International Journal of Clinical Trials
Sharma J et al. Int J Clin Trials. 2026 May;13(2):177-187
http://www.ijclinicaltrials.com
pISSN 2349-3240 | eISSN 2349-3259
DOI: https://dx.doi.org/10.18203/2349-3259.ijct20261382

Original Research Article

Scientific validation of Dhoopa formulations through fumigation in a healthcare setup

Jyoti Sharma[1]*, Ajay K. Ahirwar[2], Madhav Varshney[3], Ravi K. Agrawal[4], Vinod K. Sewariya[2], Suman Jain[1]

[1]School of Studies in Pharmaceutical Science, Jiwaji University, Gwalior, Madhya Pradesh, India
[2]S. M. S. Government Model Science College, Gwalior, Madhya Pradesh, India
[3]Defence Research AND Development Establishment, Gwalior, Madhya Pradesh, India
[4]ICAR-Indian Veterinary Research Institute, Izathnagar, Bareilly, Uttar Pradesh, India

Received: 21 February 2026
Revised: 28 March 2026
Accepted: 02 April 2026

***Correspondence:**
Dr. Jyoti Sharma,
E-mail: jyotisharma2706@gmail.com

ABSTRACT

Background: Microbial contamination of the air inside a healthcare facility, especially in the outpatient department (OPD) and the operating theatres (OT), is a contributing factor to hospital-acquired infections. Air disinfection is commonly achieved using chemical fumigants, which are associated with health hazards and limited short-term effectiveness, prompting the need to identify safer alternatives. This research was to determine the antimicrobial and air safety of two herbal Dhoopa formulations (DF-I and DF-II) in a medical facility. Aimed at evaluating their performance in reducing airborne bacterial and fungal loads in OPD and OT areas, and at comparing their performance with that of a chemical fumigant (Envipure).
Methods: Dhoopa formulations were made of herbs, cow ghee, camphor, neem, drumstick, mustard seeds, and guggul resin. Fumigation was done in the OPD and OT rooms. The quantity of the bacterial and fungal loads carried by the air was measured by the passive settle plate technique every hour until four hours after the fumigation was carried out. To determine the safety of the air, respirable particulate matter (PM2.5 and PM10) was measured.
Results: Both the DF-I and DF-II exhibited statistically significant ($p<0.001$) time-dependent decrease in the number of microbes in the air, with the best effect at the fourth hour. Envipure was initially highly efficacious and subsequently declined. The level of particulate matter came back to acceptable levels in four hours of herbal fumigation.
Conclusions: Herbal Dhoopa formulations exhibited long-term antimicrobial activity and acceptable air quality and thus could be used as environmentally friendly alternatives to chemical fumigants in hospitals.

Keywords: Dhoopa formulations, OPD, OT, Antimicrobial activity, Chemical agent, Respirable particulate matter

INTRODUCTION

Healthcare-associated infections (HAIs) remain a major issue of patient safety that has been associated with increased morbidity, mortality, and healthcare expenses worldwide. Common chemical fumigants and disinfectants, though effective, have drawbacks, including toxicity, respiratory irritancy, environmental pollution, and corrosivity to infrastructure.[1] Such concerns have led to a desire for low-risk, natural alternatives in environmental sanitation, particularly in healthcare settings.

In Ayurveda, medicinal fumigation is an ancient form of medicine in which medicinal herbs, plant resin and other natural substances are smouldered to produce fumigating smoke, which is thought to purify air and eliminate pathogens.[2] In Ayurvedic literature, Dhoopa has been used to purify the environment and treat diseases, and it is traditionally administered in both therapeutic and

family contexts. Although this insight is based on traditional knowledge, it remains a concept with limited scientific evidence to support its use in healthcare settings.[3]

Dhoopa is an Ayurvedic traditional practice of medicinal fumigation. During this process, herbs, plant resins, powders, and other natural products are selected and burned to produce smoke believed to purify the atmosphere and eliminate harmful microorganisms.[4] Dhoopa has been used in therapeutic, religious, and domestic settings to promote cleanliness, reduce odours, and maintain a healthy environment. The application of this relies on the concept that smoke from the aroma contains bioactive substances that can create a sterile or purified environment, potentially helping prevent infections.[5]

Dhoopa is a process that requires the selection of herbs and drugs with care, mixing them and burning them under control to release the volatile compounds.[6] A range of compounds can be present in these compounds, such as essential oils, phenols, and other plant secondary metabolites, which are antimicrobial, antifungal and insect-repelling.[7] Dhoopa fumigation can be used to reduce airborne pathogen concentrations, occasionally sanitize surfaces, and enhance indoor air quality through the diffusion of these bioactive compounds.[8]

Existing healthcare organisations may adopt a combination of natural fumigation techniques, such as Dhoopa, to supplement traditional cleaning procedures.[9] In addition to mitigating microbial contamination, Dhoopa can help establish a therapeutic and relaxing environment, thereby making the patient and staff feel more comfortable. Nevertheless, for traditional practices to become widely used in hospitals or clinics, they must be scientifically validated with respect to safety, efficacy, and optimal methods of use.[10]

Dhoopa formulations in Ayurveda are prepared using mixtures of herbs and natural resins with known medicinal properties. The applications of these formulations for environmental cleaning and maintaining asepsis have traditionally been presented, thereby explaining the theoretical basis for their use in contemporary disinfection plans.[11]

One of the most important studies was conducted which evaluated the antimicrobial efficacy of a traditional Ayurvedic smoke preparation, Vishaghn Dhoop, in which medicinal resins and herbs were combined to form a complex. The authors of this study showed that Gram-positive and Gram-negative bacteria, as well as the pathogenic *Candida albicans*, grew more slowly in agar plates and in liquid culture when fumigated with Vishaghn Dhoop. It is interesting to note that environmental microbial loads in unsanitised rooms subject to VD fumes for over 30 minutes had reduced by an order of magnitude, a result that was an initial demonstration that the standard Dhoopa fumigation could be used to reduce airborne microbial contamination in an actual indoor environment without apparent cytotoxicity to human lung epithelial cells.[12]

Consistent with these results, a second clinical assessment developed a new 16-component herbal fumigant, Shodashanga Dhupa, and quantified its effect on microbial air contamination in hospital rooms. The present study, using the passive settled plate method, revealed statistically significant decreases in the numbers of colony-forming units of bacteria and fungi in an outpatient paediatric department and a Panchakarma therapy room following 1 hour of Dhupana fumigation. These findings imply that readily available herbs and spices, rather than exclusively classical foodstuffs, can be successfully used in Dhoopa activities to cleanse the environment in medical facilities.[13]

These studies provide emerging scientific evidence of the antimicrobial effectiveness of herbal Dhoopa fumigation. Nonetheless, standard procedures for establishing formulations, exposure durations, and comparative efficacy against traditional fumigants in clinical practice have yet to be developed. It is evident that Dhoopa should be systematically validated in a healthcare setting to determine whether it is useful as a complementary or alternative method of disinfection.

The current research fills this research gap by scientifically assessing the antimicrobial efficacy of two new formulations of herbal Dhoopa formulations (DF-I and DF-II) in actual healthcare settings. This study differs from earlier conceptual or laboratory-based research, which evaluated the effect of time on microbial decontamination in OPD and OT environments and compared herbal fumigation with a commonly used chemical disinfectant (Envipure). Additionally, air safety was assessed using respirable particulate matter (PM2.5 and PM10) levels to determine potential health risks associated with fumigation.

Aim of the study

The objective of the current research was to scientifically assess the antimicrobial and air safety activity of two herbal Dhoopa formulations (DF-I and DF-II) as antimicrobial fumigants in medical facilities and compare the activity of the same with a widely used chemical fumigant (Envipure) under the conditions of the outpatient department (OPD) and operating theatre (OT) environments.

Objectives of the study

Objectives were: to compare the antimicrobial efficacy of two herbal Dhoopa formulations (DF-I and DF-II) in the reduction of airborne bacterial and fungal loads in the OPD and OT healthcare settings; to determine the efficacy of herbal Dhoopa fumigation, with a chemical fumigant

(Envipure) under the same conditions, in regard to the time effect on the reduction of microbial load; and to determine the air safety status during and after the fumigation process through the level monitoring of respirable particulate matter (PM2.5 and PM10) in treated healthcare settings.

METHODS

Study design and sampling technique

This research was an experimental study that used passive air sampling to measure bacterial and fungal contamination in health care settings. The passive method involves the use of petri dishes to trap the microorganisms that settle through gravity in air expressed in colony-forming units on settle plates using the 1/1/1 scheme (exposure of 1 hour, 1 m above the floor, and 1 m off walls or obstacles) - an environmental monitoring standard of the index of microbial air contamination (IMA).

Chemicals and instruments

Air sampling was conducted in sterile 90 mm petri dishes, parafilm, 70% isopropanol swabs to sterilize and disposable gloves. A gel icebox was used to maintain the samples' temperature during transport.

In case of microbial culture, nutrient agar (NA) was applied in bacteria and Sabouraud dextrose agar (SDA) in fungi. Distilled water was used in the media because autoclave was used to sterilize and it was poured under a laminar flow cabinet to prevent contamination. An electronic analytical balance was used in order to have precise reagent measurements.

An air quality detector (SMILEDRIVE) was used to measure environmental parameters, including temperature, relative humidity, PM2.5, and PM10, at each sampling point.

Study duration and study site

The study was conducted from January 2025 to July 2025. The samples taken were air samples of the Ear, Nose, and Throat (ENT) OPD and OT of Government Civil Hospital, Hazira, Gwalior, Madhya Pradesh, India (26.2331 °N, 78.1777 °E). Laboratory examination was done at the Centre of Ayurvedic Translational Research, Jiwaji University, Gwalior, Madhya Pradesh, India (26.2117 °N, 78.2041 °E).

Plant material collection and authentication

The medicinal garden of Jiwaji University supplied medicinal plant material, which was validated by a taxonomist at CSIR -National Institute of Science Communication and Policy Research (NIScPR). Formulations was screened with materials. Other ingredients, camphor, cow ghee, guggul, and mustard yellow seeds, were bought locally.

Preparation of Dhoopa formulations

The Dhoopa formulations (DF-I and DF-II) were made of the *Moringa oleifera* leaves and bark, *Azadirachta indica* leaves and bark, *Commiphora wightii* (guggul), *Brassica juncea* (mustard seeds), *Cinnamomum camphora* (camphor) and cow's ghee.

Plant parts were washed, dried in the shade, milled to a fairly coarse powder, filtered through a 60-mesh sieve, and combined in equal amounts (Figure 1).

Figure 1: Preparation of Dhoopa formulations (DF-I and DF-II).

Fumigation procedure

The room with a volume of 1000 ft^3 was fumigated with the help of the combustion of 10 g of Dhoopa formulations (DF-I and DF-II) using an electric burner. The room was sealed during fumigation. Prior to fumigation, air samples were taken at 1-hour intervals to a maximum of 4 hours after the fumigation.

Envipure (11% hydrogen peroxide + 0.01% silver nitrate, diluted 1:4 with water) was applied as a chemical

fumigation control using a commercial fumigator with 1 litre of the product. Timelines used in sampling were the same as in the Dhoopa formulations.

Air sample collection

Pre- and post-fumigation samples of microorganisms were done as NA and SDA plates in the middle and corners of the OPD and OT using the 1/1/1 passive settle plate method (1-hour exposure, 1 m height, 1 m in front of walls/ obstacles) as is required by the formula of IMA.

Environmental parameter monitoring

At the sampling points, temperature, relative humidity, PM2.5, and PM10 were measured with the help of the SMILEDRIVE detector. Triplicate measurements were provided to be able to see the reliability of measurements, which serves as a context for interpreting the changes in the microbial loads.

Sample transportation and preservation

Immediately after sampling, air-exposed plates were covered with a parafilm cover to stop an icebox under constant cold conditions by adding gel ice packs so as to avoid premature microbial growth before subjecting them to laboratory analysis.

Microbial culturing and colony enumeration

The bacterial plates (NA) and fungal plates (SDA) were incubated at 37 °C and 25 °C, respectively, and allowed to develop optimally, which is 24-48 hours and 2-5 days, respectively. After incubating, a colony counter was used to count microbial colonies.

Airborne microbial concentration was calculated based on the Omeliansky equation, using the number of microbial colonies.[14]

$$N = \frac{5a \times 10^4}{b.t}$$

Where N=CFU/m^3 or of indoor air, a=number of colonies on a petri dish, b=area of the dish in cm^2, and t=time of exposure in minutes. Passive sampling does not directly quantify CFU concentrations in aerosols; however, such conversion is common in comparative studies to estimate CFU/m^3 on settle plates as an indoor air monitor.

Additionally, the index of microbial air contamination (IMA) was calculated as CFU per plate per hour.

Statistical analysis

Statistical analysis of variances in levels of respirable PM2.5 and PM 10 and percentage of fumigation effect or mean reduction of bacterial and fungal CFU count during different set of experiments with time intervals were done by one-way ANOVA with post hoc analysis by Dunnett's test to compare the mean of all control to the means of all treatments with time intervals at different sites through GraphPad Prism Version 8.0.2 (263) and MS Excel-2019.

RESULTS

Physical parameters

The physical parameters, including temperature and relative humidity, were measured throughout the experiment under OPD and OT conditions. The temperature was between 30.7±0.7°C and 33.0±0.6 °C, whereas the relative humidity was between 40.3±0.9 and 67.3±3.5% in all time periods. These parameters were relatively constant throughout the study, indicating consistent environmental conditions during fumigation and air sampling.

PM2.5 and PM10 of OPD and OT

OPD

Opportunities to measure respirable particles matter (PM2.5 and PM10) in the OPD were taken before fumigation, during fumigation, and at hourly intervals up to four hours after fumigation (Table 1).

The average concentration of PM2.5 was 13.7±0.9 µg/m^3 (DF-I), 11.0±0.6 µg/m^3 (DF-II) and 32.0±1.2 µg/m^3 (Envipure) during the control phase. The fumigation process resulted in a significant concentration of PM2.5 to 315.7±0.9 µg/m^3 (DF-I), 306.0±2.6 µg/m^3 (DF-II), and 524.7±16.2 µg/m^3 (Envipure). After that, the PM2.5 levels decreased steadily between the 1st and 4th hour, 11.0±0.6 µg/m^3 (DF-I), 12.0±0.6 µg/m^3 (DF-II), and 80.7±4.3 µg/m^3 (Envipure).

Likewise, the PM10 control was 15.3±1.2 µg/m^3 (DF-I), 15.7±0.9 µg/m^3 (DF-II) and 40.3±1.2 µg/m^3 (Envipure). The highest PM10 concentrations during fumigation were 360.0+4.4 µg/m^3 (DF-I), 345.7+2.0 µg/m^3 (DF-II), and 630.3+9.2 µg/m^3 (Envipure), then the concentration decreased gradually. All the changes were statistically significant ($p<0.001$).

The level of PM2.5 and PM10, too, also was statistically significantly different after fumigation ($p<0.001$). The concentrations of PM2.5 at the baseline were 28.7±2.4 µg/m^3 (DF-I), 27.0±0.6 µg/m^3 (DF-II), and 40.7±1.8 µg/m^3 (Envipure). The PM2.5 concentrations rose to 190.7±0.9 µg/m^3 (DF-I), 187.0±1.5 µg/m^3 (DF-II), and 611.0±18.8 µg/m^3 (Envipure), and gradually went down to almost balanced concentrations of DF-I and DF-II by the 4th hour during fumigation.

In the case of PM10, the control values were 35.7/1.9 µg/m^3 (DF-I), 33.0/0.6 µg/m^3 (DF-II), and 45.3/0.9 µg/m^3 (Envipure). During fumigation, the highest value was 225.0±0.6 µg/m^3 (DF-I), 211.3±5.2 µg/m^3 (DF-II) and

720.3±15.3 µg/m^3 (Envipure), then steadily decreased with time.

Interpretation

The following table indicates how the levels of respirable particulate matter (PM2.5 and PM10) in the OPD changed during the intervals of fumigation at different times. Fumigants led to a considerable short-term increase in the level of particulate matter ($p<0.001$). DF-I and DF-II, however, showed a rapid reduction of the PM levels to near-controlling levels in four hours, but higher levels of PM levels were recorded in the case of the chemical fumigant implying a longer exposure to particulate matter.

And the dynamics of particulate matter in the OT setting after fumigation. Despite the fact that the levels of PM2.5 and PM10 rose dramatically during the fumigation period of all agents ($p<0.001$), recovery to reasonable air quality levels was more rapid with herbal formulations (DF-I and DF-II). Conversely, the chemical fumigant sustained relatively high levels of particulate matter up to four hours of time.

Fumigation efficacy in OPD

Dhoopa formulation-I (DF-I) and Dhoopa formulation-II (DF-II)

DF-I caused statistically significant reduction ($p<0.001$) in the count of airborne bacteria and fungi in the OPD (Table 2 and Figures 2 and 3). After the 4th hour, the average bacterial counts dropped, the bacteria count of the sample dropped to 175.6±4.5 CFU/m^3 as compared to a control of 5861.0±155.6 CFU/m^3 which equates to a 97.00 percent fumigation effect. The reduction in the number of fungi dropped to 7.8±0.9 CFU/m^3 (62.79) and was 94.93% at the 4th hour.

DF-II also exhibited statistically significant ($p<0.001$) decrease in the number of microbes in the OPD (Table 2 and Figures 2 and 3). The number of bacteria decline was 2776.9 CFU/m^3 to 203.5 CFU/m^3, which is a 92.67% change. The number of fungi dropped to 195.6-4.5 CFU/m^3 to 7.0-1.1 CFU /m^3 with a 96.43% fumigation efficacy at the 4th hour.

Interpretation

This table shows dynamics of particulate matter in the OT setting after fumigation. Despite the fact that the levels of PM2.5 and PM10 rose dramatically during the fumigation period of all agents ($p<0.001$), recovery to reasonable air quality levels was more rapid with herbal formulations (DF-I and DF-II). Conversely, the chemical fumigant sustained relatively high levels of particulate matter up to four hours of time. DF-II had a strong effect of reducing bacteria as well as fungal loads in the OPD environment. Reduction of microbes improved with time with maximum effectiveness in the 4th hour ($p<0.001$). The findings suggest that DF-II has high and long-lasting antimicrobial potential similar to that of DF-I.

Envipure chemical

Envipure chemical showed statistically significant ($p<0.001$) decrease in the counts of microbes in the OPD (Table 3 and Figures 2 and 3). The mean number of bacteria dropped to 1967.2 23.5 CFU/m^3, 579.1 12.4 CFU/m^3 at the 1st and 4th hour, respectively. The trend for the fungal counts was the same as at the 1st hour (63.12%), the efficacy was the highest and then decreased with the change in time.

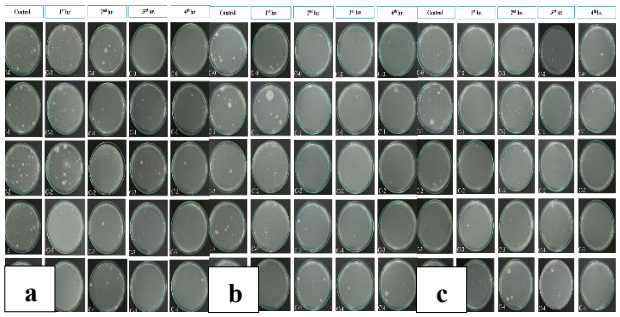

Figure 2: Fumigation effect of (a) DF-I, (b) DF-II, and (c) Envipure chemical with reduction in bacterial colony on nutrient agar (NA) in OPD centre (C-0), corner-1 (C-1), corner-2 (C-2), corner-3 (C-3) and corner-4 (C-4) with time intervals (1 to 4 hours).

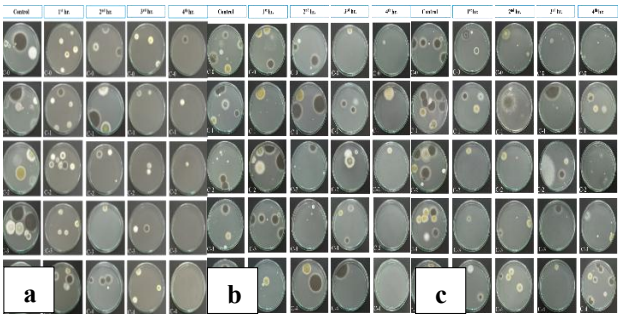

Figure 3: Fumigation effect of (a) DF-I, (b) DF-II, and (c) Envipure chemical with reduction in fungal colony on Sabouraud dextrose agar (SDA) in OPD centre (C-0), corner-1 (C-1), corner-2 (C-2), corner-3 (C-3) and corner-4 (C-4) with time intervals (1 to 4 hours).

Interpretation

As Table 3 shows, Envipure chemical was able to accomplish a quick initial reduction of the microbial counts in the OPD. But the level of microbes rose as time elapsed leading to decreased residual activity.

The antimicrobial effect was less lasting than the effect of herbal formulations, although they were statistically significant ($p<0.001$).

Fumigation efficacy in OT

Dhoopa formulation-I (DF-I) and Dhoopa formulation-II (DF-II)

DF-I led to a statistically significant ($p<0.001$) and time-dependent synthesize airborne microbial load in the OT (Table 4 and Figures 4 and 5). The number of bacteria reduced 1083.0±9.5 CFU/m^3 to 75.1±3.2 CFU/m^3, which is a 93.06% drop. The count of fungus reduced to 174.7 CFU/m^3 to 16.6 CFU/m^3 with 90.50% fumigation effect obtained at the 4th hour. DF-II exhibited statistically significant ($p<0.001$) decrease in the number of microbes in the OT (Table 4). The number of bacteria decreased to 87.3±3.7 CFU/m^3 and the number of fungi was also reduced to 7.9±1.6 CFU/m^3 with the highest decrease in counts at the 4th hour.

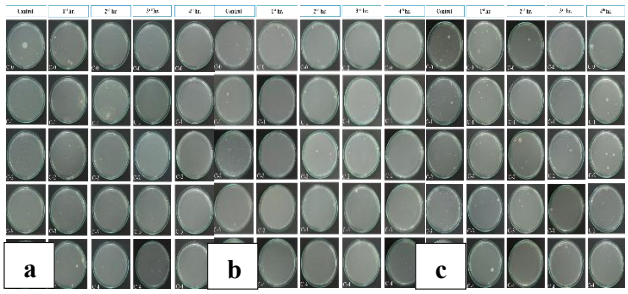

Figure 4: Fumigation effect of (a) DF-I, (b) DF-II, and (c) Envipure chemical with reduction in bacterial colony on nutrient agar (NA) in OT centre (C-0), corner-1 (C-1), corner-2 (C-2), Corner-3 (C-3) and corner-4 (C-4) with time intervals (1 to 4 hours).

Interpretation

As demonstrated in this table, DF-I is indeed effective in the OT, with a significant and steady decrease in bacterial and fungal loads in all periods of time ($p<0.001$). The highest reduction in microbes was at the 4th hour, which proved antimicrobial prolonged effects in the critical clinical setting. DF-II was effective in the OT to reduce airborne microbial contamination. Statistically and progressively significant decreases in both bacterial and fungal loads were noted with the maximum effect at the 4th hour ($p<0.001$), which is a confirmation of long-term antimicrobial efficacy.

Envipure chemical

The bacterial and fungal counts in the OT also decreased statistically significantly ($p<0.001$) in Envipure chemical (Table 5 and Figures 4 and 5). The bacterial counts dropped to 919.8±19.2 CFU/m^3 to 260.3±5.5 CFU/m^3 after the 1st and 4th hours, respectively. The highest decontamination was in the 1st hour (66.33%), and there was less efficacy at the later hours.

Interpretation

As revealed in this table, Envipure chemical was able to offer an immediate microbial reduction in the OT, though, there was an incremental recovery in the bacteria and fungi counts as the time went on. The degrading fumigation effect indicates that the effect of the antimicrobial long-term persistence is low in relation to herbal formulations of Dhoopa.

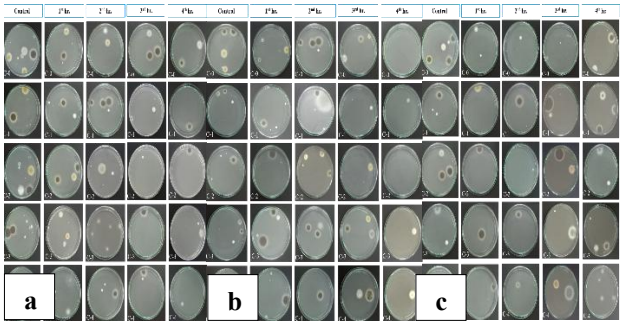

Figure 5: Fumigation effect of (a) DF-I, (b) DF-II, and (c) Envipure chemical with reduction in fungal colony on Sabouraud dextrose agar (SDA) in OT centre (C-0), corner-1 (C-1), corner-2 (C-2), corner-3 (C-3) and corner-4 (C-4) with time intervals (1 to 4 hours).

Table 1: Effect of Dhoopa fumigation on PM 2.5 and PM 10 in OPD and OT.

Time interval	Control	Fumigation	1st hour	2nd hour	3rd hour	4th hour
Effect of Dhoopa fumigation on PM 2.5 and PM 10 in OPD						
Respirable particulate matter PM 2.5 (µg/m^3)						
DF-I PM 2.5	13.7±0.9	315.7±0.9	101.7±1.5	54.3±0.9	23.3±0.9	11.0±0.6
DF-II PM 2.5	11.0±0.6	306.0±2.6	91.0±1.5	43.7±0.9	23.0±0.6	12.0±0.6
Envipure chemical PM 2.5	32.0±1.2	524.7±16.2	120.0±1.2	110.3±1.2	98.3±1.5	80.7±4.3
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Respirable particulate matter PM 10 (µg/m^3)						
DF-I PM 10	15.3±1.2	360.0±4.4	116.0±2.3	58.3±3.5	26.0±1.7	14.3±0.9
DF-II PM 10	15.7±0.9	345.7±2.0	105.7±2.0	48.0±2.3	25.0±1.5	14.0±0.6
Envipure chemical PM 10	40.3±1.2	630.3±9.2	141.3±1.5	126.3±2.3	115.3±1.8	96.3±2.2
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Continued.

Time interval	Control	Fumigation	1st hour	2nd hour	3rd hour	4th hour
Effect of Dhoopa fumigation on PM 2.5 and PM 10 in OT						
Respirable particulates matter PM 2.5 (µg/m³)						
DF-I PM 2.5	28.7±2.4	190.7±0.9	103.7±1.5	91.3±2.2	64.0±2.5	27.0±2.5
DF-II PM 2.5	27.0±0.6	187.0±1.5	98.0±2.6	77.7±2.4	61.7±1.5	26.7±1.5
Envipure chemical PM 2.5	40.7±1.8	611.0±18.8	131.0±1.5	115.3±0.9	105.3±1.2	91.3±0.9
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Respirable particulates matter PM 10 (µg/m³)						
DF-I PM 10	35.7±1.9	225.0±0.6	120.0±1.2	93.3±2.4	73.3±2.4	29.3±2.4
DF-II PM 10	33.0±0.6	211.3±5.2	111.7±3.5	89.7±1.8	71.0±1.7	31.0±1.5
Envipure chemical PM 10	45.3±0.9	720.3±15.3	150.3±0.9	133.0±1.5	123.3±1.8	103.0±1.2
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 2: Reduction of mean viable bacterial and fungal colony count CFU/m³ and fumigation effect of DF-I and DF-II in OPD.

Time interval	Control	1st hour	2nd hour	3rd hour	4th hour
Fumigation agent	**DF-I**				
Viable bacterial colony count CFU/m³ in OPD (mean±SEM)					
Centre (C-0)	5652.0±999.1	2376.0±526.2	1245.0±89.2	432.4±60.0	187.8±44.4
Corner-1 (C-1)	5599.0±954.0	2166±344.5	1214.0±61.6	449.9±66.1	179.1±41.7
Corner-2 (C-2)	6110.0±1429.0	2241.0±401.8	1249.0±97.3	463.0±76.1	161.6±8.7
Corner-3 (C-3)	6350.0±1665.0	2193.0±360.9	1419.0±276.1	454.2±79.4	170.3±27.3
Corner-4 (C-4)	5595.0±906.3	2153.0±295.0	1118.0±45.6	489.2±113.3	179.1±41.7
Mean±SEM	5861.0±155.6	2226.0±40.5	1249.0±48.6	457.7±9.3	175.6±4.5
Percentage of fumigation effect	0	62.02	78.69	92.19	97.00
P value	<0.001	<0.001	<0.001	<0.001	<0.001
Viable fungal colony count CFU/m³ in OPD (mean±SEM)					
Centre (C-0)	148.5±11.6	91.7±7.6	52.4±7.6	43.7±4.4	8.7±4.4
Corner-1 (C-1)	157.2±13.1	109.2±8.7	61.1±8.7	39.3±7.6	4.4±4.4
Corner-2 (C-2)	157.2±7.6	100.4±4.4	48.0±8.7	30.6±4.4	8.7±4.4
Corner-3 (C-3)	148.5±8.7	91.7±7.6	56.8±4.4	30.6±4.4	8.7±4.4
Corner-4 (C-4)	161.6±15.7	96.1±11.6	61.1±4.4	34.9±4.4	8.7±4.4
Mean±SEM	154.6±2.6	97.8±3.3	55.9±2.5	35.8±2.5	7.8±0.9
Percentage of fumigation effect	0	36.73	68.86	76.83	94.93
P value	<0.001	<0.001	<0.001	<0.001	<0.001
Fumigation agent	**DF-II**				
Viable bacterial load CFU/m³ in OPD (mean±SEM)					
Centre (C-0)	2908.8±421.8	2000.0±97.3	869.2±104.3	502.3±76.5	231.5±15.8
Corner-1 (C-1)	2782.1±416.7	1913.0±94.5	794.9±126.9	449.9±136.2	205.3±26.6
Corner-2 (C-2)	2795.2±591.7	1913.0±174.5	816.7±104.9	471.7±107.2	187.8±11.6
Corner-3 (C-3)	2751.6±565.3	1922.0±170.1	742.5±143.0	480.5±163.0	192.2±48.7
Corner-4 (C-4)	2646.8±542.3	1865.0±87.7	711.9±138.7	458.6±160.1	200.9±19.0
Mean±SEM	2776.9±42.0	1923.0±21.8	787.0±27.7	472.6±9.1	203.5±7.6
Percentage of fumigation effect	0	30.75	71.66	82.98	92.67
P value	<0.001	<0.001	<0.001	<0.001	<0.001
Viable fungal load CFU/m³ in OPD (mean±SEM)					
Centre (C-0)	209.6±15.1	148.5±11.6	87.3±11.6	48.0±4.4	8.7±4.4
Corner-1 (C-1)	200.9±11.6	148.5±4.4	83.0±4.4	52.4±00	8.7±4.4
Corner-2 (C-2)	192.1±8.7	126.6±4.4	78.6±7.6	39.3±13.1	4.4±4.4
Corner-3 (C-3)	192.1±11.6	126.6±8.7	78.6±7.6	34.9±4.4	8.7±4.4
Corner-4 (C-4)	183.4±7.6	113.5±4.4	69.9±11.6	30.6±4.4	4.4±4.4
Mean±SEM	195.6±4.5	132.7±6.9	79.5±2.9	41.0±4.0	7.0±1.1

Continued.

Time interval	Control	1st hour	2nd hour	3rd hour	4th hour
Percentage of fumigation effect	0	32.16	59.37	79.02	96.43
P value	<0.001	<0.001	<0.001	<0.001	<0.001

Table 3: Reduction of mean viable bacterial and fungal colony count CFU/m^3 and fumigation effect of Envipure chemical in OPD.

Time interval	Control	1st hour	2nd hour	3rd hour	4th hour
Fumigation agents	Envipure chemical				
Viable bacterial load CFU/m^3 in OPD (Mean ± SEM)					
Centre (C-0)	2004.7±421.2	594.0±319.7	620.2±319.6	690.1±344.5	773.1±335.2
Corner-1 (C-1)	2026.6±421.9	589.6±335.2	646.4±326	716.3±345.4	825.5±327.9
Corner-2 (C-2)	1917.4±422.6	545.9±317.7	611.5±312.4	677.0±326.4	799.3±329.7
Corner-3 (C-3)	1908.6±407.1	554.7±326.0	615.8±317.7	703.2±347.5	812.4±329.7
Corner-4 (C-4)	1978.5±418.5	611.5±337.7	650.8±324.0	733.8±343.7	799.3±348.2
Mean ± SEM	1967.2±23.5	579.1±12.4	628.9±8.2	704.1±9.9	801.9±8.7
Percentage of fumigation effect	0.0	70.56	68.03	64.20	59.23
P value	<0.001	<0.001	<0.001	<0.001	<0.001
Viable fungal load CFU/m^3 in OPD (mean±SEM)					
Centre (C-0)	152.8±23.1	65.5±15.1	69.9±11.6	87.3±8.7	100.4±4.4
Corner-1 (C-1)	157.2±15.1	65.5±13.1	74.2±4.4	87.3±4.4	100.4±4.4
Corner-2 (C-2)	165.9±17.5	56.8±15.7	65.5±7.6	83.0±4.4	100.4±4.4
Corner-3 (C-3)	152.8±11.6	48.0±11.6	65.5±7.6	87.3±4.4	104.8±0.0
Corner-4 (C-4)	152.8±4.4	52.4±7.6	78.6±7.6	83.0±11.6	104.8±7.6
Mean±SEM	156.3±2.5	57.6±3.5	70.7±2.5	85.6±1.1	102.2±1.1
Percentage of fumigation effect	0.0	63.12	54.74	45.25	34.61
P value	<0.001	<0.001	<0.001	<0.001	<0.001

Table 4: Reduction of mean viable bacterial and fungal colony count CFU/m^3 and fumigation effect of DF-I and DF-II in OT.

Time interval	Control	1st hour	2nd hour	3rd hour	4th hour
Fumigation agents	**DF-I**				
Viable bacterial load CFU/m^3 in OT (mean±SEM)					
Centre (C-0)	1113.7±114.2	742.5±70.3	436.8±86.0	222.7±79.0	78.6±20.0
Corner-1 (C-1)	1096.3±113.8	742.5±71.9	449.9±73.5	231.5±68.7	83.0±20.0
Corner-2 (C-2)	1065.7±106.0	716.3±75.8	423.7±77.3	209.6±66.0	65.5±7.6
Corner-3 (C-3)	1074.4±94.5	711.9±60.7	441.1±53.1	257.7±68.7	78.6±7.6
Corner-4 (C-4)	1065.7±107.3	703.2±64.4	419.3±45.4	248.9±59.1	69.9±4.4
Mean±SEM	1083.0±9.5	723.3±8.1	434.2±5.6	234.1±8.7	75.1±3.2
Percentage of fumigation effect	0	33.21	59.91	78.38	93.06
P value	<0.001	<0.001	<0.001	<0.001	<0.001
Viable fungal load CFU/m^3 in OT (mean±SEM)					
Centre (C-0)	187.8±30.6	100.4±11.6	69.9±11.6	52.4±7.6	21.8±4.4
Corner-1 (C-1)	179.0 ±21.8	96.1±4.4	69.9±4.4	43.7±8.7	17.5±4.4
Corner-2 (C-2)	174.7±11.6	83.0±4.4	56.8±8.7	39.3±7.6	13.1±0.0
Corner-3 (C-3)	174.7±17.5	91.7±7.6	56.8±4.4	30.6±4.4	17.5±4.4
Corner-4 (C-4)	157.2±20.0	83.0±4.4	56.8±4.4	34.9±4.4	13.1±0.0
Mean±SEM	174.7±5.0	90.8±3.5	62.0±3.2	40.2±3.8	16.6±1.6
Percentage of fumigation effect	0	48.00	64.49	77.00	90.50
P value	<0.001	<0.001	<0.001	<0.001	<0.001
Fumigation agents	**DF-II**				
Viable bacterial load CFU/m^3 in OT (mean±SEM)					
Centre (C-0)	934.6±127.1	620.2±117.5	283.9±30.6	179.0±34.1	87.3±11.6
Corner-1 (C-1)	904.1±114.2	615.8±96.6	292.7±15.7	196.5±33.0	91.7±13.1

Continued.

Time interval	Control	1st hour	2nd hour	3rd hour	4th hour
Corner-2 (C-2)	952.1±86.0	637.7±83.3	297.0±8.7	187.8±15.7	87.3±4.4
Corner-3 (C-3)	904.1±87.2	598.3±76.5	283.9±11.6	174.7±15.7	74.2±4.4
Corner-4 (C-4)	960.9±97.5	607.1±94.0	283.9±8.7	183.4±27.3	96.1±19.0
Mean±SEM	931.2±11.8	615.8±6.6	288.3±2.8	184.3±3.8	87.3±3.7
Percentage of fumigation effect	0	33.87	69.04	80.21	90.62
P value	<0.001	<0.001	<0.001	<0.001	<0.001
Viable fungal load CFU/m³ in OT (mean±SEM)					
Centre (C-0)	122.3±19.0	82.0±11.6	65.5±7.6	43.7±4.4	8.7±4.4
Corner-1 (C-1)	122.3±17.5	91.7±13.1	65.5±13.1	34.9±4.4	13.1±0.0
Corner-2 (C-2)	113.5±28.6	74.2±15.7	56.7±11.6	34.9±4.4	4.4±4.4
Corner-3 (C-3)	104.8±20.0	69.9±11.6	52.4±7.6	30.6±4.4	4.4±4.4
Corner-4 (C-4)	109.2±17.5	74.2±8.7	61.1±8.7	39.3±7.6	8.7±4.4
Mean±SEM	114.4±3.5	78.6±3.9	60.3±2.5	36.7±2.2	7.9±1.6
Percentage of fumigation effect	0	33.29	47.33	67.94	93.13
P value	<0.001	<0.001	<0.001	<0.001	<0.001

Table 5: Reduction of mean viable bacterial and fungal colony count CFU/m³ and fumigation effect of Envipure chemical in OT.

Time interval	Control	1st hour	2nd hour	3rd hour	4th hour
Fumigation agents	**Envipure chemical**				
Viable bacterial load CFU/m³ in OT (mean±SEM)					
Centre (C-0)	882.2±163.4	249.0±73.0	275.1±79.0	318.8±64.3	353.8±60.5
Corner-1 (C-1)	952.2±137.5	270.8±88.7	279.5±97.3	327.6±85.9	366.9±76.8
Corner-2 (C-2)	864.8±196.7	249.0±87.2	279.5±84.4	327.6±73.0	358.1±66.1
Corner-3 (C-3)	943.4±161.0	275.2±94.5	292.6±98.7	345.0±78.7	384.4±73.5
Corner-4 (C-4)	956.5±104.8	257.7±61.6	279.5±63.5	310.1±61.2	345.1±56.8
Mean±SEM	919.8±19.2	260.3±5.5	281.2±3.0	325.8±5.8	361.7±6.7
Percentage of fumigation effect	0.0	71.70	69.43	64.58	60.68
P value	<0.001	<0.001	<0.001	<0.001	<0.001
Viable fungal load CFU/m³ in OT (mean±SEM)					
Centre (C-0)	91.7±15.1	30.6±4.4	39.3±0.0	56.8±4.4	74.2±4.4
Corner-1 (C-1)	87.3±19.0	26.2±7.6	39.3±7.6	56.8±8.7	69.9±8.7
Corner-2 (C-2)	87.3±23.1	30.6±11.6	34.9±8.7	56.8 ±11.6	65.5±13.1
Corner-3 (C-3)	91.7±20.0	30.6±8.7	34.9±11.6	48.0±11.6	61.1±11.6
Corner-4 (C-4)	96.1±24.3	34.9±4.4	48.0±8.7	56.8±11.6	65.5±13.1
Mean±SEM	90.8±1.6	30.6±1.4	39.3±2.4	55.0±1.8	67.2±2.2
Percentage of fumigation effect	0.0	66.33	56.75	39.40	25.96
P value	<0.001	<0.001	<0.001	<0.001	<0.001

DISCUSSION

The current research determined the antimicrobial activity and air quality of two herbal Dhoopa formulations (DF-I and DF-II) in the hospital indoor settings and compared them with a frequently used chemical fumigant (Envipure). Findings showed that, although all fumigants caused a significant rise in the concentrations of the particulate matter during the application process, the herbal formation generated a faster return to baseline PM2.5 and PM10, together with prolonged microbial decreases, than did the chemical agent. These results are in line with larger evidence regarding the relationship between airborne particulate matter and microbial contamination of healthcare facilities. Meta-analyses have indicated the presence of significant correlations in the findings of particle mass concentration and airborne microbial counts in hospitals, which argues the significance of both the control of particulates and reduction of microbes in the air as an infection control measure.[15]

DF-I and DF-II were found to reduce bacterial and fungal loads in both OPD and OT by >90 percent at the 4th hour of fumigation time, though the action of Envipure was found to decrease with time, with its maximum effect apparent in the first hour and then gradually decreasing. This temporal reduction in chemical fumigant efficacy is in harmony with the prior findings of temporary antimicrobial activity of chemical disinfectants in air especially when it is not reinforced by ventilation measures. HVAC system and HEPA filtration studies

carried in hospital air have demonstrated that mechanical controls are more effective at airborne bacteria and fungi; however, air disinfection technologies frequently have to be used to complement ventilation and not in their place.[16]

The principles of comparative studies of herbal fumigation can substantiate the idea that the use of fumigation materials of plant origin is possible to decrease microbial air loads. Significant decreases in airborne bacterial and fungal counts were reported by Tavhare and Lakhani due to herbal fumigation of several hospital units, but not operation theatres, which could be because of a lower contamination level at the beginning or variability in air flow patterns.[17] Similarly, research assessing Unani herbal medicines found a wide range of in vitro antimicrobial activity and reductions in aerial organisms following herb fumigation. The results are consistent with the current research observation that standard herbal Dhoopa formulations have the potential to produce long-term effects on microbial reduction in the clinical setting.

The findings of PM2.5 and PM10 indicate that herbal fumigation momentarily increases the levels of particulate matter as compared to the chemical fumigants, and the herbal levels of particulate matter were smaller in magnitude and returned to almost normal levels within the fourth hour. The occupational exposure has its consequences as in the studies of the dynamics of particulates and the concentrations of bioaerosols, the long-term high levels of particulates have been pointed to respiratory irritation and higher overall bioaerosol carriers in hospitals. Smaller particulates, as suggested by prior studies, may serve as vectors for microorganisms and affect their survival in indoor air; therefore, both microbial and particulate metrics should be considered when assessing fumigation effectiveness.[18]

Though conventional fumigation has been extensively applied in the Indian healthcare environment, in the past scientific validation was based upon small-scale or *in vitro* research. A study of Ayurvedic fumigation with garlic peel, turmeric, carom seeds, and loban reported a significant reduction in airborne bacterial CFU counts and suggested that several herbal constituents possess antimicrobial activity. The present study further provides quantitative, time-specific amounts in real-world hospital settings, thereby strengthening the evidence base for herbal fumigation as a supplementary air sanitation approach.[19]

Generally, the relative efficacy of DF-I and DF-II, regarding the antimicrobial effectiveness and the recovery of the particles, implies herbal Formulations can provide an alternative to traditional chemical agents in situations whereby extended contact with the particulates and chemical residues are of a concern. Future studies on the long-term occupation outcomes, optimization of formulations ingredients using phytochemical antimicrobial profiles, and compatibility with mechanical ventilation systems to maximize the indoor air hygiene and minimize the potential health hazard should be investigated.

CONCLUSION

The current study proves that herbal Dhoopa formulations (DF-I and DF-II) are effective air-sanitizers in medical facilities, with a longer antimicrobial action and a relatively safer particulate matter content compared to the fumigant considered. Both of these formulations statistically significantly reduced ($p<0.001$) the airborne bacterial and fungal loads in OPD and OT environments, and the maximum efficacy was achieved after the 4th hour of fumigation. Quite on the contrary, fumigant chemical exhibited a prompt slump and overall rapid increase in count of microorganisms, at the start, with slow recovery, suggesting that there was little residual antimicrobial effect.

Despite an overall increase of PM2.5 and PM10 during the application of all the fumigation agents, the concentrations of the particles related to DF-I and DF-II decreased fast and returned to the reasons levels within four hours, with a chemical agent, the concentration of particles remained high. This distinction has an essential occupational health implication because long-term exposure to high concentrations of particulates can have a respiratory hazard to medical workers and patients.

The general implications of the findings are that herbal Dhoopa formulations offer an alternative but effective and less damaging to the environment compared to chemical fumigants to be used in disinfecting indoor air in healthcare facilities. Their capacity to attain sustained microbial control in addition to reducing the long-term exposure to particulate matters emphasize the possibility of their use as a complementary or alternative approach to hospital air sanitation. More research on long-term safety, formulations standardization and compatibility with current ventilation systems is suggested to help facilitate expanded clinical use.

ACKNOWLEDGEMENTS

The authors would like to acknowledge late Prof. G. B. K. S. Prasad, Centre of Ayurvedic Translational Research, Gwalior, Madhya Pradesh, India, for continuous support, motivation, and supervision. They also acknowledge the Director, ICAR-IARI, Dr. A. K. Singh, and Dean and Joint Director (Education), Dr. Anupama Singh, Indian Agricultural Research Institute (IARI) at PUSA, New Delhi, India.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Moura J, Baylina P, Moreira P. Exploring the real costs of healthcare-associated infections: an international review. Int J Healthc Manag. 2018;11(4):333-40.
2. Sabu I, Sathya KR, Pozhamkandathil PK, Panda P, Meda MR. Exploring the multidimensional potentials of Ayurveda fumigation formulations. J Res Tradit Med. 2021;7(1):21.
3. Chouragade B. Dhoopan: therapeutics of herbal fumigation in Ayurvedic texts. Interdiscip J Yagya Res. 2021;4(1):1-8.
4. Bisen N, Inchulkar SR, Kaushik Y, Chauhan NS. Dhoopan: an Ayurvedic fumigation strategy to control and prevent air-borne infections. Maharaja Sayajirao Univ Baroda Sci Technol. 2025;59:23-8.
5. Bhagwat PS, Vijaykumar SJ, Ibrahim SS, Dharashive VM. Preparation and evaluation of herbal dhoopbatti for cleansing the air. Int J Herb Med. 2016;4(6):98-103.
6. Vachaspati A. An in-vitro study to evaluate the anti-bacterial and anti-fungal effect of dhupana yoga in hospital area. Rajiv Gandhi Univ Health Sci. 2024.
7. Marsin AM, Muhamad II, Anis SN, Lazim NA, Ching LW, Dolhaji NH. Essential oils as insect repellent agents in food packaging: a review. Eur Food Res Technol. 2020;246(8):1519-32.
8. Gupta R. A study on the effect of Maheshwara Dhoopa as disinfectant [dissertation]. Rajiv Gandhi Univ Health Sci (India);
9. Joshi P, Jogdand SB, Ingole AC, Laghate JK. Role of dhupa as fumigation therapy in intramural environmental cleansing and sustainable conservation: a case study. Asian J Complement Altern Med. 2015;3(8).
10. Dharini AV. A study to evaluate the antimicrobial effect of Vishaghna Gana Dhupa in hospital area [dissertation]. Rajiv Gandhi Univ Health Sci (India). 2023.
11. Sawarkar P, Sawarkar G. Dhoopana: a concept of fumigation in Ayurveda. J Pharm Res Int. 2021;33(36A):225-48.
12. Balkrishna A, Yagyadev S, Vipradev S, Singh K, Varshney Y, Rastogi S, et al. Vishaghn dhoop, nano-scale particles with detoxifying medicinal fume, exhibits robust anti-microbial activities: implications of disinfection potentials of a traditional ayurvedic air sterilization technique. J Evid Based Integr Med. 2022;27:2515690X211068832.
13. Sourabh DS, Pant C. Effect of dhupana (medicated fumigation) on the microbial concentration in indoor air in Kumaragara (pediatric OPD and IPD): an experimental study. J Indian Syst Med. 2024;12(2):90-8.
14. Viani I, Colucci ME, Pergreffi M, Rossi D, Veronesi L, Bizzarro A, et al. Passive air sampling: the use of the index of microbial air contamination. Acta Biomed. 2020;91(Suppl 3):92.
15. Hiwar W, King MF, Shuweihdi F, Fletcher LA, Dancer SJ, Noakes CJ. What is the relationship between indoor air quality parameters and airborne microorganisms in hospital environments? A systematic review and meta-analysis. Indoor Air. 2021;31(5):1308-22.
16. Dai R, Liu S, Li Q, Wu H, Wu L, Ji C. A systematic review and meta-analysis of indoor bioaerosols in hospitals: the influence of heating, ventilation, and air conditioning. PLoS One. 2021;16(12):e0259996.
17. Tavhare SD, Lakhani K. Validation of hospital environmental disinfection efficiency of herbal fumigants. Int J Ayurvedic Med. 2023;14(2):376-9.
18. Taushiba A, Dwivedi S, Zehra F, Shukla PN, Lawrence AJ. Assessment of indoor air quality and their inter-association in hospitals of northern India: a cross-sectional study. Air Qual Atmos Health. 2023;16(5):1023-36.
19. Bhatwalkar SB, Shukla P, Srivastava RK, Mondal R, Anupam R. Validation of environmental disinfection efficiency of traditional Ayurvedic fumigation practices. J Ayurveda Integr Med. 2019;10(3):203-6.

Cite this article as: Sharma J, Ahirwar AK, Varshney M, Agrawal RK, Sewariya VK, Jain S. Scientific validation of Dhoopa formulations through fumigation in a healthcare setup. Int J Clin Trials 2026;13(2):177-87.