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Short Communication

Validation of environmental disinfection efficiency of traditional Ayurvedic fumigation practices

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ABSTRACT

Environmental disinfection greatly reduces the occurrence of nosocomial or healthcare associated infections (HCAIs) which are the major healthcare problems worldwide. In India, Ayurvedic traditional fumigation with natural plant products is used to disinfect environment. In the present study, environmental disinfection efficiency of traditional fumigation practice has been evaluated by using natural plant products such as garlic (*Allium sativum*) peel, turmeric (*Curcuma longa*) powder, Carom (*Trachyspermum ammi*) seeds (Ajwain) and Loban (resin of *Styrax benzoin* and *Boswellia* species). The efficiency of traditional fumigation using these natural products to disinfect air and surface was evaluated. The effect of traditional fumigation on the microbiological quality of air was revealed by active air sampling. In addition, the ability of the traditional fumigation using garlic peel to disinfect inanimate surface was evaluated using three strains of methicillin resistant *Staphylococcus aureus* (MRSA). Glass slide was artificially contaminated with the bacteria and fumigated whereas non-fumigated slide served as control. The control and fumigated slides were analyzed for surviving bacteria and subjected to scanning electron microscopy (SEM) analysis. Traditional fumigation performed separately with three grams of garlic peel, turmeric, carom seeds and loban powder reduced the average air borne bacterial colony forming units (cfu)/m^3 compared to non-fumigated control. The SEM analysis showed reduced number of bacteria in garlic peel fumigated surface samples. The results of the study strongly suggested that the traditional Ayurvedic fumigation with natural plant products is effective in reducing air-borne bacteria and in disinfecting inanimate surfaces. The traditional fumigation with herbal products has huge potential to address the problem of nosocomial infections.

1. Introduction

Nosocomial infections or health care-associated infections (HCAIs) are currently one among the biggest problems of health care system. HCAIs result in increased antibiotic resistance, financial burden, morbidity and mortality [1]. Acceptable microbiological air and environmental quality in modern hospitals and operation theaters (OTs) is controlled by plenum ventilation system, laminar air-flow system with HEPA filters and positive air pressure [2]. The ventilation systems are designed in such a way that the filtered air (conventional and HEPA filters do not allow particles of >5 μm and >0.3 μm size respectively) enters the OTs through diffusers and exit from the floor vents maintaining positive pressure and reducing the aerial microorganisms in OTs. Different types of ventilation systems are available based on the entry direction and flow of filtered air with their own advantages and disadvantages. The operational and maintenance cost of these ventilation systems is very high [3]. Thus, underdeveloped and developing countries lack such expensive technologies and regular conventional chemical fumigation is used for environmental disinfection of healthcare environment, which is associated with

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toxic side effects [4]. Therefore, there is a necessity to investigate novel and unconventional methods of disinfecting health care environment. Cahill et al. [5] reported a novel method of using cold air plasma for decontamination of inanimate surfaces.

Fumigation using mainly plant based products and to certain extent inorganic and animal origin substances is not only described in Ayurveda but is practiced all over the world across various cultures and civilizations [6]. Fumigation referred as *Dhoopana* in the two fundamental texts of Ayurveda, *Caraka Samhitha* and *Sushrutha Samhitha*, is prescribed for treatment of various ailments and disinfection of environment and inanimate objects [7,8]. The effectiveness of fumes which emanate by burning plants products in rituals (*yagnas* or *havan*-offering of various material including plants products through fire) to reduce airborne bacteria and as a part of prescribe procedure for treatment of bacterial infections has been reported [9–14]. In India, Ayurvedic traditional fumigation practices using Garlic (*Allium sativum*) peel, Turmeric (*Curcuma longa*), Carom (*Trachyspermum ammi*) seeds (Ajwain) and Loban (resin of *Styrax benzoin* and *Boswellia* species) powders have been mentioned for disinfection. Traditionally fumigation of mothers, newborns and their room using garlic peel, turmeric, loban and ajwain is not only done as part of postpartum care to avoid infections but also to help healing. The fumigation of infants and mothers with these plant products is currently practiced widely in many households and communities. However, the effectiveness of fumigation with these plant products for environmental disinfection to enhance human health has not been validated scientifically. Therefore, we validated the ability of traditional Ayurvedic fumigation method using these plant products to improve microbiological quality of air and to disinfect surface revealing its potential as unconventional alternative to challenges of chemical fumigation and would like to share the exciting results.

2. Materials and methods

Two fundamental experiments were designed to evaluate the efficiency of traditional fumigation practices for environmental disinfection: one for improving microbial air quality (measured in colony forming units (cfu)/m^3 by active air sampling [15]) and the second for surface disinfection. The active air sampler compels certain volume of air on to the media plate present in the sampler. The media plate is incubated to obtain the cfu/m^3 of air which is an indicator of the microbial burden of the air. First, we analyzed base line cfu/m^3 readings (control without any fumigation) of a room (18 ft × 28 ft × 10 ft) using an active air sampler (Himedia ® Model: LA637) which is sensitive enough to detect <5 cfu/m^3. The air was sampled for 10 min at a flow rate of 10 L/min at the beginning of the day before any activity using Luria–Bertani (LB) plates and cfu/m^3 were calculated after overnight incubation of the plates at 37 °C. The room was then fumigated by adding 3 g of each plant product i.e. garlic peel, turmeric powder, loban powder, ajwain powder independently and separately to hot embers to produce fumes, as done traditionally. The room was thereafter sealed for 12 h. After the treatment, we assessed microbiological air quality using an active air sampling at the beginning of the following day, also before any activity.

To asses surface disinfection ability of traditional fumigation we used the method mentioned by Cahill et al. [4], in brief 10 μl of 0.5 McFarland overnight cultures of three methicillin resistant *Staphylococcus aureus* strains (MRSA 1–3) were spotted on glass slides and allowed to dry. The slides were kept in a 0.03 m^3 box and fumigated with 1 gm of garlic peel. The box was sealed and incubated overnight and non-fumigated slides served as control. After the treatment, the spot was re-suspended in 100 μl of LB media and plated on LB agar plate and incubated overnight at 37 °C to count cfu which would indicate the number of surviving bacteria. All the experiments were performed thrice independently, and unpaired student's t-test was performed using SigmaPlot, version 10. Further, similar glass slides were subject to SEM analysis after gold sputter coating at an acceleration voltage of 20 kV on Nova NanoSEM 450. The SEM images were analyzed using MATLAB (Math Works) image analysis software to obtain correlation and difference between treated and control samples.

3. Results

Active air sampling which measures the airborne bacterial count in cfu/m^3 of air was utilized to validate the efficiency of herbal fumigation in improving microbiological air quality. The airborne bacterial count without fumigation (control) was 75.66 ± 4.93 cfu/m^3 that got reduced to 21.55 ± 3.34, 30.55 ± 8.07, 30.33 ± 1.2 and 30.66 ± 6.33 cfu/m^3 upon fumigation with garlic peel, turmeric, ajwain seed and loban powder respectively (Table 1a). Fig. 1A is a graphical representation of the data indicating that fumigation with these botanical agents significantly decreased cfu/m^3 of air. These Ayurvedic fumigants significantly improved microbiological air quality which is associated with reduced HCAIs [3]. The observed higher efficiency of garlic peel in improving microbiological air quality compared to other herbal products that were tested may be linked to the sulfur containing chemicals (Allicin and diallyl sulfides) of garlic that have been identified to exhibit antibacterial activity against *S. aureus* and MRSA bacteria [16]. Elemental analysis of garlic peel indicates the presence of sulfur and ethyl acetate extract of garlic peel exhibits antibacterial activity against *Colletotrichum acutatum* [17,18]. MRSA is the most common bacteria found in hospital environment causing HCAIs [5]. Therefore, the better performance of garlic peel to disinfect air was followed up by testing its potential to disinfect surface using MRSA.

In order to investigate the efficiency of garlic peel fumigation on disinfecting inanimate surfaces, MRSA 1, 2 and 3 (different strains) were spotted on a glass slide. The mean cfu value of control slide with no fumigation was more than 300 for all the three strains. It was observed that fumigation of glass sides with garlic peel reduced the mean cfu of MRSA1, 2 and 3 to 12.6 ± 6.43; $P = 1 \times 10^{-7}$), 10.33 (SD, 9.5; $P = 5 \times 10^{-10}$) and 9.66 (SD, 1.53; $P = 7 \times 10^{-7}$) respectively (Table 1b). To further assess the effect of garlic peel fumigation on surface bacteria, the fumigated and control glass slides spotted with MRSA bacteria were subjected to SEM analysis. The results shown in Fig. 1B clearly indicate that after fumigation the numbers of bacteria were reduced and the morphology of the bacteria also changed indicating that garlic peel fumigation is an effective surface disinfectant. Further, the SEM images were analyzed using MATLAB image analysis software to quantify the difference in the surface bacteria upon fumigation with garlic peel. Fig. 1C shows the analysis which indicates that correlation of control sample is higher with taller peaks everywhere indicating relatively uniform bacteria on the slide. Upon fumigation with garlic peel, the correlation value was significantly reduced with reduced peak heights which indicates that the treatment resulted in decreased bacteria on the slide. The color of peaks in control sample is more towards red with higher correlation compared to treated sample where the color of peaks is towards blue with decreased correlation. The mean correlation values of the control sample and treated sample are 0.0794 and 0.0329 respectively which quantitatively indicates that the bacteria was significantly reduced upon garlic peel fumigation. Altogether our study clearly suggests that even a little quantity of a simple and unassuming natural product such as garlic peel has significant surface sterilization potential against MRSA which can survive on surfaces from days to months and tolerate high temperatures.

Table 1
Effect of traditional fumigation on microbial air quality and surface disinfection evaluated by number of colony forming units (cfu).

a) Microbial air quality: Number of cfu/m^3 without (control) vs. with fumigation		b) Surface disinfection: Number of cfu of MRSA bacteria on untreated surface (control) compared to garlic peel fumigated surface		
Treatment	Mean	Bacterial strain	Control Mean	Garlic peel fumigation Mean
Control	75.66 ± 4.93	MRSA 1	>300	12.67 ±6.43
Garlic peel	21.55 ± 3.34	MRSA 2	>300	10.33 ± 9.5
Tumeric powder	30.55 ± 8.07	MRSA 3	>300	9.66 ±1.53
Carom seeds	30.33 ± 1.2			
Loban	30.66 ± 6.33			

Abbreviations: SD, standard deviation.
Mean cfu are calculated from three independent experiments.

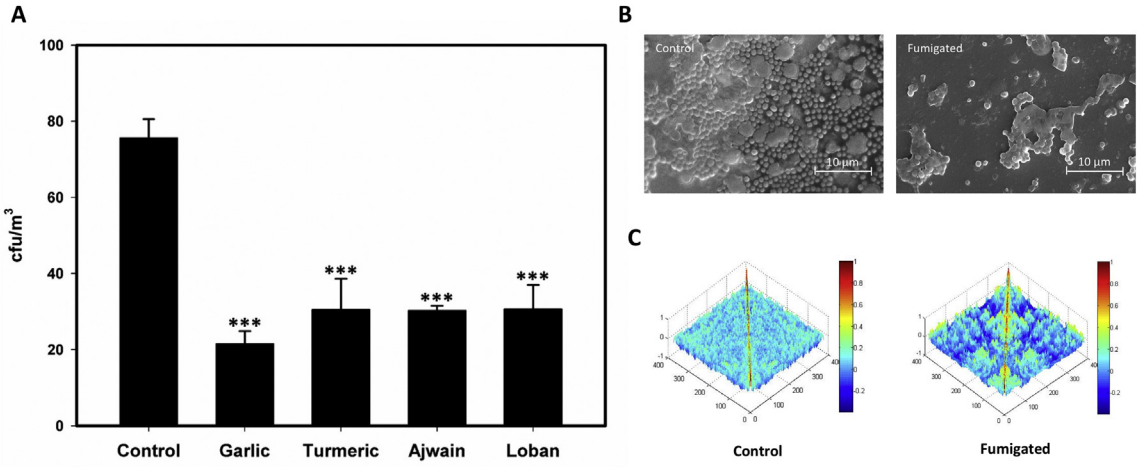

Fig. 1. (A) Evaluation of fumigation with plant products to improve microbial air quality. Graphical representation of the effect of traditional natural plant fumigating agents on microbiological air quality. The graph shows statistically significant (***p ≤ 0.001) improvement of microbiological air quality in terms of cfu/m^3 when fumigated with natural plant products mentioned in the graph compared to control or baseline readings. The data represents the average of experiments performed thrice in triplicates. (B) SEM analysis of surface disinfection of fumigation with garlic peel. The SEM figure shows the effect of garlic peel fumigation on MRSA3 strain compared to control slide at 5000× magnification. (C) MATLAB correlation images of control and fumigated sample's SEM images are shown as labeled. The correlation values are represented through colors. The value of correlation increases from blue to red as shown in color bar scale. In control sample there are peaks almost everywhere while in treated sample the height of peaks reduces significantly and many regions peaks are disappeared completely (blue region) which indicates there is reduction in correlation factor. The mean correlation values are 0.0794 and 0.0329 for control and treated samples respectively. The decrease in mean correlation values confirms the distortion of bacteria in treated sample.

4. Discussion

Fumigation in Ayurveda is described as *Dhoopana*, which has been mentioned in various ancient Ayurvedic texts and practiced traditionally, to disinfection environment with various herbs. In addition, fumigation using various herbal, mineral and animal products has been prescribed for treatment of central nervous system (CNS) disorders and various bacterial infections [19,6]. A meta-analysis reported that traditional Chinese fumigation when combined with western medicine improved symptoms of diabetic peripheral neuropathy [20]. The effectiveness of fumigation in combination with Ayurvedic oral medication has been reported to reduce infection rate after vaginal delivery with episiotomy [21]. Fumigation treatment for vitiligo is very popular in Southeast Asia, but its efficacy is yet to be validated scientifically. Many studies have reported the use of plant essential oils for grain fumigation to control insect pests [22]. Especially the use of allicin (primary sulfur compound present in garlic) has been reported as a potential alternative fumigant to control insect [23] and its application effectively disinfected eggs compared to formaldehyde fumigation [24].

The objective of the present study was to validate the effectiveness of Ayurvedic traditional fumigation methods using garlic peel, turmeric, ajwain seed and loban powder as environmental disinfectants and reveal their potential as an alternative to conventional chemical fumigation. Although, traditional fumigation with these herbal products is widely used in India, to best of our knowledge this is the first study that scientifically validated their disinfection efficiency. Reports published by Nautiyal et al. [11] and Bisht et al. [9] evaluated the efficiency of fumigation with various herbs to reduce airborne bacteria. Nautiyal et al. demonstrated that 500 g *havan samagri* (mixture of more than 50 odiferous and medicinal plants) reduced the airborne bacteria by 94% within 1 h of fumigation. However, in the present study use of only 3 g of four herbal materials resulted in nearly 60–70% reduction in airborne bacteria in more than 300 times bigger space. Bisht et al. reported that among various herbs tested 15 g of *Devadāru* (*Cedrus deodara*) resulted in around 96% reduction in airborne bacteria whereas in the present study only 3 g of garlic peel reduced the airborne bacterial by 71% in a much larger room (eight times). Both the studies have not tested fumigation with garlic, turmeric, ajwain seed and loban powder that are routinely used to fumigate newborns and mothers after massage and bathing to avoid infections and facilitate healing and require validation [25]. The effectiveness of these agents in reducing infections could be due to the antibacterial phytochemicals present in these plants [26–28]. The data of the present study clearly indicated that fumigation with each of these herbal products has significantly improved the

microbiological quality of air by significantly reducing the cfu/m^3 of air. Furthermore, in this study the efficiency of garlic peel fumigation on surface disinfection was established using MRSA which is drug resistant bacteria as well as clinically relevant in terms of nosocomial infections. The SEM analysis suggests that garlic peel fumigation could most likely disrupt the bacterial membranes and cell wall which is supported by the report that garlic changes the composition of bacterial membranes [29]. The findings of this study not only complements and substantiates previous studies regarding the efficiency of herbal fumigation in reducing air borne bacteria but also established the effectiveness of fumigation in significantly reducing surface bacteria using modern tools such as SEM. The present study adds four new herbal products to the list of fumigants that can be used for environmental disinfection. The use of garlic peel which is waste product has been validated as a disinfection when fumigated and can be potentially used in rural settings to improve health and hygiene. The cost of formaldehyde fumigation, which is toxic, ranges from 10 to 25 Indian Rupees (INR)/cubic meter whereas other methods such as hydrogen peroxide fogging or spraying requires equipment which cost between 50 and 100 thousand INR, whereas garlic fumigation could be done almost free of cost. A recent review highlights the potential use of herbal waste (which is usually discarded) generated by the Ayurvedic hospitals in fumigation for disinfection [30]. Although the present study indicates that fumigation with the tested herbs is efficient in disinfection, more research should be carried out to understand the effect of various parameters such as humidity, temperature and exposure time on the disinfection ability. Additional research taking into consideration the outcomes of basic research is required in real hospital settings to adapt these methods as alternative and complementary therapy. If additional studies are successful, garlic, and other botanical agents can potentially replace or provide an alternative to conventional chemical fumigating agents.

5. Conclusion

The study comprehensively conclude that fumigation with garlic peel, turmeric powder, ajwain seed powder and loban significantly reduces air borne bacteria. In addition, garlic peel is effective in disinfecting surfaces of drug resistant MRSA bacteria which is one of the main bacteria responsible for nosocomial infections. The ease of availability and acceptance of these plant products could be utilized as a cheap and effective alternative in treating throat or respiratory tract infections. In addition, they have the potential to be used as alternative to harmful and toxic conventional chemical fumigation for healthcare environmental disinfection.

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