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AN EXPERIMENTAL STUDY TO COMPARE THE ANTIMICROBIAL EFFECT OF FORMALDEHYDE FUMIGATION WITH THAT OF SURAKSHA DHUPA ON AIRBORNE MICROBES IN DISSECTION HALL

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ABSTRACT

Every host does not have an optimum level of immunity to defend himself against the disease-causing pathogens. Hence, the concept of fumigation or sterilization is very much important. Modern methods generally used for sterilization are using chemicals like Formalin solution, Potassium Permanganate, Hydrogen peroxide, silver nitrate, etc. Even though they have an excellent success rate, some side effects of using them include eye irritation, nasal irritation, headache, respiratory damage, unpleasant neurological signs, carcinogenic nature, etc. It is also expensive. This scenario demands an Ayurveda procedure that is cost-effective, safe and convenient to use like Dhupana Karma. Reducing the microbial load to a non-pathogenic level is the purpose of Ayurveda techniques like Dhupana, Homa, Havana, etc. Considering all these, in the present study, an attempt is made to estimate the anti-microbial effect of "Suraksha Dhupa" compared to Formaldehyde fumigation in Dissection Hall. Fumigation is done with Suraksha dhupa and formalin, keeping one week between the procedures. The efficacy is checked by counting the number of CFU, and the results are compared. After fumigation with Suraksha Dhupa and formaldehyde, a decrease in the colony is observed, which is statistically insignificant ($p > 0.05$). However, slightly more reduction in the colony is observed in the formaldehyde group, which is statistically insignificant.

Keywords: Antimicrobial effect, airborne microbes, Formaldehyde, fumigation, Suraksha Dhupa, dhupana, dissection hall.

INTRODUCTION

Dissection hall is where the medical students are exposed to the dissection of cadavers to gather knowledge of Shareera. But still, it has been the prone area for all kinds of airborne microbes entering from the cadavers, the remnants after the dissection, and the air entering from outside to the dissection hall. Pathogenic microorganisms, insects, and pests are major health obstacles in working areas like the hospital and the dissection hall. This place acts as a reservoir for microbes. The diversity of microbial species associated with infections is also growing. Inhalation of airborne microorganisms can lead to adverse health effects, including infection, allergic reaction, inflammation, and respiratory diseases. Inhaled microorganisms are directly deposited on the moist surface of the respiratory tract. Hence, the lungs are more susceptible to infection than the GIT. However, unlike viruses that require a host, bacteria can grow on various surfaces where physical and nutritional factors are suitable. Hence, bacteria can be released into the air from multiple sources like natural or anthropogenic environments. These infectious bacteria are transported via bioaerosols to susceptible hosts when released into the air.

Numerous gram-positive and gram-negative bacteria are commonly found in the air. Gram-positive bacteria like *Staphylococcus sp.* and gram-negative bacteria like *E.coli* are widely disseminated from the skin, oral and nasal surfaces, and the hair of humans and human cadavers[1]. Fungal spores present in the air in various indoor environments cause contamination when they colonize indoor substrates. Whenever fresh air is introduced from outside, it could be the source of fungal spores inside the room. Some of the common fungus found in the dissection hall includes - *Aspergillus niger, Aspergillus japonicus, Cladosporium colocasiae, Hyphodermella corrugate, Penicillium sp., Penicillium oxalicum, Pseudocercospora pallida, Trichoderma atroviride,* etc.[2]

Cleaning staffs of the dissection hall, teachers, medical students, and other healthy volunteers are at risk of developing infections and diseases from this. Therefore, effective fumigation is essential to prevent the infection by airborne microbes more effectively and make the dissection hall a safer place.

Because of its cost-effective procedure, formaldehyde is the 'drug of choice' for embalming cadavers, fumigation of OT, and dissection hall. But one cannot deny that chemicals like formaldehyde, which kills microorganisms or insects, are toxic to human beings. It can cause harmful effects ranging from burning of the eyes to carcinomas.[3]

The fumigation carried out using fine powder of selected drugs with the intention of nirjantukarana (disinfection) is called

Dhupana.[4] Dhupana is an inseparable part of Ayurveda when discussing man's internal and external purification and surroundings. This method uses animal, herbal and herbo-mineral drugs for fumigation to heal various diseases like vrana, arsas, yoni vyapats, or disinfect vranagara, kumaragara, sutikagara, bheshajagara, shastrakarmagara, etc. Since the Vedic period, Homa-Havana and Yagnya, sterilization of air by Agnihotra, sterilization of house and places around it by Dhupana, is going on traditionally[5]. Based on the opinion of Kasyapa, other paediatricians have classified dhupana into two based on its origin, i.e., jangama (animal origin) and sthavara (plant origin).

Since ancient times, naturally growing plants have played an essential role in discovering new therapeutic agents. Almost all antibiotics are subjected to the problem of bacterial resistance. Therefore, more unique herbal antibacterial compounds from plants and their semi-synthetic derivatives to overcome the resistance are under invstigation[6]. In Ayurveda, many drugs have an antimicrobial or antibacterial effect. In this concern, effective herbal fumigation (Suraksha Dhupa) containing drugs – Nimba (*Azadirachta indica)*, Nirgundi (*Vitex negundo)*, Vasa (*Adhatoda vasica)*, Tulsi (*Ocimum sanctum),* Haridra (*Curcuma longa)* and Shallaki niryasa (*Boswellia serrata)* possessing antimicrobial and germicidal action is selected to see the effectiveness against airborne microorganisms. Acharyas have explained these herbs as Krumigna individually.[7] Now, as a combination of dhupa or fumigation, how effective the above drugs prove as Krumigna compared to formaldehyde vapour is carried out in this work. This combination or formulation mainly consists of various drugs that show a synergistic effect and helps in propagating the activity of the main antimicrobial drug. Most of these drugs possess volatile components, which help lower the microbial contamination in the air and on difficult to reach surfaces.

Hence, keeping all these in mind, this study is done to see the efficacy of Suraksha dhupa in airborne microbes compared to formalin.

MATERIALS AND METHODS

The study was done to find out different types of microorganisms present in the dissections hall and evaluate the efficacy of Suraksha dhupa as a fumigating agent compared to formaldehyde vapour.

The Suraksha Dhupa vatakas/cakrikas were prepared, and the efficacy was checked by fumigating them in the Dissection Hall of the Department of Rachana Shareera (Department of Anatomy), SKAMCH & RC, Bangalore, Karnataka, India. The efficacy was compared with Formaldehyde vapour.

The identified raw drugs, i.e., Nimba patra, Nirgundi patra, Vasa patra, Tulsi patra, Haridra churna and Shallaki niryasa, required for the study were purchased from the approved vendors of Sri Kalabyraveshwara Swamy Ayurvedic Medical College Hospital and Research centre. Raw drugs were authenticated by the experts in the department of Dravya Guna, SKAMCH & RC, Bangalore. Other required materials were a grinder, cloth for drying, burning charcoal, mud plate, and ghee. Formalin was bought from the approved vendor of SKAMCH & RC, Bangalore, Karnataka, India.

Only the airborne microbes from the three most contaminated places in the dissection hall were included in the study. Microbes are present elsewhere, and the less contaminated areas are excluded from the study.

The ingredients used for making Suraksha Dhupa and their quantities are mentioned in Table 1.

Table 1: Ingredients for Suraksha Dhupa

Ingredients	Quantity
Nimba patra	1 part
Nirgundi patra	1 part
Vasa patra	1 part
Tulsi patra	1 part
Haridra churna	½ part
Shallaki niryasa	½ part
Tulasi swarasa	Q.S.

The above-mentioned wet drugs were washed in clean water under aseptic conditions. The dry drugs (Haridra churna and Shallaki niryasa) were cleaned for physical impurities. Then each of the wet drugs was weighed and crushed separately. These wet drugs were mixed with the appropriate quantity for dry medicines. It was made into a fine paste with the help of a sufficient quantity of Tulsi swarasa. Then, it was made into cakrikas (herbal cookies) or vatakas of equal size weighing approximately 5 gram each. These were dried in the sunlight for five days. About 60 cakrikas were prepared, which weighed around 300 grams in total. The cakrikas prepared were black after drying under sunlight and had the odour of the used drugs. These were stored in an airtight container, and the container was kept in a dry, moisture-free place.

The study was done in 2 groups. The study areas were – I) Near the entrance, II) Near the wet specimen Rack, and III) Near the dead body preserving tank in the dissection hall of Sri Kalabyraveshwara Ayurvedic Medical College, Hospital and Research Centre, Bangalore. The collected air samples with the petri dishes before and after dhupana with Suraksha Dhupa and formaldehyde vapour were sent for cultural analysis at Azyme Biosciences Pvt Ltd. lab, Bangalore, to see if Suraksha Dhupa is as effective as formalin.

Group A: 3 Luria Bertani (LB) agar petri plates prepared by Azyme Bioscience Lab under aseptic conditions were exposed to the air for 24 hours at the three mentioned study areas to collect the 'before fumigation' samples. Then, dhupana was done with 60 dried cakrikas (20 cakrikas were burnt in each study area) weighing 5-10 grams each and 300 grams in total by using charcoal and a mud plate for burning with the help of ghee. After about 4 hours, when the smoke disappeared completely, another three petri dishes were exposed for 24 hours to take the 'after fumigation' air samples from the same areas.

An interval of 1 week was given between the fumigation with Suraksha Dhupa and formaldehyde.

Group B: After one week, 3 LB agar petri dishes were exposed to the air for 24 hours at the 3 study areas to collect the 'before fumigation' samples. Later, 2 litre of formalin was diluted with 1L of water and fumigation was done with the fumigation machine. After 3-4 hours, when the dissection hall was ready to be used, another three petri plates were exposed to air to collect the 'after fumigation' samples.

The 12 petri dishes with nutrient agar media were prepared by Azyme Biosciences Pvt. Ltd. Lab. All the air exposure samples were sent to the laboratory as soon as they were collected to assess Bacterial colonies. The dishes were incubated for 24 hours, and the colonial growth was studied.

Luria Bertani (LB) agar media (Tryptone 10g, sodium chloride 10g, yeast extract 6g, agar 15g, distil water 1000mL) was prepared and autoclaved at 121 °C for 15 minutes. 20mL of the media was poured into the sterilized petri plate and allowed for solidification. The LB agar plates were Air Exposed in the testing yard, respectively. The plates were incubated at 37 °C for 24 hours.

RESULT

After 24 hours, the plates were observed, and the colony-forming units (CFU) are listed in Table 2.

After fumigation with Suraksha Dhupa, a decrease in Colony was observed, which is statistically insignificant (p=0.05) (Table 3).

After fumigation with Formaldehyde, a decrease in Colony was observed, which is statistically insignificant (p=0.05) (Table 4).

Insignificant results between the groups, i.e., were observed; however, reduction in the colony is slightly better in Formaldehyde than in Suraksha Dhupa. (Table 5)

DISCUSSION

Formaldehyde is widely used for disinfection of the dissection hall, but it also has numerous side effects, from respiratory diseases to carcinoma. Hence, this study was done to prepare a formulation in the form of dhupa, containing the Krumigna property of drugs and checking its efficacy towards airborne microbes. The drugs were in a fresh, healthy state and unaffected by insects. They predominantly contain katu, tikta, kashaya rasa, laghu and ruksha guna. These qualities of the drugs help in spreading rapidly and in quick combustion.

The description of the properties of these drugs are given in Samhitas. According to that, katu rasa is mentioned to have krumigna property[8]; tikta rasa has vishagna and krumigna property[9] and kashaya rasa has ropana effect[10]. Most of the drugs that are chosen, have volatile components. The drugs' volatile nature helps lower the air's microbial contamination and the surfaces that are difficult to reach manually.

Nimba leaves have antibacterial properties and could be used for controlling airborne bacterial contamination on the residential premises[11]. One of the most promising natural compounds is Azadirachtin, an active compound extracted from the *Azadirachta indica* (Neem tree) whose antiviral, antifungal, antibacterial and insecticidal properties have been known for several years. It also contains phenols, unsaturated sterols, triterpenes and saponin, phenolic diterpenoids, c-seco meliacins, c-seco limonoids, polysaccharides, etc.

Various pharmacological studies and clinical practices have demonstrated that Tulsi possesses anti-oxidative and antimicrobial functions, including antibacterial, antifungal, antimalarial and anti-helminthic. Some of the phytochemical constituents of Tulsi are ursolic acid flavonoids such as apigenin, polyphenols, anthocyanins and luteolin, eugenol, thymol, or sesquiterpene alcohols proved to be effective against a wide variety of gram-positive and gram-negative bacteria, fungi and viruses. Aqueous and ethanolic extracts have immunomodulatory properties, and leaves of the plant, when taken, can induce cytokine secretions.[12]

Table 2: Colony-forming units (CFU)

Air Exposure Samples		Colony-Forming Units	
		Before Fumigation	After Fumigation
Group A	I	1.24×10^3	0.218×10^3
	II	0.25×10^3	0.029×10^3
	III	0.33×10^3	0.032×10^3
Group B	I	2.06×10^3	0.097×10^3
	II	1.024×10^3	0.107×10^3
	III	0.178×10^3	0.019×10^3

I – Near the Entrance; II – Near Wet Specimen Rack; III – Near Dead Body Preserving Tank

Table 3: Changes observed in the Colony treated with Suraksha Dhupa

Analysis	BT	AT	Mean	SD	SEM	t	df	p
I	1.24×10^3	0.218×10^3	513.67	441.91	255.137	2.013	2	>0.05
II	0.25×10^3	0.029×10^3						
III	0.33×10^3	0.032×10^3						

BT: Before Treatment, AT: After Treatment

Table 4: Changes observed in the Colony treated with formaldehyde

Analysis	BT	AT	Mean	SD	SEM	t	df	p
I	2.06×10^3	0.097×10^3	1013	905.823	522.977	1.937	2	>0.05
II	1.024×10^3	0.107×10^3						
III	0.178×10^3	0.019×10^3						

BT: Before Treatment, AT: After Treatment

Table 5: Comparison between the Colony treated with Suraksha Dhupa and formaldehyde

Analysis	$AT_{(A)}$	$AT_{(B)}$	Mean (A)	Mean (B)	S.D.	S.E.	t	df	p
I	0.218×10^3	0.097×10^3	93	74.33	83.79	48.376	0.273	4	>0.05
II	0.029×10^3	0.107×10^3							
III	0.032×10^3	0.019×10^3							

Figure 1: Near the entrance

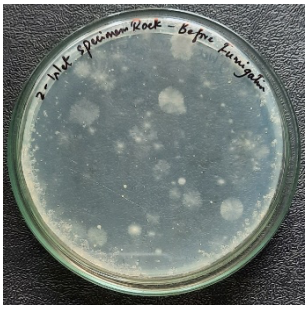

Figure 2: Near wet specimen rack

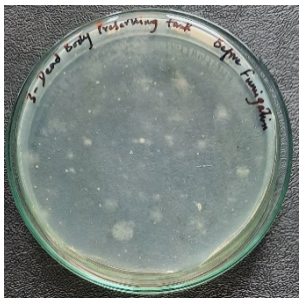

Figure 3: Near Dead Body Preserving Tank

Group A: Before Fumigation with Suraksha Dhupa

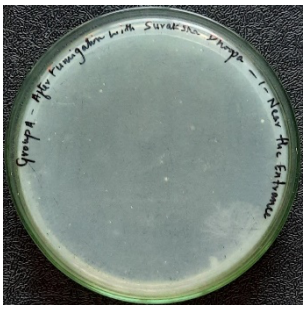

Figure 4: Near the entrance

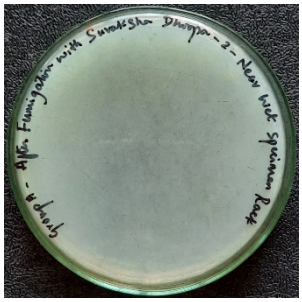

Figure 5: Near wet specimen rack

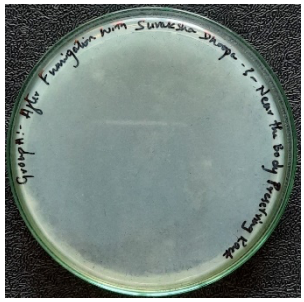

Figure 6: Near Dead Body Preserving Tank

Group A: After Fumigation with Suraksha Dhupa

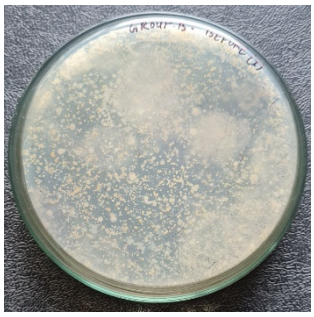

Figure 7: Near the entrance

Figure 8: Near wet specimen rack

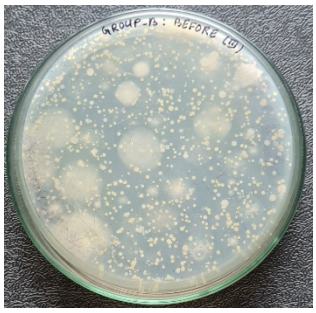

Figure 9: Near Dead Body Preserving Tank

Group B: Before Fumigation with formaldehyde

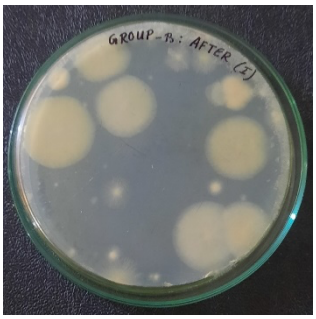

Figure 10: Near the entrance

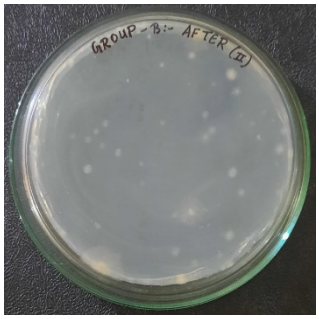

Figure 11: Near wet specimen rack

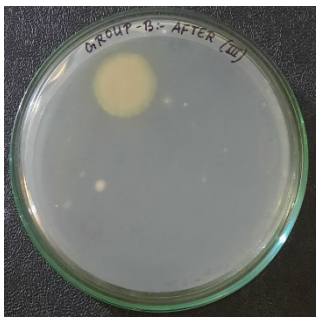

Figure 12: Near Dead Body Preserving Tank

Group B: After Fumigation with formaldehyde

Adhatoda vasica leaves extract would-be replacement for synthetic/artificial antimicrobial agents and need further studies and clinical trials. The phytochemical screening indicated the presence of secondary metabolites such as alkaloids, flavonoids, tannins and phenol in the extracts, making them have antibacterial potentials[13].

The fresh and aqueous extracts of *Vitex negundo* leaves in various dilutions were found to have antibacterial activity against the three bacteria viz., *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae*[14]. The chemical components like camphene, phenolic acids, citral, alkaloids and, alpha-pinene in

nirgundi and essential oils, demethoxycurcumin in haridra are also found to be the main antimicrobial components.

Hence, the drugs present in the formulation Suraksha Dhupa show a synergistic effect and help propagate the main antimicrobial activity.

CONCLUSION

It is a study entitled "An experimental study to compare the antimicrobial effect of Formaldehyde with that of Suraksha Dhupa on airborne microbes in dissection Hall". Disinfection or fumigation is not a new thing in the traditional medicine Ayurveda. It is used to disinfect vranagara, kumaragara, sutikagara, bheshajagara, shastrakarmagara, etc. Since the Vedic period, Homa-Havana and Yagnya, air sterilisation by Agnihotra, houses and places around it by Dhupana, etc., were done regularly.

By considering all these, after exploring the knowledge of ancient science and modern technology and taking the references from Ayurveda classical texts, in this study, we tried to demonstrate that the drugs which possess krumigna property individually in the Ayurveda classics can be used together in a combination which can give a synergistic effect as anti-microbial. It can be used as a fumigation as all the drugs have volatile components. The study showed that Suraksha dhupa and Formaldehyde have an antimicrobial effect which is statistically insignificant. Further studies are required to assess the effect of Suraksha Dhupa on species of microbes.

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