% sodium hypochlorite solution for 5 min, and the same solution was allowed to stay in the tubing water system for 10 min. Sterile water was flushed for another 5 min. This procedure was done before the commencement of the study. Samples were collected from different dental units on solid nutrient blood agar in Petri dishes using the passive air sampling method.[6-8]

#### *Before treatments*

One agar plate was placed in proximity to the dental chair 1 h before the commencement of the treatment and kept open for an hour. Later, it was closed and kept for incubation at 37°C for 48 h. This was done to evaluate the general microbial count in bioaerosols near dental units before the commencement of treatments.

#### *During treatments without essential oil vapors*

Four agar plates were placed at four different places near the dental chair at 0.5 m, 1 m, 1.5 m and 2 m distance. The plates were placed at the height of 1–1.5 m from the floor. The plates were opened when the treatment started and was closed after two h. After prior intimation to the patients, brief clinical history was taken before the commencement of the treatments.

#### *During treatments with essential oil vapors*

EO vapors were introduced near the dental unit 1 h before the next treatment session using a commercially available ultrasonic diffuser and continued for 3 h. After 1 h of introducing EO vapors, four agar plates were again placed at four different places near the dental chair at 0.5, 1, 1.5 and 2 m distance. The collected samples were incubated at 37°C for 48 h.

After incubation of the collected samples, microbial colonies developed on the blood agar plates (Figures 1-4). The bacterial colonies were counted using digital colony counter. The values of the bacterial count were given as colony-forming unit (cfu)/plate. The obtained values were calculated as cfu per cubic meter (cfu/$m^3$) using the following formulae.[8]

Formula to obtain CFU/$m^3$ – Omeliansky formula

$$N = 5a \times 10^4 (bt)^{-1}$$

where "*N*" is microbial CFU/$m^3$ of indoor air, "a" is the number of colonies per petri dish, "b" is dish surface ($cm^2$), "t" is the exposure time (minutes).

The final index of bacterial air contamination (IBA)[8] results was obtained and taken for statistical analysis using the MannWhitney test.

## RESULTS

### Before treatments

The IBA contamination among 30 samples collected near the dental units 1 h before the commencement of the treatment and without introducing EO vapors is ranging between 262 and 3262 cfu/$m^3$ of indoor air. The baseline IBA mean is 1345.8 ± 757.3689212 cfu/$m^3$ of indoor air.

### During treatments without essential oil vapors

During treatments the samples collected near the dental units at 0.5, 1, 1.5, and 2 m distance for a period of 2 h are indicating an average bacterial count of 2266.1 ± 1004.7 cfu/$m^3$, 2228.5 ± 1051.9 cfu/$m^3$, 2055 ± 1031.8 cfu/$m^3$ and 1834.2 ± 848.5 cfu/$m^3$, respectively. There was no significant difference between the cfu/$m^3$ recorded at 0.5, 1, 1.5, and 2 m distance.

### During treatments with essential oil vapors

After introducing EO vapors near the dental units and during the treatments the mean bacterial count (IBA) is 1191.07 ± 719.6 cfu/$m^3$, 1188.5 ± 816.9 cfu/$m^3$, 1139.66 ± 755.6 cfu/$m^3$ and 1176.5 ± 726.8 cfu/$m^3$ of indoor air at 0.5, 1, 1.5, and 2 m distance, respectively.

### Comparision of index of bacterial air without essential oil vapors and with essential oil vapors

The IBA at four distances without and with EO vapors are compared using the MannWhitney test [Table 1]. The IBA has significantly increased from the baseline (before dental treatments) during treatments and significantly decreased (43.625%) after introducing EO vapors at different distances [Table 2 and Graph 1].

**Table 1: Comparision of the bacterial colony-forming unit per cubic meter of indoor air during treatments at four distances without and with essential oil vapours**

| Statistics | Bacterial cfu/$m^3$ of indoor air during treatments at 0.5 m | | Bacterial cfu/$m^3$ of indoor air during treatments at 1 m | | Bacterial cfu/$m^3$ of indoor air during treatments at 1.5 m | | Bacterial cfu/$m^3$ of indoor air during treatments at 2 m | |
|---|---|---|---|---|---|---|---|---|
| | Without essential oil vapours | With essential oil vapours | Without essential oil vapours | With essential oil vapours | Without essential oil vapours | With essential oil vapours | Without essential oil vapours | With essential oil vapours |
| Mean±SD | 2266.1±1004.74 | 1191.07±719.632 | 2228.53±1051.9 | 1188.5±816.939 | 2055±1031.89 | 1139.67±755.673 | 1834.2±848.483 | 1176.53±726.812 |
| Mean difference | 1075.03333 | | 1040.03333 | | 915.33333 | | 657.66667 | |
| Percentage of reduction | 47.44 | | 46.67 | | 44.54 | | 35.85 | |
| *Z* | −4.31 | | −3.999 | | −3.859 | | −3.445 | |
| *P* | 0 | | 0 | | 0 | | 0.001 | |

SD: Standard deviation, cfu/$m^3$: Colony-forming units per cubic meter

## DISCUSSION

A safe working environment is an essential aspect of the delivery of dental health care. Microbes that are airborne and liberated through bioaerosols are an ongoing problem in dental clinics. The control and minimization of microorganisms contained in the aerosol are of great importance to the health of dental personnel. Reports by Gupta *et al*., have associated these aerosols with respiratory infections, ophthalmic and skin infections, tuberculosis and hepatitis which showed that both the professional and the patient are exposed to high amounts of bacteria.[9] To reduce the risk of cross-infection and biological hazards caused due to the bioaerosols chemical fumigation is preferred which is unfortunately proved to be a health hazard.[10]

Hence, an alternate method using EO vapors extracted from medicinal plants can be used near dental units. Not

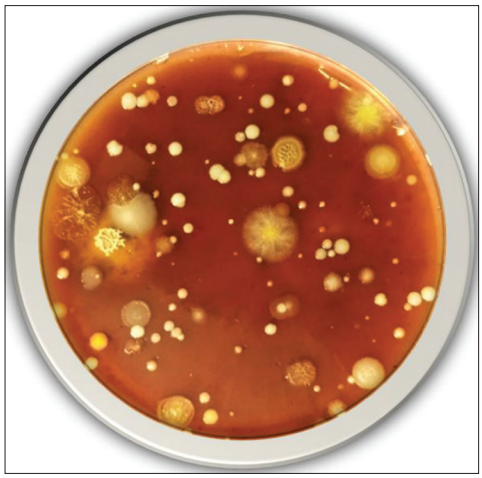


**Figure 1:** Different Microbial colonies that developed after incubation at 37°C for 48 h

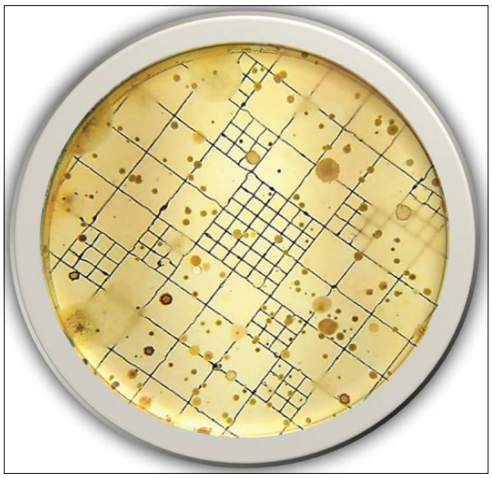


**Figure 2:** Microbial colonies formed on blood agar plate placed near dental before treatments without essential oil vapors

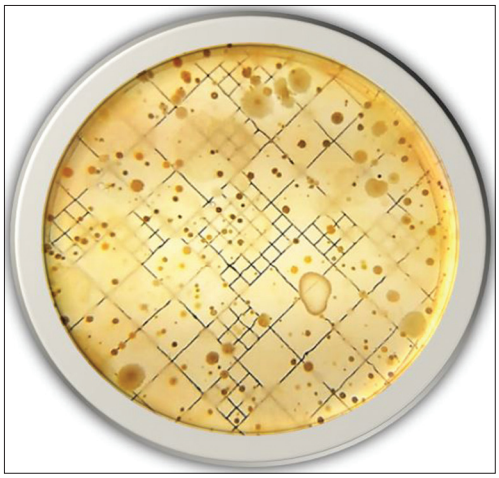


**Figure 3:** Microbial colonies formed on blood agar plate placed near dental units during treatments without essential oil vapors

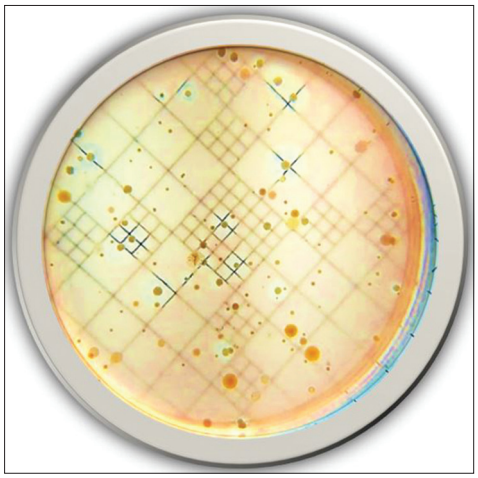


**Figure 4:** Microbial colonies formed on blood agar plate placed near dental units during treatments with essential oil vapors

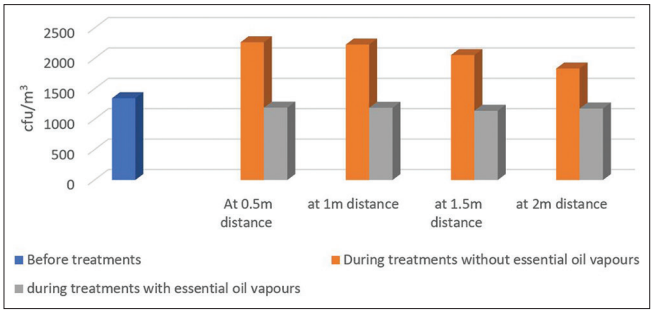
**Table 2: Mean bacterial count at different distances with and without essential oil vapours**

| Distance | Mean | *P* |
|---|---|---|
| Before treatments | 1345.8 | 0.000 |
| During treatments without essential oil vapors at 0.5 m distance | 2266.1 | 0.000 |
| During treatments without essential oil vapors at 1 m distance | 2228.53 | 0.000 |
| During treatments without essential oil vapors at 1.5 m distance | 2055 | 0.000 |
| During treatments without essential oil vapors at 2 m distance | 1834.2 | 0.000 |
| During treatments with essential oil vapors at 0.5 m | 1191.07 | 0.002 |
| During treatments with essential oil vapors at 1 m | 1188.5 | 0.007 |
| During treatments with essential oil vapors at 1.5 m | 1139.67 | 0.002 |
| During treatments with essential oil vapors at 2 m | 1176.53 | 0.005 |

many studies have been done on using plant extracts as alternate fumigation method near the dental units. The antibacterial properties of EOs and their dispersion into air may help to reduce contamination.[11] EO vapors of Neem (*Azadirachta indica*), Clove (*Syzygium aromaticum*), Cinnamon bark (*Cinnamomum verum*), *Eucalyptus* (*Eucalyptus globulus*), Lemon Grass (*Cymbopogon*) and Thyme (*Thymus vulgaris*) are ought to have antibacterial effects on various airborne bacteria.[12-14] Till date studies using all these six EOs in combination against airborne bacteria and bacteria in bioaerosols liberated during various dental procedures have not been done. Hence, the present study was conducted to assess the IBA contamination near dental units and evaluate the effects of EO vapors on the bacterial count in the bioaerosols liberated during dental treatments.

## Index of bacterial air contamination during treatments without essential oil vapours

The bacterial count observed at different distances was 2266.1 ± 1004.7 cfu/$m^3$ at 0.5 m distance, 2228.5 ± 1051.9 cfu/$m^3$ at 1 m distance, 2055 ± 1031.9 cfu/$m^3$ at 1.5 m distance and 1834.2 ± 848.5 cfu/$m^3$ at 2 m distance and the difference between the bacterial counts at different




**Graph 1:** Bacterial counts all through the study

distances was not statistically significant ($P > 0.005$). Similarly, in a study by Rautemaa *et al.*, the mean bacterial density at distances <1 m from the patient was 823 cfu/m$^2$/h. At distances >1.5 m from the patient was 1120 cfu/m$^2$/h, for which the difference was not statistically significant.[6]

In the present study, the mean IBA during dental treatments was 319.98 cfu/plate/2 h, whereas in a study by Zemouri *et al.* 2020 has found a mean IBA of 655 cfu/plate/30 min during dental treatments.[15]

### Index of bacterial air contamination during treatments with essential oil vapours

After diffusing the vapors of the EO blend of Neem (*A. indica*), Clove (*S. aromaticum*), Cinnamon bark (*C. verum*), *Eucalyptus* (*E. globulus*), Lemon Grass (*Cymbopogon*) and Thyme (*Thymus vulgaris*), the mean bacterial counts near the dental units during treatment procedures were found to be 1191.07 ± 719.6 cfu/m$^3$ at 0.5 m distance, 1188.5 ± 816.9 cfu/m$^3$ at 1 m distance, 1139.66 ± 755.6 cfu/m$^3$ at 1.5 m distance and 1176.5 ± 726.8 cfu/m$^3$ at 2 m distance. There was a significant reduction ($P < 0.05$) in the bacterial count at different distances. The reduction is about 47.44% at 0.5 m distance, 46.67% at 1 m distance, 44.54% at 1.5 m distance and 35.85% at 2 m.

Studies have been done on EOs either by using them directly or by vaporizing. If the gaseous form was used, techniques like the Tube dilution technique and Disc and hole-plate diffusion were performed to evaluate the inhibitory effects of EOs in the laboratories. Very few studies have been done on the antibacterial effects of EO vapors on the bacterial count in indoor air. Since very few studies have been conducted on the effects of EOs on bacterial counts in bioaerosols, the present study was compared with the studies by Lanzerstorfer *et al.*[16] and Chao *et al.*[11] in a hospital environment and invitro, respectively. Lanzerstorfer *et al.* stated that dispersion of EOs of Lemon (*Citrus limon*) and Silver fir (*Abies alba*) in a hospital environment had reduced the bacterial count by about 40%.[16] A study by Chao *et al.* stated that diffusion of EO blend of *Cinnamomum* zeylanicum, *Rosmarinus officinalis*, *S. aromaticum*, *E. globulus* labill and *C. limon* into the hood containing different bacterial strains at different times had reduced the bacterial count to about 30% in first 3 min, and 90% after 6 min.[11]

It can be concluded that Vaporization of EO blend of Neem, *Eucalyptus*, Clove, Cinnamon bark, Thyme and Lemongrass near the dental units during treatments at 5 µl/min has significantly ($P < 0.05$) reduced the bacterial count in bioaerosols near the dental units by nearly 43.625% at all distances.

The mechanism of action of EOs was stated by Man *et al.* that the hydrophobicity of the EOs is responsible for the disruption of bacterial structures by the degradation of the cell wall and cytoplasmic membrane of bacteria, cytoplasm coagulation and diffusion through the double lipid layer of the membrane, together with alteration of its permeability.[17] Previous studies have proved that Azadirachtin in Neem, Cinnamaldehyde in Cinnamon bark, neral and geranial in Lemongrass, Carvacrol in Thyme, Cineole and terpineol in *Eucalyptus* and eugenol in Clove are the major constituents that have an antibacterial effect.[12-14] It was stated that the antibacterial activity of EO vapors might also be an attractive alternative disinfection method of the hospital environment due to their ability in preventing biofilm formation.[18] The present study shows that the EO vapors could control the biological hazard by decreasing the bacterial count by about 43% but not wholly eradicating the bacteria. Hence, these EO vapors can be used as an adjuvant to the disinfection measures followed in the dental clinical establishments but not as a disinfection method alone. Since the present study evaluates only the bacterial count irrespective of the type of bacteria, further research on the kind of bacteria and effects of these EO vapors on different types of bacteria should be conducted to conclude more specifically. Apart from EO vapor effects on the bacteria, the antimicrobial effect of EO vapors on microbes like fungi and viruses liberated in the bioaerosols should also be evaluated.

## CONCLUSION

To conclude the EO vapors are said to have an effect on the bacterial count in bioaerosols by reducing the actual count to a certain extent (43%) but not wholly eradicating the bacteria. Hence, apart from chemical fumigation, EO vapour diffusion can be introduced as an adjuvant for disinfection and can be considered as a novel method of fumigation to reduce bacterial count and also to prevent chemical hazards from chemical fumigation. Since the

present study evaluates only the bacterial count irrespective of the type of bacteria, further research on the kind of bacteria and effects of these EO vapors on different types of bacteria should be conducted to conclude more specifically. Apart from EO vapor antibacterial effects on the bacteria, the antimicrobial effect on microbes like fungi and viruses liberated in the bioaerosols should also be evaluated.

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## Conflicts of interest

There are no conflicts of interest.

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