ORIGINAL ARTICLE
J Mycol Infect 2025; 30(2): 58-65
pISSN:3058-423X, eISSN:3058-4302
https://dx.doi.org/10.17966/JMI.2025.30.2.58

JMI

Effects of Inhaling the Cypress Essential Oil Complex on the Nasal Microbiome

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Background: Aromatherapy using natural essential oils has been proposed as a complementary strategy to prevent respiratory infections, particularly in elderly populations vulnerable to microbial colonization and antibiotic resistance.

Objective: To evaluate the effect of inhaling the cypress essential oil complex on the nasal microbiome.

Methods: A clinical study was conducted with 14 participants (mean age: 73 years) who were randomly assigned to either a treatment group ($n = 8$) or placebo group ($n = 6$). The treatment group was exposed to a natural essential oil complex containing cypress, lavender, eucalyptus, pine needle, rosemary, German chamomile, and bergamot oils for 3 hours daily over 4 weeks. The placebo group received a 1/100 dilution of the formulation. Nasal swabs were collected before and after treatment and were analyzed for changes in microbial composition. Statistical significance was assessed using the paired *t*-test.

Results: The treatment group exhibited a significant reduction in the genus *Staphylococcus* (1.92% to 0.62%, $p < 0.05$) and a decrease in *Streptococcus pneumoniae* (1.04% to 0.87%). In contrast, the placebo group experienced minimal or no adverse changes. No adverse effects were reported during the study period.

Conclusion: Inhalation of the cypress essential oil complex was associated with a reduction in nasal colonization by potential respiratory pathogens in older adults. These preliminary findings suggest a possible microbiome-modulating effect that warrants further investigation in larger, outcome-based clinical studies.

Key Words: Aged, Aromatherapy, Microbiota, Respiratory tract infection, *Staphylococcus aureus*

Received: May 1, 2025 **Revised:** June 2, 2025 **Accepted:** June 9, 2025

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INTRODUCTION

Infections of the respiratory tract remain a major cause of morbidity and mortality in the elderly population, primarily due to age-related immunosenescence and a higher prevalence of chronic comorbidities[1]. Among these infections, pneumonia is particularly prevalent and frequently associated with hospitalization and adverse outcomes[2]. The nasal cavity, as a primary entry site for airborne pathogens, plays a pivotal role in the pathogenesis of lower respiratory tract infections[3]. In healthy adults, the microbiota of the nasal cavity is predominantly composed of commensal genera such as *Corynebacterium*, *Staphylococcus*, and *Propionibacterium*, which play key roles in maintaining mucosal homeostasis and suppressing the overgrowth of pathogenic organisms[4]. Colonization of the nasal mucosa by pathogenic bacteria, notably *Staphylococcus aureus* and *Streptococcus pneumoniae*, is a known risk factor for respiratory infections[5,6]. The composition of the nasal microbiome has thus emerged as a relevant factor that influences an individual's susceptibility to infection[7]. Modulating the nasal microbial environment to suppress colonization by respiratory pathogens may represent a viable strategy for infection prevention, particularly in older adults[8].

Amid growing concerns regarding antibiotic resistance and the adverse effects of prolonged antimicrobial use, there is increasing interest in complementary nonpharmaceutical approaches to infection control[9]. Due to its antimicrobial, anti-inflammatory, and mucolytic properties, aromatherapy using natural essential oils has gained attention[10]. Essential oils contain volatile compounds such as monoterpenes and phenolics, many of which have demonstrated broad-spectrum antibacterial activity *in vitro*[10]. Cypress essential oil (*Cupressus sempervirens*), which is traditionally used in Mediterranean medicine, possesses antiseptic and respiratory-soothing effects and has demonstrated antimicrobial activity against respiratory pathogens under experimental conditions[11]. Although clinical data in humans remain limited, its therapeutic potential is supported by pharmacologic evidence and documented synergistic effects with other essential oils such as lavender, eucalyptus, rosemary, and chamomile[12].

This study aimed to evaluate the effects of inhaling a cypress-based essential oil complex inhalation on the nasal microbiome in older adults, with a particular focus on its ability to reduce colonization by pathogenic bacteria such as *S. aureus* and *S. pneumoniae*.

MATERIALS AND METHODS

1. Preparation of the essential oil complex

The test formulation, referred to as the Cypress Essential Oil Complex No. 8, was prepared by blending seven natural essential oils with known antimicrobial properties. The essential oil composition was as follows: cypress essential oil (*C. sempervirens*, 50 g), lavender essential oil (*Lavandula angustifolia*, 20 g), eucalyptus essential oil (*Eucalyptus globulus*, 10 g), pine needle essential oil (*Pinus sylvestris*, 10 g), rosemary essential oil (*Rosmarinus officinalis*, 5 g), German chamomile essential oil (*Matricaria chamomilla*, 3 g), and bergamot essential oil (*Citrus bergamia*, 2 g). All essential oils were purchased from a certified commercial supplier and blended in a sterile facility to ensure consistency and purity.

2. Study design and participants

All participants were community-dwelling older adults with no clinical signs of upper respiratory tract infection at the time of enrollment. The final cohort consisted of 14 participants (mean age: 73.4±10.3 years; range: 55~89 years), including 5 men and 9 women. All participants were in generally stable health. Documented chronic conditions included 2 participants with hypertension, 2 with type 2 diabetes, 1 with hyperlipidemia, and 1 with both hypertension and hyperlipidemia. No participants had clinical evidence of immunosuppression, recent antibiotic use, or active respiratory illness at enrollment. We randomly assigned participants to either a treatment group (n = 8) or a placebo group (n = 6). The treatment group was exposed to the cypress essential oil complex for 3 hours daily over a 4-week period, whereas the placebo group received an aromatherapy product diluted to 1/100th the concentration of the active formulation.

3. Application protocol

Each participant received two identical glass containers filled with either the active formulation or placebo. They were instructed to place the containers in the room where they spent the most time (typically bedroom or living room) and to ensure passive inhalation exposure for at least 3 hours daily for 4 consecutive weeks. To maintain consistent aromatic exposure throughout the study period, the containers were replaced when the remaining liquid level fell below 0.5 cm.

4. Microbial analysis

We collected nasal swabs from all participants before and after the 4-week intervention period. We obtained samples from the anterior nares by gently rotating a sterile swab approximately 1 to 2 cm into each nostril, following standard nasal screening protocols commonly used for microbial carriage studies. We extracted genomic DNA using a QIAamp DNA Stool Mini Kit (QIAGEN), and the V4 region of the 16S rRNA gene was amplified for microbial profiling. Sequencing was performed on the Illumina iSeq 100 platform, and taxonomic classification was conducted using the EZBioCloud pipeline and Illumina BaseSpace. The raw sequencing generated approximately 100,000 reads per sample. After quality

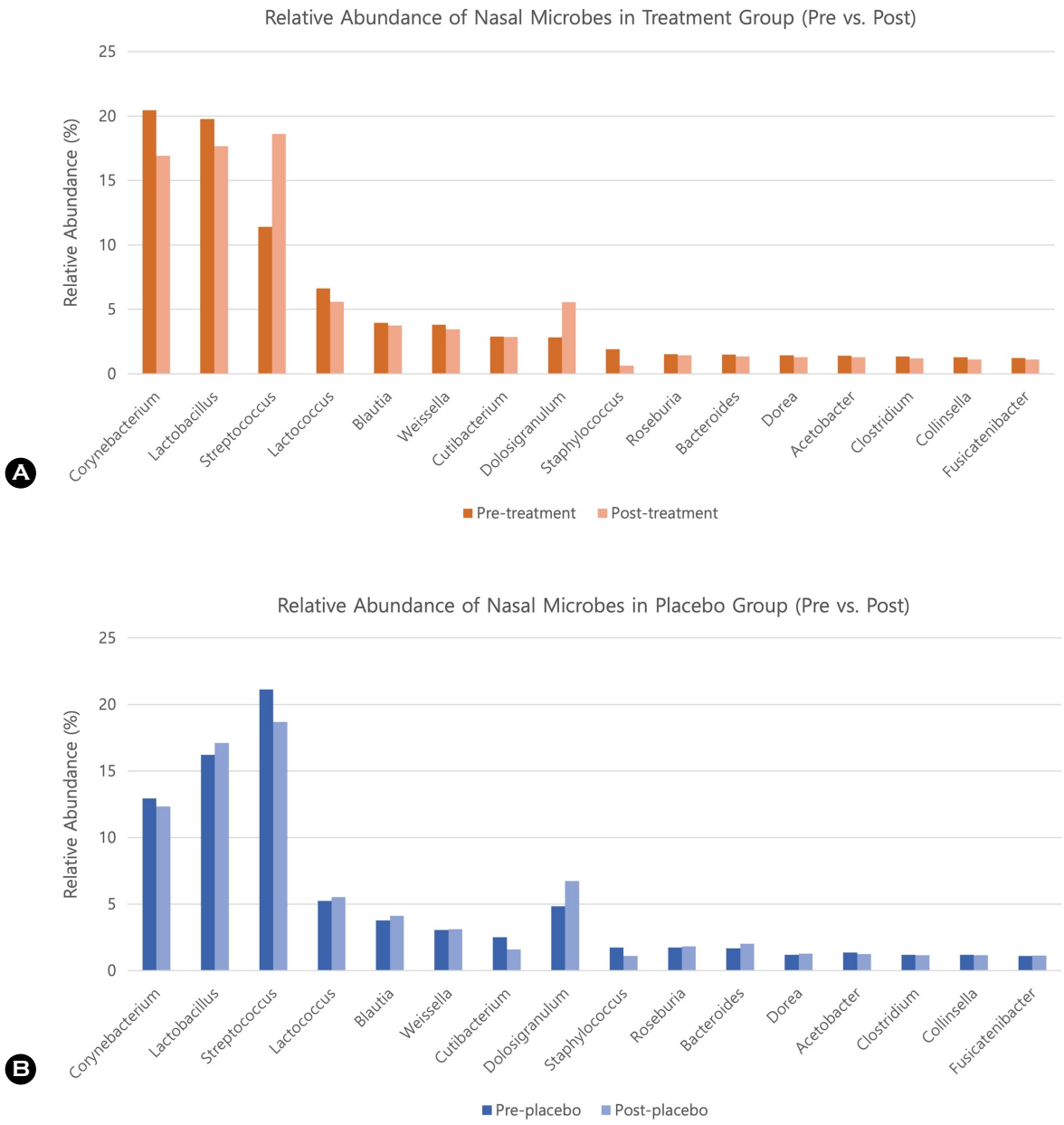

Fig. 1. Relative abundance of nasal microbial genera in the treatment group (A) and placebo group (B), pre and postintervention. Bar charts illustrate the relative abundance (%) of selected bacterial genera before and after a 4-week intervention. In the treatment group (A), *Staphylococcus* showed a marked decrease, whereas other genera such as *Dolosigranulum* showed an increase. The placebo group (B) exhibited minimal or inconsistent changes.

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control and filtering, the average read count for downstream analysis was approximately 42,831 (range: 26,868~55,982). All samples passed the quality thresholds and were included in the final analysis. Although relative abundance at the genus level was reliably assessed, the interpretation at the species level (e.g., *S. aureus*, *S. pneumoniae*) should be made with caution due to the inherent resolution limits of 16S rRNA analysis.

5. Statistical analysis

The primary outcome was the change in the relative abundance of target bacterial genera and species pre and postintervention. Within-group differences were evaluated using paired *t*-tests, and between-group comparisons were conducted using independent sample *t*-tests. We considered a p value of <0.05 to be statistically significant.

6. Ethical considerations

The Institutional Review Board of Kyungpook National University Hospital reviewed and approved the study protocol (IRB No. KNUH 2019-06-007-001). All participants provided written informed consent before enrollment in accordance with the Declaration of Helsinki.

RESULTS

1. Reduction of nasal microbes at the genus level

As shown in Table 1 and Fig. 1, the treatment group exhibited a notable reduction in the relative abundance of potentially pathogenic bacterial genera after 4 weeks of essential oil exposure. The genus *Staphylococcus* decreased from 1.92% at baseline to 0.62% postintervention in the treatment group, whereas the placebo group showed a smaller reduction from 1.74% to 1.11%. For the genus *Clostridium*, the treatment group experienced a slight decrease from 1.33% to 1.19%, whereas the placebo group experienced a minimal change from 1.18% to 1.16%. The bar chart in Fig. 1 visually illustrates these pre and postintervention shifts in microbial composition at the genus level.

Table 1. Reduction of nasal microbes at the genus level

Genus	Treatment group		Placebo group	
	Pre-treatment	Post-treatment	Pre-placebo	Post-placebo
Corynebacterium	20.47	16.91	12.94	12.33
Lactobacillus	19.76	17.68	16.22	17.10
Streptococcus	11.40	18.61	21.13	18.68
Lactococcus	6.64	5.60	5.23	5.54
Blautia	3.96	3.74	3.78	4.11
Weissella	3.81	3.46	3.07	3.11
Cutibacterium	2.90	2.86	2.52	1.58
Dolosigranulum	2.82	5.55	4.83	6.74
Staphylococcus	1.92	0.62	1.74	1.11
Roseburia	1.52	1.44	1.75	1.81
Bacteroides	1.48	1.34	1.67	2.02
Dorea	1.42	1.27	1.20	1.29
Acetobacter	1.39	1.28	1.37	1.24
Clostridium	1.33	1.19	1.18	1.16
Collinsella	1.27	1.11	1.19	1.16
Fusicatenibacter	1.22	1.10	1.10	1.13

2. Reduction in nasal microbes at the species level

As presented in Table 2 and Fig. 2, there was a statistically significant decrease in *S. aureus* in the treatment group ($p < 0.05$), whereas we observed no meaningful reduction in the placebo group. In addition, the relative abundance of *S. pneumoniae* decreased from 1.04% to 0.87% in the treat-

Table 2. Reduction of nasal microbes at the species level

Species	Treatment group		Placebo group	
	Pre-treatment	Post-treatment	Pre-placebo	Post-placebo
Corynebacterium accolens group	16.78	11.57	9.02	6.38
Lactobacillus fermentum group	7.32	6.21	5.56	6.29
Lactococcus lactis group	6.63	5.59	5.23	5.54
Streptococcus danieliae	4.88	12.35	14.30	10.72
Lactobacillus delbrueckii group	4.38	3.61	3.31	3.50
Streptococcus salivarius group	3.92	3.40	3.26	3.66
Weissella confuse group	3.62	3.30	2.93	2.97
Corynebacterium pseudodiphtheriticum group	3.44	5.17	3.73	5.79
Colosigranulum pigrum	2.82	5.55	4.83	6.74
Cutibacterium acnes group	2.70	2.21	2.21	1.47
Lactobacillus sakei group	2.56	2.14	2.15	2.19
Lactobacillus paracasei group	2.44	2.08	2.07	2.09
Staphylococcus aureus group*	1.91	0.62	1.72	1.10
Blautia wexlerae	1.56	1.57	1.60	1.74
Lactobacillus bifermentans	1.40	1.14	1.07	1.22
Roseburia cecicola group	1.27	1.27	1.57	1.61
Collinsella aerofaciens group	1.26	1.09	1.17	1.13
Fusicatenibacter saccharivorans	1.22	1.10	1.10	1.13
Blautia obeum	1.15	1.01	0.95	1.07
Anaerostipes hadrus group	1.14	1.07	1.09	1.07
Romboutsia timonensis group	1.04	0.94	1.00	0.96
Streptococcus pneumoniae group	1.04	0.87	0.94	1.68
KQ235806_s group	0.96	0.85	0.76	0.83
Dorea longicatena	0.88	0.84	0.72	0.80
Acetobacter aceti group	0.88	0.81	0.84	0.74
PAC001136_s	0.85	0.78	0.74	0.70
Streptococcus sanguinis group	0.77	0.64	0.56	0.61
Ruminococcus faecis	0.73	0.71	0.67	0.67

*$p < 0.05$

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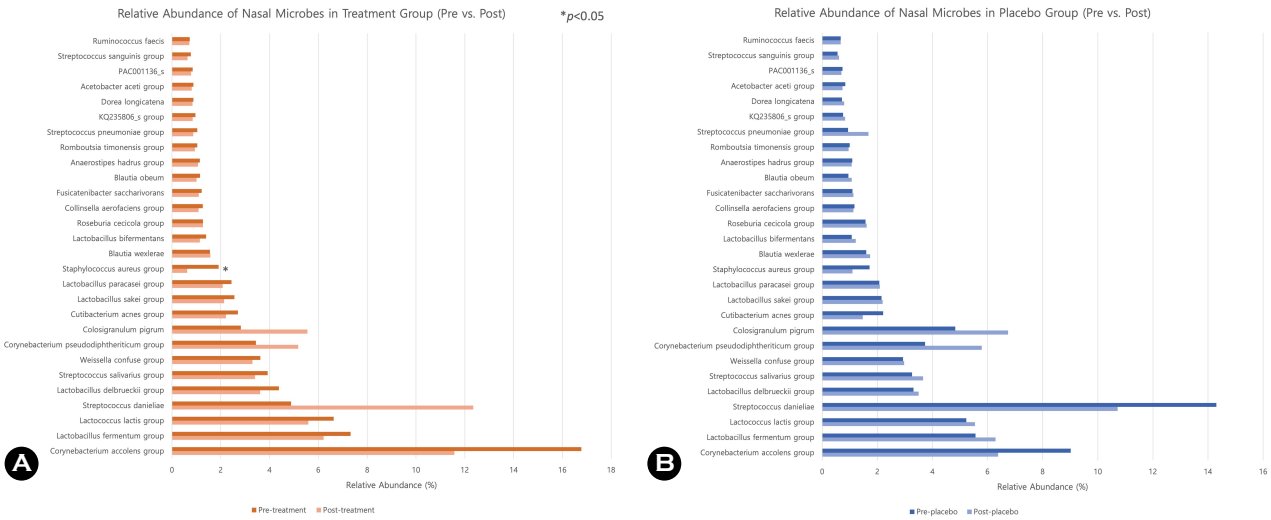

Fig. 2. Relative abundance of nasal microbial species in the treatment group (A) and placebo group (B), pre and postintervention. Each bar represents the relative abundance (%) of individual bacterial species detected in nasal swabs collected before and after the intervention. A significant reduction in *Staphylococcus aureus* was observed in the treatment group ($p < 0.05$), as indicated by an asterisk. Other species such as *Streptococcus danieliae* and *Corynebacterium accolens* also showed notable shifts.

ment group. Conversely, the placebo group exhibited an increase in *S. pneumoniae* from 0.94% to 1.68%. Fig. 2 provides a visual comparison of these changes at the species level, highlighting the differential microbial responses between groups. No adverse events were reported in either group during the study period.

DISCUSSION

In this study, we demonstrated that the inhalation of a cypress-based essential oil complex was associated with a measurable reduction in the relative abundance of nasal pathogens, particularly *S. aureus* and *S. pneumoniae*, among elderly participants. These findings suggest that essential oil-based aromatherapy is a safe and noninvasive approach to modulating the nasal microbiome in populations at high risk of respiratory infections. The antimicrobial effects of essential oils are thought to involve multiple mechanisms, including disruption of microbial cell membranes, increased permeability, and leakage of intracellular contents[13]. Certain components, such as thymol and carvacrol, may also interfere with quorum sensing and inhibit the formation of biofilm[13]. These actions likely contribute to the observed reduction in nasal colonization by *S. aureus* and *S. pneumoniae* following essential oil inhalation.

The antimicrobial activity observed in this study is consistent with previous *in vitro* research highlighting the efficacy of cypress essential oil (*C. sempervirens*) against common respiratory pathogens[14]. The essential oil blend used in the present study contained not only cypress oil but also other botanicals such as lavender, eucalyptus, rosemary, and chamomile, which are known to exert complementary or synergistic antimicrobial effects[12]. These combinations may enhance the permeability of the membrane or the stability of active compounds, potentially contributing to the overall reduction in bacterial load.

Of particular interest is the significant reduction in *S. aureus* incidence in the treatment group. Nasal colonization by *S. aureus* is a well-established risk factor for subsequent respiratory tract infections, and decolonization strategies have been explored primarily in hospital and long-term care settings[15]. The findings from this study support the potential use of aromatherapy as a community-based adjunct for reducing nasal carriage of this organism, particularly in older adults who may be more susceptible to infections and complications.

No adverse effects were reported throughout the intervention, further supporting the safety profile of the essential oil complex when used in a passive inhalation format. The simplicity of administration and low risk of systemic toxicity render aromatherapy an attractive candidate for integration into nonpharmacologic preventive strategies, especially in vulnerable elderly populations.

Nonetheless, we must acknowledge several limitations of

this study. The small sample size reduced the statistical power and limited the generalizability of our findings. The 4-week duration might not have been sufficient to assess the long-term microbiome stability or clinical outcomes. Although we collected demographic and health information, the study design could not fully control for potential confounders such as environmental exposure, hygiene habits, and individual immune status. Because of the lack of individual-level microbial data, between-group statistical comparisons were not feasible; as such, our interpretations are limited to within-group trends. Furthermore, nonparametric methods and multiple comparison adjustments (e.g., false discovery rate correction) were not applied. Because the changes in relative abundance were modest—often less than 1%—caution is warranted when interpreting their clinical relevance. Future studies with larger cohorts, extended follow-up, and improved analytical approaches are needed to confirm these preliminary findings.

In conclusion, this preliminary study suggests that the inhalation of a cypress-based essential oil complex may be associated with a reduction in nasal colonization associated with potentially pathogenic bacteria in elderly individuals. Although the findings highlight a possible microbiome-modulating effect, further large-scale studies are needed to confirm its clinical relevance and potential role in supporting respiratory health.

CONFLICT OF INTEREST

In relation to this article, we declare that there is no conflict of interest.

DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ETHICAL APPROVAL STATEMENT

The study was approved by the Institutional Review Board of (IRB No. KNUH 2019-06-007-001). This study was conducted in accordance with the principles of the Declaration of Helsinki.

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