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Research Article

AN EXPERIMENTAL STUDY TO EVALUATE THE ANTI-MICROBIAL EFFECT OF FUMIGATION WITH *SARSHAPANIMBADI DHOOPA*

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ABSTRACT

Nowadays rising airborne diseases are one of the major health concerns of the entire world. Prevalence of various respiratory diseases are increasing in persons exposing to contaminated environment. Therefore, disinfection of our environment is most important. In *Ayurveda, Dhoopana* is a method of disinfection. *Sarshapanimbadidhoopa* mentioned in *Sushruta Samhita* with ingredients *Sarshapa, Nimba, Saindhava* and *Ghrita* was selected for the study to evaluate its antimicrobial activity. Primarily Staphylococcus aureus and Candida albicans were identified from different treatment procedure rooms of hospital by placing petri dishes open for one hour with suitable medium. Fumigation of identified microbes were done within a glass chamber with and without adding *Dhoopanadravya* to red hot charcoal. In addition to that one set of microbial plate of bacteria and fungus were kept as such without fumigation for the assessment of efficacy of fumigation. Fumigation was done with varying doses (15g, 20g, 25g and 30g) and time span (5minutes, 10minutes, 15minutes and 20minutes). Results obtained were statistically analyzed using independent t-test, ANOVA test and paired t-test. Reduction in Colony Forming Unit (CFU) of bacteria and fungi were found to be significant and fumigation with adding *Dhoopachoorna* was found to be more significant than without adding *Dhoopachoorna* to charcoal. Complete cessation of bacterial CFU was found on fumigation with 30g *Choorna* for 20minutes and fungal CFU was found on fumigation of 25g *Choorna* for 15minutes. The study concluded that *Sarshapanimbadi Dhoopa* has antimicrobial effect.

INTRODUCTION

The atmosphere of earth consists of a variety of gases, dust particles, water vapour and micro-organisms. These airborne micro-organisms, known as bio-aerosols include bacteria, viruses, fungi as well as airborne toxins. Respiratory ailments such as allergic manifestations and pathogenic infection may occur due to these pathogenic airborne microbes. Sneezing, coughing, talking and breathing by infected persons leads to the release of pathogens into atmosphere. It is hard to prevent such a method of transmission. Both short and long term exposure to them have their own health impacts and is a major threat to the health and wellbeing of entire population, especially those with weaker immunity. In current circumstances like COVID-19 and other pandemic, air borne diseases are major health challenge to the community. Pollution is also a contributory factor that adversely affect the quality of air. All countries are equally affected by outdoor pollutants and suffering from the environmental health hazards. Ambient (outdoor) air pollution was estimated to cause 4.2 million premature deaths worldwide including both cities and rural areas per year in 2019; major cause of this mortality is the exposure to fine particulate matter. Such exposure results in various cardiovascular abnormalities and respiratory diseases, sometimes even malignancies[1]. Thus, proper disinfection and sterilization is essential.

Ayurveda focus not only on the curative aspects but on the maintenance of positive health of individuals also. Our Classical texts have described about various *Krimi* and diseases caused by *Krimi*[2]. In addition to that different methods to prevent the diseases caused by *Krimi* are also mentioned. *Acharya*

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Charaka has explained about *Vikrutavatha, Vikrutajala, Vikrutadesha* and *Vikrutakala* in the context of *Janapadodhwamsa*[3]. Purification methods of *Vishayuktavayu* are described in various classics as fumigation of drugs (*Dhoopana*), medicated flags (*Oushadhapataka*), sprinkling methods (*Prokshana*) and medicated musical instruments[4]. A group of drugs having *Krimighna* property is described as *Rakshoghnagana.*[5] Curative as well as preventive aspects of *Dhoopana* was mentioned in classical texts. *Dhoopana* can be practised in *Bheshajagara*, *Soothikagara*, *Sastrakarmagraha* and *Kumaragara as* preventive aspect[6]*.*

In *Ayurveda, Dhoopana* is an effective disinfection method which is natural and biocompatible. Nowadays, exposure to conventional disinfectants can potentially result in lung or respiratory illnesses because they contain a complex blend of chemicals. As *Dhoopana* is done with various medicinal drugs, this method is of less adverse effect, cost effective and has medicinal value too. The *Dhoopanayoga* which is selected for study is having four ingredients which are easy to collect and convenient to use as routine measure. The present study is an attempt to assess the anti-microbial effect of *Sarshapanimbadidhoopa* mentioned by *Acharya Susrutha* in *Vranithopasaneeya Adhyaya*[7].

Table 1: Ingredients of *Sarshapanimbadi dhoopa choorna*

Sl.no	Sanskrit Name	Malayalam name	Botanical name / English name	Family	Parts used
1	*Sarshapa*	*Vellakkaduku*	*Brassica alba* / Mustard	Cruciferae	Seeds
2	*Arishta*	*Veppu*	*Azadirachta indica* / Neem tree	Meliaceae	Leaves
3	*Ghritha*	*Neyyu*	Ghee	-	-
4	*Saindhava*	*Induppu*	Rock salt	-	-

AIM

To study the anti-microbial effect of fumigation with *Sarshapanimbadidhoopa.*

Objectives

1. To identify the microbes obtained through settle plate method.
2. To standardize the concentration of the *Sarshapa nimbadidhoopa* in repeated trials with varying concentration and exposure time for effective elimination of microbes within a glass chamber.

Study Design

Experimental study

Study Setting

- Microorganisms were identified from treatment procedure rooms and general wards of Govt Ayurveda college Hospital Tripunithura.
- Fumigation study on identified microorganisms was done in PVT Merit Biolabs, Vennala (NABL accredited).

Material and Methods

a. Preparation of *Sarshapanimbadidhoopa*

Four ingredients were used in the preparation of *Sarshapanimbadidhoopa* which include *Sarshapa, Nimba, Ghrita* and *Saindhava.*

Good quality *Sarshapa*, *Ghrita* and *Saindhava* were collected from market and analysed for genuineness. Fresh leaves of *Nimba* were collected from Govt. Ayurveda College Tripunithura. *Sarshapa* and *Nimba patra* were sun-dried. Dried *Nimba patra*, dried *Sarshapa* and *Saindhava* were powdered and kept in airtight bottles separately.

Table 2: Quantity of each ingredients taken

Dose of *Sarshapa nimbadi dhoopa*	Quantity of *Sarshapa* added	Quantity of *Nimba* added	Quantity of *Saindhava* added	Quantity of *Ghritha* added
15g	3.75g	3.75g	3.75g	3.75ml
20g	5g	5g	5g	5ml
25g	6.25g	6.25g	6.25g	6.25ml
30g	7.5g	7.5g	7.5g	7.5ml

For the preparation of *Dhoopachoorna* for different trials of fumigation, required quantity of powdered *Sarshapa, Nimba* and *Saindhava* were mixed thoroughly and kept in airtight packets. *Ghrita* was added to this mixture at the time of fumigation in the dosage as mentioned in Table 2. The amount of charcoal taken was 5g for all sessions of fumigation either with adding *Dhoopadravya* or without adding *Dhoopadravya.*

b. Media and reagents

i. Nutrient agar
ii. Sabouraud agar
iii. Distilled water
iv. Baird Parker Agar (BPA)
v. Dichloran Rose Bengal Chloramphenicol Agar (DRBCA)

c. Other requirements

i. Sterile petri dishes
ii. Sterile test tubes
iii. Sterile conical flasks
iv. Antiseptic solution
v. Glass chamber (50cm*40cm*40cm)
vi. Mud *Sarava* for fumigation
vii. Electric charcoal burner stove
viii. Bunsen Burner

d. Preparation of agar solution

For identification of microorganisms from the hospital environment, Nutrient agar and Sabouraud agar were used in petri plates. For the sensitivity study of fumigation Baird Parker Agar and Dichloran Rose Bengal Chloramphenicol Agar were used. Powders of 2.8g nutrient agar, 6.5g sabouraud agar, 6.3g BPA and 3.16g DRBCA were suspended separately in 100 ml of distilled water, stirred well and mixed it thoroughly to dissolve the powder in the liquid. Then the mixture is sterilised by autoclaving at 121°C for 15 minutes. The prepared liquids were transferred to required number of petri dishes and allowed to solidify.

Nutrient agar medium was used for bacterial identification. Sabouraud agar was used for fungal identification. BPA was used for bacterial sample and DRBCA was used for fungal sample in sensitivity study of fumigation.

e. Preparation of petri dishes

Initially petri dishes were cleaned well and wrapped in kraft paper. They were autoclaved at 121°C for about 20 minutes. Plates were dried in a drier before use. The sterilized plates were kept on the cleaned laminar air flow table. The mouth of the flask containing sterilized media was shown near the flame of bunsen burner. Lid of the petri dish was slightly opened and around 20 ml of Nutrient agar, Sabouraud agar, BPA and DRBCA was transferred in to the separate petri dishes. The plates were closed and allowed to solidify.

f. Preparation of fumigation chamber

A completely closed glass box (50cm* 40cm *40cm) was selected for fumigation. The chamber was disinfected using 70% ethanol prior to use.

g. Procedure

Step 1: Collection of microbes from different rooms of hospital

Four petri dishes with nutrient agar solution and four petri dishes with sabouraud agar medium kept in different position of the treatment procedure rooms, general wards of hospital before sterilisation. These plates were kept open for one hour, after which plates were closed with lids and taken to microbiology lab.

Step 2: Identification of microbes from microbiology lab

Bacterial plates were incubated for 24 hours at 37°C and fungal plates were incubated for five days at 25°C. After incubation period, microbial colonies were identified based on morphological characters. Then confirmatory tests like Gram staining, Catalase test, Tube coagulase test were conducted. Staphylococcus aureus and Candida albicans were identified and confirmed.

Step 3: Sensitivity study of fumigation at microbiology lab

Microorganisms selected for the study were Staphylococcus aureus and Candida albicans. For easy counting and calculating the number of colonies, 10^{-7} serial dilution was used to get an average colony forming unit of 100 to 120. Spread plate method was used to spread the microbial samples from a liquid medium to estimate the number of colonies.

Before fumigation, the glass chamber and surface of mud *Sarava* were sterilised using 70% ethanol. Charcoal was burned using electric charcoal burner stove and made to red hot. This red hot charcoal was transferred to mud *Sarava* and placed inside glass chamber. One open bacterial plate was also kept inside the closed chamber for 5 minutes. After 5 minutes the petri dish was taken out and closed with its lid. Then kept inside the incubator. The same procedure was repeated for another bacterial plate of same sample with adding 15g *Sarshapa nimbadi dhoopa choorna* to red hot charcoal. After 5 minutes, plate was taken out, closed with lid and placed in incubator. The glass chamber was kept open to ensure that there were no fumes in the chamber and sterilization was done. Then bacterial plate was fumigated with red hot charcoal 10 minutes, 15 minutes and 20 minutes. Fumigation was also done with adding 20g, 25g and 30g of *Sarshapa nimbadidhoopa choorna* to red hot charcoal for 10 minutes, 15 minutes and 20 minutes respectively. Plates were incubated. Same procedure was repeated for fungal sample too. For more accuracy of the experiment, a second and third trial of fumigation was done in both bacterial and fungal plate with and without adding the *Dhoopanachoorna* to red hot charcoal.

After serial dilution one staphylococcal sample was inoculated into a petri dish containing Baird Parker Agar medium and one Candida sample was inoculated into a petri dish containing Dichloran Rose Bengal Chloramphenicol Agar medium. These petri dishes were neither fumigated with charcoal nor with *Dhoopachoorna*. After inoculation they were kept in the incubator for assessing the microbial growth without fumigation.

h. Period of incubation

For the support of microbial growth, different time duration and temperature were selected according to the microorganisms. Bacterial plates were incubated for 48 hours at 35°C and fungal plates were incubated for five days at 25°C. After incubation of BPA

for 48 hours at 37°C, total colonies in each plate were counted and compared with the control plates. After incubation, DRBCA for 5 days at 25°C, total colonies in each plate were counted and compared with the control plates.

i. Estimation

After the incubation period, both bacterial and fungal plates were taken out and the number of colonies were observed, counted with naked eyes and documented.

Ethical Clearance

Ethical clearance was obtained from the Institutional Ethics Committee (IEC), Govt Ayurveda College Tripunithura dated 10/08/2022. Ethics Committee reference number 03/SV/IEC/2022

Observation, Analysis and Interpretation

Staphylococcus aureus and Candida albicans were identified from hospital environment

Observation of Bacterial plate fumigation without adding *Dhoopachoorna*

Table 3: CFU of Bacterial plates post fumigation

Duration	0min	10 min	10 min	15min	20min
Trial A	103	38	32	11	11
Trial B	102	36	30	11	12
Trial C	104	39	29	12	12

Observation of Bacterial plate fumigation with adding *Dhoopachoorna*

Table 4: CFU of Bacterial plates post fumigation

Drug dosage and fumigation duration	0g 0min	15g 5min	20g 10min	25g 15min	30g 20min
Trial A	103	12	5	2	0
Trial B	102	12	6	1	0
Trial C	104	13	6	1	0

Observation of fungal plate fumigation without adding *Dhoopachoorna*

Table 5: CFU of fungal plates post fumigation

Duration	0min	5min	10min	15min	20min
Trial A	118	20	18	14	14
Trial B	117	21	17	14	12
Trial C	119	22	15	13	12

Observation of fungal plate fumigation with adding *Dhoopachoorna*

Table 6: CFU of Fungal plates post fumigation

Drug dosage and fumigation duration	0g 0min	15g 5min	20g 10min	25g 15min	30g 20min
Trial A	118	2	1	0	0
Trial B	117	2	1	0	0
Trial C	119	3	2	0	0

Analysis of microbial fumigation by statistical tests

Independent t-test, One way ANOVA test and paired t-test were used for the analysis by statistical tests.

Independent t test: Bacterial

Table 7: Difference in Colony Forming Unit (CFU) between the bacterial groups which are fumigated with drug and without adding drug

Fumigation with adding drug to charcoal				Fumigation without adding drug to charcoal				Mean Difference	T	P value
Dose and time	Mean	SD	SEM	Time	Mean	SD	SEM			
15g 5min	12.3	0.577	0.333	5 min	37.6	1.52	0.877	-25.3	26.95	0.0001
20g 10min	5.6	0.577	0.333	10 min	30.3	1.52	0.877	-24.7	26.31	0.0001
25g 15min	1.3	0.577	0.333	15 min	11.3	0.577	0.333	-10	21.22	0.0001
30g 20min	0	0	0	20 min	11.6	0.577	0.333	-11.6	34.82	0.0001

One way Anova: Bacterial

Table 8: Difference in Colony Forming Units (CFU) within the bacterial groups which are fumigated with adding drug

Dose and time	Colony forming units			F	P value
	Mean	SD	SEM		
15g 5min	12.3	0.577	0.333	370.15	0.000
20g 10min	5.6	0.577	0.333		
25g 15min	1.3	0.577	0.333		
30g 20min	0	0	0		

Paired t test: Bacterial

Table 9: Difference in colony forming unit of bacterial groups before and after fumigation

Dose and time	Colony forming units			Paired mean difference	T value	P value
	Mean	SD	SEM			
0g 0min	103	1	0.577	103	178.4	0.00003
30g 20 min	0	0	0			

Independent t test: Fungal

Table 10: Difference in Colony Forming Unit (CFU) between the fungal groups which are fumigated with drug and without adding drug

Fumigation with adding drug to charcoal				Fumigation without adding drug to charcoal				Mean Difference	T	P value
Dose and time	Mean	SD	SEM	Time	Mean	SD	SEM			
15g 5min	2.3	0.577	0.333	5min	21	1	0.577	-18.7	28.05	0.0001
20g 10min	1.3	0.577	0.333	10min	16.6	1.52	0.877	-15.3	16.29	0.0001
25g 15min	0	0	0	15min	13.6	0.577	0.333	-13.6	40.82	0.0001
30g 20min	0	0	0	20min	12.6	1.15	0.66	-12.6	18.97	0.0001

One-way Anova: Fungal

Table 11: Difference in Colony Forming Units (CFU) within the fungal groups which are fumigated with adding drug

Dose and time	Colony forming units			F	P value
	Mean	SD	SEM		
15g 5min	2.3	0.577	0.333	23.16	0.0003
20g 10min	1.3	0.577	0.333		
25g 15min	0	0	0		
30g 20min	0	0	0		

Paired t test: Fungal

Table 12: Difference in colony forming unit of fungal groups before and after fumigation

Dose and time	Colony forming units			Paired mean difference	T value	P value
	Mean	SD	SEM			
0g 0min	118	1	0.577	118	204.3	0.00002
30g 20 min	0	0	0			

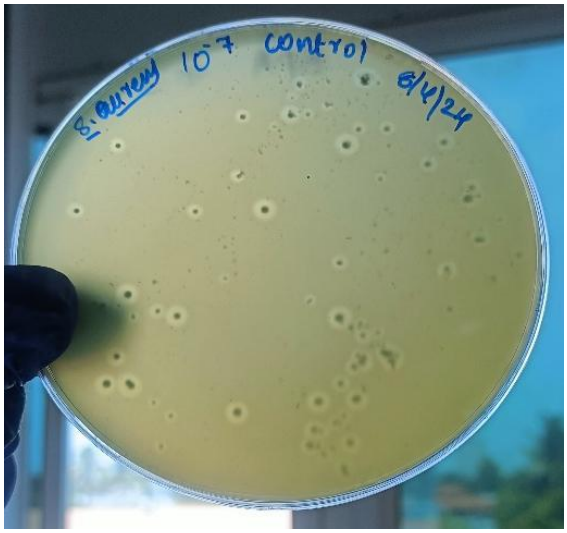

Fig 1: Bacterial plate without fumigation

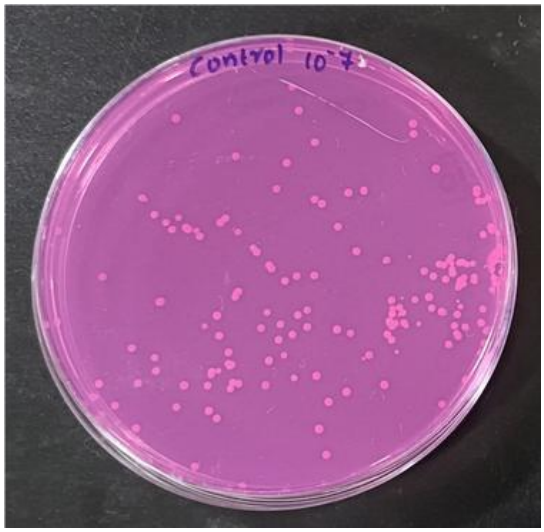

Fig 2: Fungal plate without fumigation

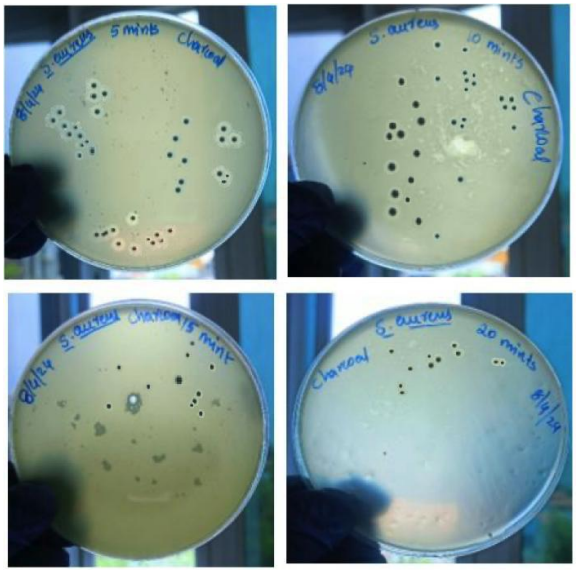

Fig 3: Bacterial plates fumigated with only charcoal (5,10,15 and 20 minutes)

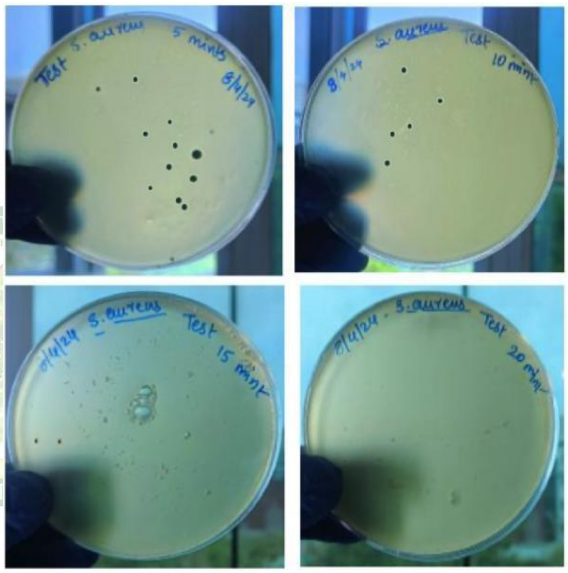

Fig 4: Bacterial plates fumigated with drug (15g 5min,20g 10min, 25g 15min, 30g 20min)

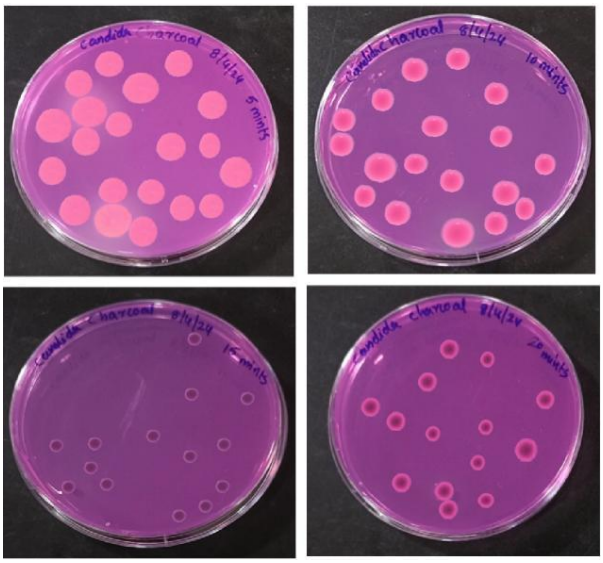

Fig 5: Fungal plates fumigated with only charcoal (5,10,15 and 20 minutes)

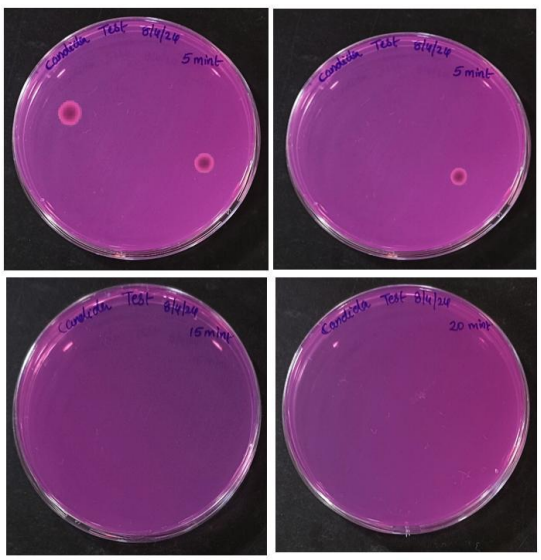

Fig 6: Fungal plates fumigated with drug (15g 5min, 20g 10min, 25g 15min, 30g 20min)

DISCUSSION

Dhoopana is a disinfection method described in *Ayurveda*. The present study drug, *Sarshapanimbadi dhoopa* contains four ingredients: *Sarshapa, Arishta, Saindhava* and *Ghrita*. Among the four ingredients, two are herbal origin, one is animal origin and other one is mineral origin. *Dhoopana* of identified bacteria and fungi was done with and without adding the test drug to charcoal. A decrease in microbial colonies were found in both sessions of fumigation. But cessation of microbial colonies was achieved only in fumigation with adding *Sarshapanimbadidhoopa* to charcoal.

Reduction in Colony Forming Units after fumigation without adding drug to charcoal may be due to the factors like increase in temperature, reduction in oxygen concentration and reduction in moisture content of the chamber during the process of fumigation. Special properties of charcoal like light weight in nature and high adsorptive power also contributes to reduction of CFU.

Fumigation with adding drug to charcoal results in cessation of both bacterial and fungal Colony forming Units. For the study, white variety of *Sarshapa* was selected, because it is superior in *Krimighna* and *Kandughna* as described in various *Nighantus*[8]. It has *Katu, Thiktha rasa* and *Laghu Snigdha guna*[9]. Both *Katu rasa* and *Thikta rasa* are *Kapha hara* in nature which results in *Krimighna karma* as *Kapha* is the factor which helps to grow. *Snigdha guna* helps in the maintenance of fumes and heat during fumigation. In addition to that *Sarshapa* is volatile in nature too. Volatile contents of the drugs are usually released into atmosphere on fumigation. Studies has proved that volatile content of atmosphere resulting from fumigation can adversely affect the bacterial growth and prevent spoilage by delaying microbial attack[10]. AITC is one of the chemical compound seen in *Sarshapa* which has potent antimicrobial activity[11]. Also *Sarshapa* shows strong antimicrobial activity, even against the study organisms Staphylococcus aureus and Candida albicans[12].

Neem is commonly used as a medicinal plant both externally and internally in India since ancient times itself. *Thiktakashaya rasa* and *Laghu rukshaguna* of *Nimba* make it *Krimighna*in nature[13]. Air purification properties of *Nimba* has been proved through various studies. Studies have shown that leaf extract of *Azadirachta indica* showed strong antimicrobial activity against many human pathogenic microorganisms including study organisms, Staphylococcus aureus and Candida albicans[14].

Saindhava is *Sookshma* in nature which indicates its penetrating nature through minutes spaces. In addition to that it is able to alter the osmotic conditions which in turn causes the weakening of microbes. Studies have proven the antimicrobial activity of *Saindhava*[15].

Ghrita the best among four *Snehadravyas,* is also an ingredient of this formulation. The *Yogavahi* and *Agni deepana* properties of *Ghrita* inside our body also reflect in the external environment by providing continuous combustion of *Dhoopachoorna*. *Ghrita* is an extensively used drug in *Agadathantra* through various routs and mode of administration such as oral, external application and external fumigation. This also implies its detoxification property.

On analysing the *Rasa* of ingredients, it is clear that they are *Akasha-Vayu-Agni mahabhuta* predominant. These *Mahabhutas* are characterised by *Laghu, Rooksha* and *Theekshnaguna* which helps in combustion, spreading of fumes and also reaching to minutes spaces. Also *Nimba* and *Sarshapa* were included under *Krimighna Kushtaghnaganas* by majority of *Acharyas* which indicates their detoxification properties. *Vishaghnarakshoghna karma* of various drugs are explained due to their *Dravyaprabhava also.*

Thus each ingredients of formulation and *Sarshapa nimbadidhoopa* as a whole contributes to the reduction of CFU of both bacterial and fungal plates and even cause the cessation of microbial colony when compared to fumigation without adding the *Dhoopa yoga* to charcoal

CONCLUSION

Airborne diseases are increasing in the present era. This negatively affects entire population especially children, old age persons and immunocompromised people. Proper disinfection and sterilization are needed to cop up with this situation. *Dhoopana* is a promising disinfection modality in the current situation of shooting airborne diseases. Antimicrobial activity of *Dhoopachoorna* was assessed in Staphylococcus aureus and Candida albicans which were identified from petri-dishes kept open on different rooms of hospital. Fumigation was done in a cuboid shaped glass chamber (50cm*40cm*40cm) with varying concentration of drug (15g, 20g, 25g, 30g) and varying the time of exposure (5 minutes, 10 minutes, 15 minutes, 20 minutes) Complete cessation of bacterial colonies were found on fumigation with 30g *Choorna* for 20 minutes and complete cessation of fungal colonies were found on fumigation with 25g *Choorna* for 15 minutes. Observations were analysed with statistical tests independent t-test, one way ANOVA test and paired t-test and was found to be significant. The overall results shows that *Sarshapa nimbadidhoopa* has anti-microbial effect on hospital-based microbes, Staphylococcus aureus and Candida albicans. Thus, null hypothesis is rejected and alternate hypothesis, fumigation with *Sarshapa nimbadidhoopa* has effect on antimicrobial activity was accepted.

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