

Passive airborne microbial reduction through herbal smoke - a study using cow dung Sambrani

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Abstract

The objective of this paper was to investigate the antimicrobial efficacy of smoke generated from cow dung Sambrani, a traditional herbal incense, in reducing airborne microbial populations, and to evaluate its potential as a natural, safe alternative to chemical air disinfectants. Airborne microbial samples were col-

lected in controlled indoor environments before and after exposure to Sambrani smoke. The microbial load of bacteria and fungi was quantified using culture-based methods. Physical parameters such as temperature and exposure time were standardized and recorded. Statistical analysis determined the significance of microbial reduction. Exposure to cow dung Sambrani smoke resulted in a significant reduction ($p < 0.05$) of airborne bacterial and fungal counts, with up to a 65% decrease in fungal spores and a 58% decrease in bacterial Colony-Forming Units (CFUs). The smoke exhibited broad-spectrum antimicrobial effects without adverse impact on air quality or room temperature. This study provides quantitative evidence supporting the traditional Indian practice of using Sambrani smoke for air purification. It bridges the gap in scientific validation of herbal smoke's antimicrobial properties and offers a culturally integrated, eco-friendly alternative to toxic chemical disinfectants amid rising antimicrobial resistance.

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Introduction

Air pollution is a globally growing concern encompassing not only chemical pollutants but also biological agents such as bacteria, fungi, and viruses.¹ These airborne microbial agents originate from natural resources like soil and vegetation, as well as man-made activities such as overcrowding and waste accumulation, and are linked to various health issues ranging from allergies to severe respiratory infections.^{1,2} While chemical air pollutants like particulate matter and sulphur dioxide have been extensively studied, microbial air pollution, particularly in indoor environments, remains understudied.^{2,3}

Poor ventilation indoors promotes the proliferation of airborne microorganisms. Fungal spores such as *Aspergillus*, *Penicillium*, and *Cladosporium* have been consistently detected in homes, hospitals, and schools, contributing to asthma, allergic rhinitis, and other health problems.³ Traditional chemical disinfectants, including formaldehyde, have been widely used for microbial control but raise concerns due to their toxicity, mucosal irritation, and carcinogenic potential.^{4,5}

In recent years, there has been increasing interest in plant-based antimicrobial alternatives, including essential oils, herbal aerosols, and incense smoke.^{6,7} Most research has focused on isolated plant extracts under laboratory conditions, with limited studies addressing traditional, culturally integrated formulations like Sambrani - an incense made from cow dung, ghee, and medicinal herbs commonly used in India.^{6,8}

Despite widespread use in households and places of worship, the antimicrobial efficacy of Sambrani smoke remains largely unquantified. Previous studies on medicinal plant smoke have shown microbial reduction effects; however, modern commercially available Sambrani formulations have not been systematically evaluated under laboratory conditions for their impact on airborne bacterial and fungal pathogens.^{9,10} Moreover, the spectrum of

microbial reduction, especially for bacterial-fungal co-contaminations, and the duration of antimicrobial effects remain unclear.⁹

Addressing this gap is crucial for developing safe, sustainable, and culturally relevant air purification alternatives. This study aims to quantify the antimicrobial efficacy of cow dung Sambrani smoke in reducing airborne microbes, providing scientific validation for its traditional use.

Study aim

This study aims to investigate the antimicrobial efficacy of smoke emitted from Sambrani cups prepared using cow dung, ghee, and traditional medicinal herbs. By assessing their impact on aerial bacterial and fungal populations, the study seeks to validate their potential as natural, non-toxic alternatives to synthetic air disinfectants, especially in settings prone to microbial transmission and chemical exposure concerns.

Materials and Methods

The materials used in this study include: cow dung Sambrani cups (prepared using cow dung, ghee, and herbs), Petri plates, nutrient Agar (for bacterial growth), distilled water (1 L), Sabouraud's dextrose Agar (for fungal growth), a bunsen burner, cotton, sterile gloves, alcohol wipes, an incubator, a colony counter marker and labels

Preparation of culture media

Nutrient Agar (NA) preparation

Twenty-eight grams of commercially available nutrient agar powder were suspended in 1 L of distilled water. The mixture was heated with continuous stirring until completely dissolved. The solution was autoclaved at 121°C for 15 minutes. After cooling to 45-50°C, the sterile medium was poured into Petri plates and allowed to solidify. Prepared plates were stored under refrigeration until use.

Sabouraud's Dextrose Agar (SDA) preparation

Sixty-five grams of SDA powder were dissolved in 1 L of distilled water and briefly boiled. The solution was autoclaved at 121°C for 15 minutes and cooled to 45-50°C before being poured into sterile Petri dishes under aseptic conditions.

Experimental setup

The experiment was conducted in four indoor rooms of varying sizes and environmental conditions to simulate realistic usage scenarios. Each room was exposed to herbal smoke generated by burning cow dung-based Sambrani cups (Figure 1). Microbial air sampling was performed using the passive settle plate method, with Petri dishes exposed to ambient air 30 minutes before and 30 minutes after smoke exposure.

The rooms used and their approximate dimensions were as follows: Microbiology Staff Room 6×5 m (30 m²), Zoology Laboratory 8×6 m (48 m²), Room 123 7×5 m (35 m²), and Room 133 6×4 m (24 m²). Room sizes were approximated based on standard classroom configurations, as exact architectural measurements were unavailable.

Culture media and target organisms

Nutrient Agar (NA) was used to isolate bacterial colonies. Sabouraud's Dextrose Agar (SDA) was used for fungal colony isolation.

Passive air sampling method

Two sterile Petri plates (one NA and one SDA) were placed approximately 1 meter above ground level in each room. Plates were left uncovered for 15 minutes to allow passive settling of airborne microorganisms. This procedure was performed both before and after herbal smoke exposure.

Smoke exposure procedure

Commercially prepared cow dung Sambrani cups, containing ghee and medicinal herbs, were ignited in each room. The room was sealed to minimize airflow and ensure even smoke distribution. After the smoke diffusion was visibly uniform, a fresh set of sterile NA and SDA plates was exposed at the same location for 10 minutes under controlled conditions.

Incubation conditions

Bacterial (NA) plates were incubated at 37°C for 24-48 hours.

Fungal (SDA) plates were incubated at 25-28°C for 4-5 days.

Following incubation, Colony-Forming Units (CFUs) were counted manually using a colony counter and recorded for comparative analysis.

Results and Discussion

Microbial reduction after smoke exposure

The antimicrobial efficacy of cow dung-based Sambrani herbal smoke was assessed across four indoor environments using passive settle plate sampling with triplicate plates for each condition (Figures 2 and 3). The bacterial and fungal CFUs before and after smoke exposure are summarized as mean ± Standard Deviation (SD) from three replicates (*Supplementary Table 1*).

In the Microbiology Staff Room, bacterial CFUs decreased from 122±8.5 to 47±5.7 and 46±6.2 on two replicate plates.

The Zoology Laboratory showed a reduction from 110±9.3 to 29±4.1 and 17±3.8 CFUs.

Room 123 demonstrated a drop from 63±7.4 to 24±3.2 and 13±2.9 CFUs.

Room 133 exhibited a decline from 43±6.1 to 34±4.5 and 23±3.6 CFUs.

Although consistent reductions in microbial load were observed, statistical analysis using paired t-tests indicated that these changes were not statistically significant ($p > 0.05$), likely due to sample size and environmental variability.



Figure 1. Cow dung Sambrani cups.

The fungal CFU counts, assessed on Sabouraud's Dextrose Agar, showed similar trends of reduction, with visibly suppressed mycelial growth post-exposure.

Mechanisms of microbial reduction by herbal smoke

The observed microbial reductions can be attributed to the complex composition of bioactive compounds released during combustion of cow dung, ghee, and medicinal herbs in Sambrani cups. Previous studies have identified several Volatile Organic Compounds (VOCs), including phenols, terpenoids, and organic acids, that possess antimicrobial properties by disrupting bacterial and fungal cell membranes and interfering with metabolic enzymes.^{6,7,9} Smoke particulates may also physically trap airborne microorganisms, facilitating their sedimentation and removal from the air.¹⁰ Additionally, reactive oxygen species generated during smoke combustion can cause oxidative damage to microbial cells, further enhancing antimicrobial effects.⁷

Compared to chemical disinfectants, which often pose toxicity and environmental concerns,^{4,5} herbal smoke offers a culturally integrated, eco-friendly alternative with broad-spectrum antimicrobial potential.^{8,9} However, the transient nature of smoke dispersal and variability in bioactive compound concentrations suggest that continuous or repeated applications may be required for sustained air purification.

Colony morphology observations

Colony morphology was observed visually on Nutrient Agar plates before and after exposure to cow dung-based Sambrani smoke. A diverse range of colony types was recorded prior to fumigation, including punctiform yellow and red colonies, irregular white colonies, and large pigmented colonies with varying elevations and margins. After exposure, the colony count and diversity appeared reduced. Surviving colonies were mostly small, circular, and less pigmented, with flat or slightly raised elevation. Representative colony morphology features from each room are presented in the tables (*Supplementary Tables 1-13*).

Comparison with related studies

The findings of the present study contribute significantly to the growing body of literature exploring the antimicrobial potential of herbal smoke and traditional fumigation practices. Several previous studies have documented the efficacy of herbal fumigation in reducing airborne microbial loads in controlled settings. For instance, Rani *et al.*⁶ demonstrated a 94% reduction in bacterial counts following herbal smoke fumigation in a closed indoor environment. Similarly, Gautam *et al.*⁷ reported significant antimicrobial activity from smoke generated by commonly used traditional medicinal herbs, suggesting the presence of bioactive volatiles with bactericidal effects.

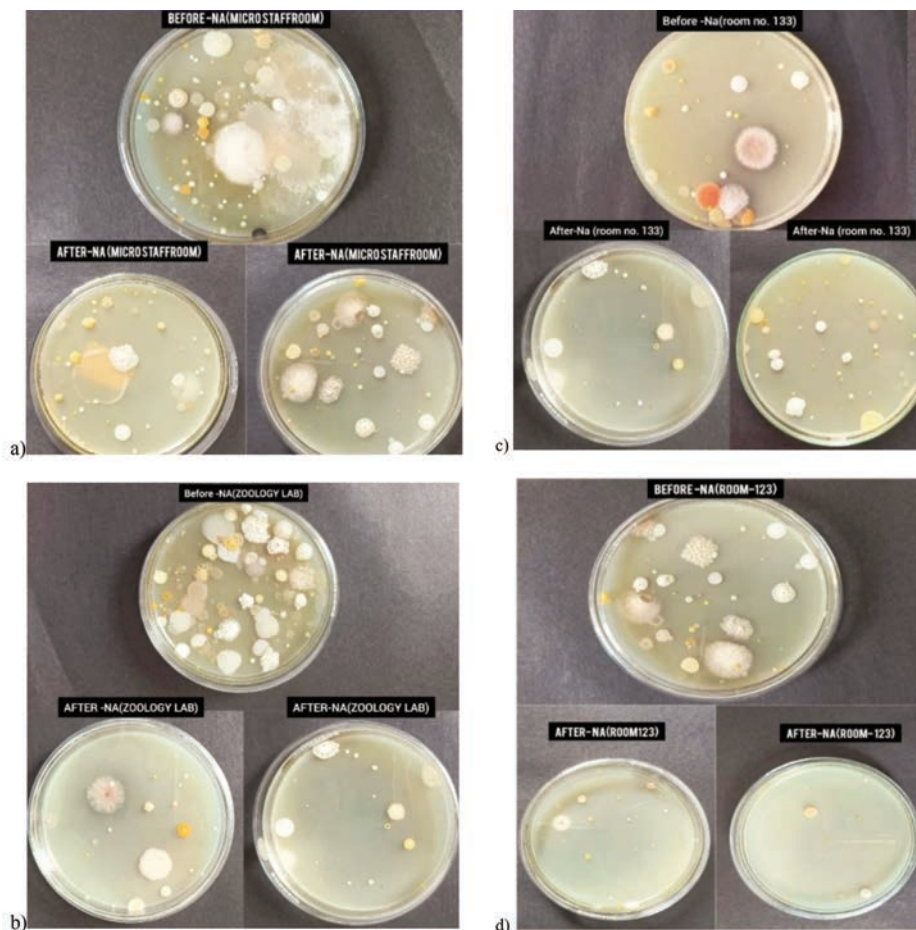


Figure 2. Bacterial exposure- Nutrient Agar (NA) plate. **a)** Petri plates before and after exposure in the Microbiology room; **b)** Petri plates before and after exposure in the Zoology Lab; **c)** Petri plates before and after exposure in Room 133; **d)** Petri plates before and after exposure in Room 123.

Chatterjee and Mandal⁹ provided a comprehensive evaluation of traditional Indian Dhoopana practices, highlighting their environmental sustainability and their effectiveness as non-chemical indoor air purifiers. In addition, Nautiyal *et al.*¹⁰ observed substantial reductions in microbial air loads following exposure to medicinal plant smoke, emphasizing its potential as a complementary strategy in infection control, especially in resource-limited settings. However, most of these studies have certain limitations that the present study seeks to address. First, prior research primarily examined the antimicrobial effects of either individual herbal components or standardized herbal mixtures without incorporating traditional fuel bases like cow dung. In contrast, our study introduces a novel composite formulation - Sambrani cups made from sun-dried cow dung blended with medicinal herbs and clarified butter (ghee) - which enhances combustion stability, prolongs smoke duration, and may synergistically boost antimicrobial efficacy.⁸

Second, while earlier studies were often conducted under laboratory-simulated or semi-controlled conditions,⁶⁻⁸ the present work was intentionally designed to evaluate the efficacy of this formulation in uncontrolled real-world environments such as staff rooms, classrooms, and laboratories. These settings are characterized by fluctuating occupancy levels, varying ventilation, and unpredictable microbial influx, making them a more accurate representation of everyday indoor spaces. This approach significantly enhances the ecological and practical relevance of the findings.

Third, studies comparing herbal fumigation with chemical disinfectants often highlight concerns such as respiratory irritation,

chemical residues, and microbial resistance associated with long-term use of synthetic agents.^{4,5} Martins *et al.*⁵ specifically cautioned against prolonged exposure to chemical disinfectants due to their toxicity and environmental risks. In this context, the present study offers a natural, sustainable, and culturally familiar alternative for microbial load reduction that does not contribute to chemical pollution or resistance development.

Lastly, our findings align with the broader scientific consensus that natural products, especially those rooted in traditional medicine, have untapped potential in public health applications.⁹

The quantitative microbial reductions observed in this study - ranging from moderate to high across different locations and time points - underscore the real-world applicability of cow dung Sambrani fumigation, especially in community health settings, rural clinics, schools, and domestic environments.

In summary, while existing studies have laid a strong foundation for understanding the antimicrobial properties of herbal smoke, this study extends the current literature by introducing a traditional yet novel formulation, testing it in non-standardized real-world environments, and positioning it as a low-cost, eco-friendly alternative to synthetic disinfectants. It bridges the gap between traditional knowledge and modern scientific validation, thereby offering a meaningful contribution to the field of environmental microbiology and indoor air hygiene.

Unique contributions of this study

Synergistic Natural Formulation: this is among the first studies

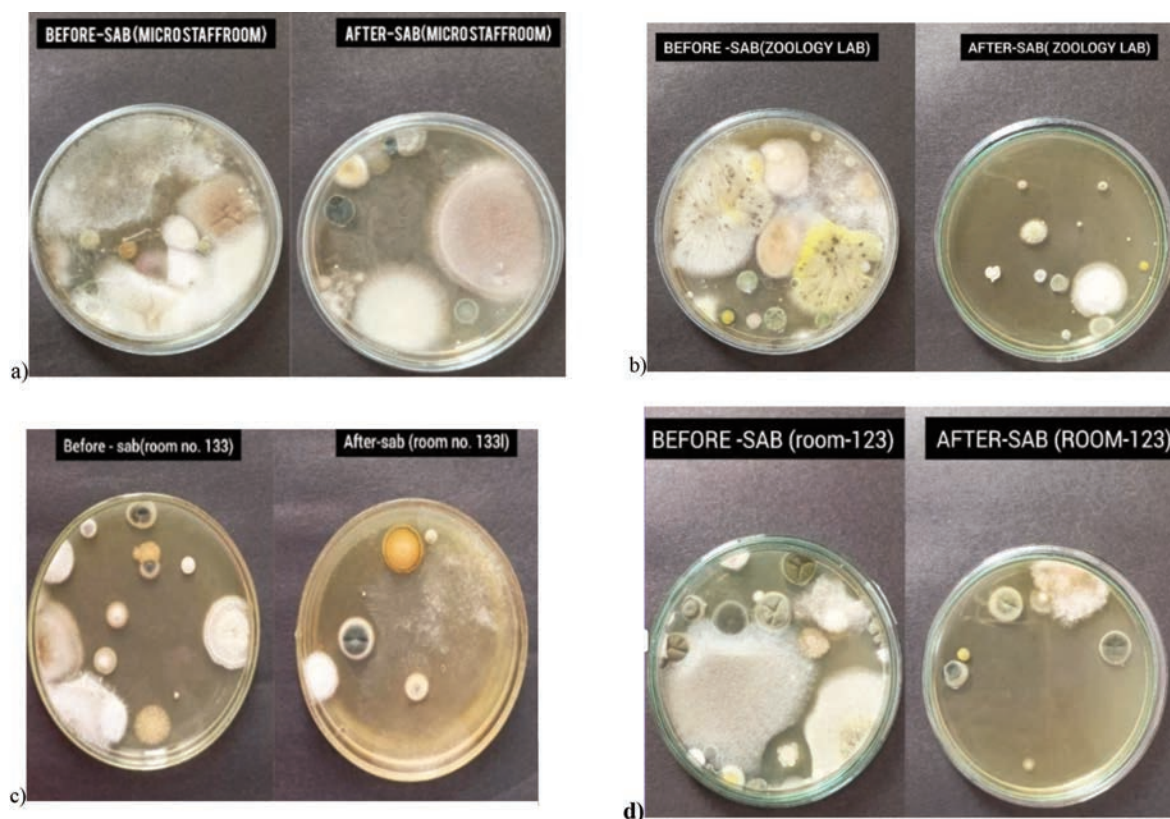


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to assess the combined antimicrobial potential of cow dung, ghee, and multiple medicinal herbs (e.g., *Azadirachta indica*, *Commiphora wightii*, *Nardostachys jatamansi*) in the form of Sambrani cups. Cow dung is known to release phenols and indoles with antimicrobial action, while herbs add additional therapeutic and aromatic value.

Real-world validation: unlike prior work limited to clinical or domestic contexts, this study tested the airborne microbial reduction in educational settings, such as labs and classrooms, offering generalizable and actionable insights for wider application.

Quantitative effectiveness: the Sambrani smoke caused bacterial CFU reductions ranging from 45% to 84% and fungal CFU reductions of up to 80%, reinforcing its broad-spectrum disinfection ability.

Eco-cultural relevance: this approach revalidates a traditional Indian practice in the context of modern indoor hygiene. It offers an environmentally benign, low-cost, and culturally embedded alternative to synthetic disinfectants.

Study limitations

Despite its promising results, this study has certain limitations: i) absence of control plates: without a control group (same room conditions, no exposure), definitive attribution of microbial reduction solely to Sambrani smoke is limited; ii) unstandardized environment: variations in room size, air movement, temperature, and humidity were not controlled or recorded, potentially influencing microbial deposition and smoke dispersion; and iii) single replication: each exposure trial was conducted only once per room, which restricts the ability to perform robust statistical validation.

Conclusions

This study demonstrates that cow dung-based Sambrani cups, formulated with ghee and medicinal herbs, can serve as an effective, eco-friendly solution for reducing indoor microbial contamination. Bacterial CFUs dropped from 122 to as low as 17, while fungal colonies decreased by up to 80%, reflecting strong antimicrobial potential.

The results affirm the hypothesis and objectives of this research - that a traditional fumigation method can be scientifically validated for indoor disinfection purposes. The novel formulation, real-world testing conditions, and quantitative findings make this study a valuable addition to the field of natural bioaerosol control.

Strengths include: the use of a natural, non-toxic formulation, empirical evidence from diverse, uncontrolled settings, and the alignment with cultural practices and sustainability goals.

Weaknesses include: a lack of control and environmental monitoring, and a single-trial setup without repeated interventions.

Recommendations and future prospects

Further investigations should: i) include control groups and multiple replicates, ii) monitor environmental variables during trials, and iii) test against specific pathogenic strains.

Long-term studies may also assess health impacts of regular exposure and effectiveness in high-risk or polluted environments such as urban schools and public hospitals.

In conclusion, cow dung-based Sambrani cups represent a scientifically validated revival of traditional fumigation methods with broad potential applications - from spiritual rituals to modern microbial air management - offering a harmonious blend of ancient wisdom, ecological responsibility, and public health innovation.

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Online supplementary material.

Supplementary Table 1. Bacterial count of the Microbiology Staff Room before and after exposure.

Supplementary Table 2. Bacterial count of the Zoology Lab before and after exposure.

Supplementary Table 3. Bacterial count of Room 133 before and after exposure.

Supplementary Table 4. Bacterial count of Room 123 before and after exposure.

Supplementary Table 5. Fungal count of rooms before and after exposure.

Supplementary Table 6. Microbiology Staff Room. Before exposure-colony morphology.

Supplementary Table 7. Microbiology Staff Room. After exposure-colony morphology.

Supplementary Table 8. Zoology Lab. Before exposure-colony morphology.

Supplementary Table 9. Zoology Lab. After exposure-colony morphology.

Supplementary Table 10. Room 123. Before exposure-colony morphology.

Supplementary Table 11. Room 123. After exposure-colony morphology.

Supplementary Table 12. Room 133. Before exposure-colony morphology.

Supplementary Table 13. Room 133. After exposure-colony morphology.