Research Article

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ANTIMICROBIAL ACTION OF DHUPANA (FUMIGATION WITH HERBS) WITH RESPECT TO AIR BORNE MICROBES IN INDOOR ENVIRONMENT OF CENTRAL HOSPITAL

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ABSTRACT

Hospital has been the prone area for all kinds of organisms entering through different kind of patient who carry different infective organism. Patients, patient by standard, hospital employees, visitors and other healthy volunteers are at risk of developing infections through transmissions. Therefore an effective fumigation as disinfectant is essential to kill the airborne microbes effectively. Herbal fumigation is been carried out to detect the antimicrobial action in indoor hospital environment. Air sample was collected from reception, wards, and corridors by Hi media open petriplate exposure method and microbes were identified. The same area is subjected to herbal fumigation for stipulated period of time. Then again air sample has been collected from the same area where herbal fumigation was done. The effectiveness of herbal fumigation in its antimicrobial action was concluded after comparing before and after fumigation growth in the petriplate.

Key words: Dhupana, Antimicrobial, Haridra, Lashuna, Sarshapa, Nimba, Kaidarya, Tulsi, Chakrikas, Potato Dextrose Agar, Nutrient Agar

INTRODUCTION

Germicidal, antimicrobial, anti helmenthic property of garlic, tulsi, neem, turmeric, mustard, curry leaves are explained in detailed in Ayurvedic classics [1]. All these are natural and easily available common herbs. Phytochemical analysis of fresh and dried leaf extracts of *Ocimum gratissimum*[2] revealed presence of antimicrobial principles such as resins, tannins, glycosides, alkaloids, flavonoids saponin, anthraquinone, cardiac glycoside, steroidal ring, steroidal trepans and carbohydrates at different concentrations. Ayurvedic texts categorise Tulsi as stimulant, aromatic and antipyretic[3].

Ancient science has very clearly emphasized importance of sterile atmosphere in neonatal care unit, post natal care unit, Operation Theater and so on. In this regards it is an attempt to understand the utility of herbs such as Garlic, Turmeric, Mustard seeds, Curry leaves, Neem and Tulasi with the help of modern parameters. Ancient Acharyas have explained about these herbs as Krimighna individually[4-8]. Now as a combination in the form of fumigation how effective above drugs prove as krimighna which means to destroy worms and germs including sookshmakrimi which means microbes, was carried out in this work.

Since ancient time, naturally occurring plants have played an important role in the discovery of new therapeutic agents. Almost all antibiotics are subjected to the problem of bacterial resistance. Therefore, newer herbal antibacterial compounds from plants and their semisynthetic derivatives to overcome the resistance are under investigation. Garlic (*Allium sativum* Linn.) has an important dietary and medicinal role for centuries. Its therapeutic uses include beneficial effects on the cardiovascular system, antibiotic, anticancer, anti-inflammatory, hypoglycemic, and hormone-like effects Garlic extracts have been used to treat infections for thousands of years.

Pathogenic Microorganisms, insects and pests, are major obstacles of health in living area, densely populated and sensitive working areas like hospitals, clinical laboratories contain enormous pathogens which cause iatrogenic disease. The indoor environment of hospitals acts as a reservoir for opportunistic human pathogens people with decreased immune competence may be susceptible to normally harmless environmental microbes. This proportion of population with increased susceptibility is expanding rapidly. The diversity of microbial species associated with infections is also growing. Besides well-known nosocomial agents such as Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa and Aspergillus fumigates are emerging as causative agents[9, 10].

MATERIALS AND METHOD

Materials for dhupana: Raw herbs mentioned below, grinder, mould, cloth for drying, sunlight, burning charcoal, mud pot, ghee.

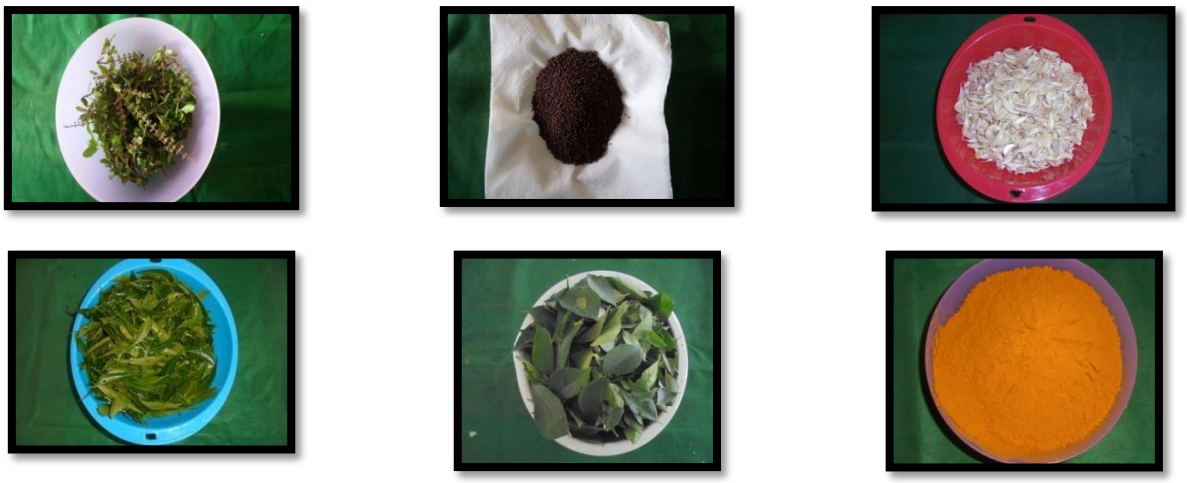
Materials for Microbial Study: Air sample of hospital, Petriplates, Chemicals and reagents to prepare agar medium, Incubators, Stains for coloring the smear, Glass slides, Compound Microscopes.

Method

The Air Sample collection of indoor environment of central hospital is collected before and after dhupana by Passive Open Air Petri Plate Exposure Method, the smear will be stained by Gram Stain method

The materials used for fumigation are as follows

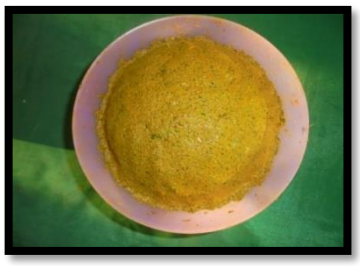
- Sarshapa (*Brassica Campestris*) : Seeds
- Lashuna (*Allium Sativum*): Cloves
- Kaidarya (*Murraya koenigiilinn.)/spreng:* Leaves *(Bergerakoenigii.spreng)*
- Nimba (*AzadirachtaIndica Linn*) : Leaves
- Haridra *(Curcuma Longa*) : Rhizome
- Tulsi (*Ocimum Sanctum*) : Leaves



Picture 1

Method of drug preparation

Above mentioned drugs are washed in clean water under aseptic condition. Then each the drug were crushed separately and then mixed and made into a fine paste. Then it is made into Chakrika (herbal cookies) of equal size weighing about 5 gram each. Then it is dried in the sunlight for 5 days.



Picture 2: Medicinal paste for the preparation of Chakrikas

Picture 3: Drying of the Chakrikas

Microbiological Study

Preparation of Culture Media

Hi-Media was used for the preparation of culture media. This was prepared as per the classical method of media preparation by using Hi-media like Nutrient agar, Potato dextrose agar for Aerobic and Thioglycolate culture media for Anaerobic Microorganisms. After dissolving in distilled water with preferred amount of culture media this was autoclaved for 15 minutes for 15 lbs pressure at 121 ^{0}C. After autoclaving sterile petriplates was poured with 15ml of Hi-media preparation in an aseptic condition, and leave it for solidification. After solidification it was stored in refrigerator at -2 ^{0}C [4-5, 75] and used for the Passive monitoring. The petri plate is opened and exposed to the area desired and the organisms in the air gets trapped in the media. After sample collection from indoor hospital environment by **Passive monitoring** (Air exposure method & Swabbing techniques) first herbal Fumigation (dhupana) would be done at 9.05 am,

It was done in the Shree Dharmasthala Manjunathaeshwara Ayurveda College and hospital campus. Three places were selected namely Entry of Hospital (Dhanwantari Statue and Reception area), IPD Wards (Male General Ward – 2) and the Ward Corridors. Then the petri plates were exposed to the air at three respective locations as given above for 20 minutes.

Picture 4: Air collection by Passive Open air Petri plate Exposure Method

Organisms found after Petri plate Exposure in the Reception area are Bacteria and Fungi as follows:

- *Staphylococcus aureus*
- *Pneumococcus.sps*
- *Pseudomonas.sps*
- *Acinetobacter.sps*
- *Penicillium.sps*
- *Aspergillus.sps*

Method of drug fumigation

Picture 5: Dhupana (Herbal Fumigation)

The herbal cookies or dried chakrikas prepared are now crushed and sprinkled to burning charcoal. Soon they start to emit fumes. These fumes are allowed to spread in the area of collection of air sample. This is called as dhupana or herbal fumigation. After 15 minutes of dhupana 30 more minutes of duration is given for germicidal action. Second air sample for microbes was collected 30 minutes after completion of Fumigation. This second sample from the same place was taken by **Passive monitoring** method. The sample specimen is incubated at 37 ^{0}C for 24 hours in incubator for Aerobic Bacteria, Identification and characterization of Microorganisms were done for Microorganisms that are seen after Fumigation.

Isolation of Airborne Bacteria

Isolation of bacteria was done in the Shree Dharmasthala Manjunatheshwara Ayurveda College and Hospital campus. Three places were selected namely Entry of hospital, IPD Wards and the Corridors. To isolate the bacterial colonies Nutrient Agar for Bacteria and Potato Dextrose Agar for Fungi are used. Then the petri plates were exposed to the air at three respective locations as given above for 20 minutes.Now the Nutrient Agar plates were kept in the incubator at 37 ^{0}C For 24 hours and Potato Dextrose Agar plates in room temperature for 48-72 hours. Now after incubation, distinct bacterial and fungal colonies are observed.

Table 1: Procedure for Fumigation and Sample collection

Number of Samples And Fumigation	**Morning At 9.00am**	**Evening At 5.00pm**
Sample 1	Culture media Exposure for 2minutes	Culture media exposure for 2 minutes
Fumigation with Dhupanadravyas	Fumigation for 15 min	Fumigation for 15 min
Sample 2	Hi-Media Exposed to air For 2 minutes after Fumigation at 9.20am	Hi-Media Exposed to air For 2 minutes after Fumigation at 5.20pm
Sample 3	Hi-Media Exposed to air For 2 minutes. 30minutes after 2nd sample taken. That is at 9.50 am	Hi-Media Exposed to air For 2 minutes. After 30 minutes that is at 5.50pm
Sample 4	Culture media Exposure for 2minutes after 30 minutes at 10.20am	Culture media Exposure for 2minutes after 30 minutes at 6.20pm

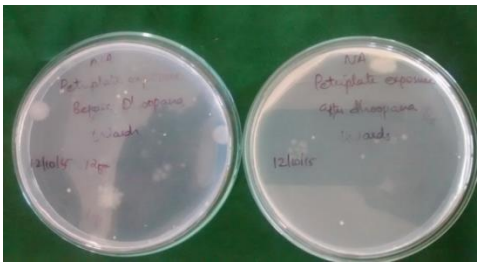
OBSERVATIONS

Before and after Dhupana, exposure of Nutrient agar plates in Entrance (Reception area)

During the exposure of Nutrient Agar plate in the Entrance area for 2 minutes, it was observed that the medium showed medium to minimal growth of organisms in the plate after 24 hours of incubation in the incubator at 37^{0}C. Afterwards Dhupana has been done for 15 minutes in the same area. Once again the Nutrient Agar plate was exposed in the same area for 2 minutes after 15 minutes of fumigation. There observed very minimal organism growth after 24 hours of culturing in the incubatorat 37^{0}C.

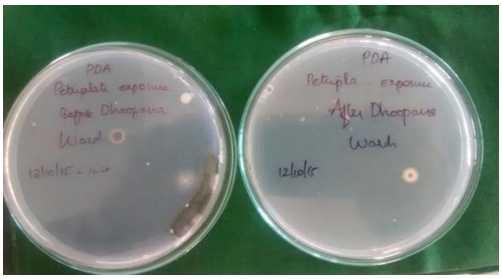
Picture 6: Before and after Dhupana, exposure of potato dextrose agar plates in Entrance (Reception area)

During the exposure of Potato Dextrose Agar plate in the Entrance area for 2 minutes, it was observed the medium to minimal growth of organisms in the plate after 48-72 hours in the room temperature. Afterwards Dhupana has been done for 15 minutes in the same area. Once again the Potato Dextrose Agar plate was exposed in the same area for 2 minutes after 15 minutes of fumigation. There observed very minimal organism growth after 48-72 hours of culturing in the room temperature.



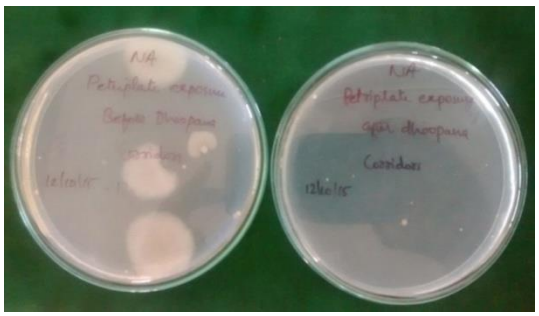
Picture 7: Before and After Dhupana, exposure of Nutrient agar plates in Ward (Male General Ward)

During the exposure of Nutrient Agar plate in the Male General Ward 2 for 2 minutes, it was observed the medium to minimal growth of organisms in the plate after 24 hours of incubation in the incubator at 37 ^{0}C. Afterwards Dhupana has been done for 15 minutes in the same area. Once again the Nutrient Agar plate was exposed in the same area for 2 minutes after 15 minutes of fumigation. There observed very minimal organism growth after 24 hours of culturing in the incubator at 37 ^{0}C.



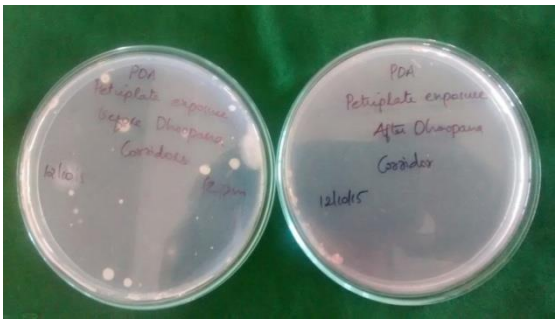
Picture 8: Before and After Dhupana, Exposure of potato dextrose agar plates in Ward

During the exposure of Potato Dextrose Agar plate in the Male General Ward 2 for 2 minutes, it was observed the medium to minimal growth of organisms in the plate after 48-72 hours of culturing in the room temperature. Afterwards Dhupana has been done for 15 minutes in the same area. Once again the Potato Dextrose Agar plate was exposed in the same area for 2 minutes after 15 minutes of fumigation. There observed very minimal organism growth after 48-72 hours of culturing in the room temperature.



Picture 9: Before and After Dhupana, exposure of Nutrient agar plates in Ward Corridors

During the exposure of Nutrient Agar plate in the Corridor area of Wards for 2 minutes, it was observed the medium to minimal growth of organisms in the plate after 24 hours of incubation in the incubator at 37 ^{0}C. Afterwards Dhupana has been done for 15 minutes in the same area. Once again the Nutrient Agar plate was exposed in the same area for 2 minutes after 5 minutes of fumigation. There observed very minimal organism growth after 24 hours of culturing in the incubator at 37 ^{0}C.



Picture 10: Before and After Dhupana, exposure of potato dextrose agar plates in Ward Corridors

During the exposure of Potato Dextrose Agar plate in the Corridor area of wards for 2 minutes, it was observed medium to minimal growth of organisms in the plate after 48-72hours of incubation in the room temperature. Afterwards Dhupana has been done for 15 minutes in the same area. Once again the Potato Dextrose Agar plate was exposed in the same area for 2 minutes after 15 minutes of fumigation. There observed very minimal organism growth after 48-72hours of incubation in the room temperature.

Table 2: Day I – Morning – Nutrient Agar plates

Day	Before Dhupana–9.00Am	After Dhupana – 9.20Am & 9.30Am	After ½ an hour after Dhupana – 9.50Am & 10.05Am	Micro organism
Day I – Entrance	Gram negative Bacilli, Gram Positive Diplococci	Gram negative Bacilli,	Gram negative Bacilli	*E.coli, Pneumococci.sps, Staphylococci,sps, Actinomyces*
Day I – Corridor	Gram positive Cocci Gram negative bacilli	Gram positive Cocci	No growth	*Staphylococci,sps, Actinomyces*
Day I – Ward	Gram negative Bacilli	Gram negative Bacilli	No growth	*E.coli, Yersenia.sps*

Table 3: Day I – Morning – Potato Dextrose plates

Day	Before Dhupana - 9.00Am	After Dhupana - 9.20Am & 9.30Am	After ½ an hour after Dhupana - 9.50Am & 10.05Am	Micro organism
Day I – Entrance	Growth seen	Growth seen	Growth seen	*Rhizopus.sps, Mucor.sps, Aspergillus fumigatus, Penicillium.sps*
Day I – Corridor	Growth seen	Growth seen	Growth seen	*Cladosporium.sps, Mucor.sps, Aspergillus fumigatus*
Day I – Ward	Growth seen	Growth seen	Growth seen	*Curvularia.sps, Aspergillus.sps*

Table 4: Day I – Evening – Nutrient Agar plates

Day	Before Dhupana – 4.00Pm	After Dhupana – 4.25Pm & 4.35Pm	After ½ an hour after Dhupana – 4.40Pm & 4.50Pm	Micro organism
Day I – Entrance	Gram negative Bacilli	No growth	Gram negative Bacilli	*E.coli, Yersenia.sps*
Day I – Corridor	Gram positive cocci	No growth	Gram positive cocci	*Staphylococci,sps,*
Day I – Ward	Gram negative Bacilli, Gram Positive Diplococci	No growth	Gram negative Bacilli, Gram positive cocci	*Staphylococci,sps, Pneumococci.sps, E.coli*

Table 5: Day I – Evening – Potato Dextrose plates

Day	Before Dhupana– 4.00Pm	After Dhupana– 4.25Pm & 4.35Pm	After ½ an hour after Dhupana - 4.40Pm & 4.50Pm	Micro organism
Day I – Entrance	Growth seen	No growth	No growth	*Rhizopus.sps, Mucor.sps, Aspergillus fumigatus, Penicillium.sps*
Day I – Corridor	Growth seen	No growth	No growth	*Mucor.sps, Aspergillus fumigatus, Cladosporium.sps Rhizopus.sps*
Day I – Ward	Growth seen	No growth	No growth	*Aspergillus fumigatus, Curvularia.sps, Penicillum.sps*

Table 6: Day II – Morning – Nutrient Agar plates

Day	Before Dhupana – 9.15Am	After Dhupana 9.50Am & 10.00Am	After ½ an hour after Dhupana – 10.05Am & 10.15Am	Micro organism
Day II – Entrance	Gram negative Bacilli,	Gram PositiveDiplococci, Gram Negative Bacilli	Gram Positivecocci, Gram Negative Bacilli, Gram Positive Diplococci	*E.coli, Yersenia.sps Staphylococci,sps, Pneumococci.sps*, Actinomyces
Day II – Corridor	Gram negative Bacilli	Gram Negative Bacilli	Actinimyces, Gram Negative Bacilli	*Aerobic spore forming bacteria, Yersenia.sps*
Day II – Ward	Gram negative Bacilli, Gram Positive Diplococci	Gram negative Bacilli,Tetrad	Gram Negative Bacilli	*Yersenia.sps Aerobic spore forming bacteria, staphylococci.sps*

Table 7: Day II – Morning – Potato Dextrose plates

Day	Before Dhupana – 9.15Am	After Dhupana– 9.50Am & 10.00Am	After ½ an hour after Dhupana - 10.05Am & 10.15Am	Micro organism
Day II – Entrance	Growth seen	No growth	Growth seen	*Cladosporium.sps Aspergillus fumigatus, Curvularia.sps, Penicillum.sps*
Day II – Corridor	Growth seen	No growth	Growth seen	*Penicillium.sps, Rhizopus.sps*
Day II – Ward	Growth seen	No growth	Growth seen	*Mucor.sps, Aspergillus fumigatus, Rhizopus.sps*

Table 8: Day II – Evening – Nutrient Agar plates

Day	Before Dhupana – 4.10Pm	After Dhupana – 4.35Pm & 4.45Pm	After ½ an hour after Dhupana– 4.50pm & 5.00Pm	Micro organism
Day II – Entrance	Gram Negative Bacilli, Gram Positive Diplococci	No growth	Gram negative bacteria,	*Staphylococci,sps, Actinomyces, Pneumococci.sps*
Day II – Corridor	Gram negative Bacilli	No Growth	Gram negative bacteria,	Actinomyces
Day II – Ward	Gram Positive Diplococci	No Growth	Gram negative bacilli, gram positive diplococci	*Yersenia.sps Pneumococci.sps,*

Table 9: Day II – Evening – Potato Dextrose Agar plates

Day	Before Dhupana– 4.10Pm	After Dhupana – 4.35Pm & 4.45Pm	After ½ an hour after Dhupana– 4.50pm & 5.00Pm	Micro organism
Day II – Entrance	Growth seen	No growth	No growth	*Rhizopus.sps, Mucor.sps, Aspergillus fumigatus,*
Day II – Corridor	Growth seen	No growth	No growth	*Aspergillus fumigatus, Penicillum.spscurvularia.sps*
Day II – Ward	Growth seen	No growth	No growth	*Mucor.sps, Cladosporium.sps Aspergillus fumigatus*

Table 10: Day III – Morning – Nutrient Agar plates

Day	Before Dhupana- 9.10Am	After Dhupana- 9.50Am &10.00Am	After ½ an hour after Dhupana – 10.05Am &10.15Am	Micro organism
Day III – Entrance	Gram negative Bacilli,	No growth	No growth	*Yersenia.sps*
Day III – Corridor	Gram Positive Diplococci	No Growth	Gram negative Bacilli, Streptobacilli	*Bacillus.sps Yersenia.sps,*
Day III – Ward	Gram Positive Diplococci, Gram negative Bacilli,	No growth	Gram negative Bacilli, Streptobacilli	*Yersenia.sps Bacillus.sps, Pneumococci.sps,*

Table 11: Day III – Morning – Potato Dextrose plates

Day	Before Dhupana- 9.10Am	After Dhupana-9.50Am & 10.00Am	After ½ an hour after Dhupana -10.05Am & 10.15Am	Micro organism
Day III – Entrance	Growth seen	No growth	Growth seen	*Rhizopus.sps, Mucor.sps, Cladosporium.sps Aspergillus fumigatus, Penicillium.sps*
Day III – Corridor	Growth seen	No growth	Growth seen	*Curvularia.sps, Penicillium.sps*
Day III – Ward	Growth seen	No growth	Growth seen	*Aspergillus fumigatus, Penicillum.spscurvularia.sps*

Table 12: Day III – Evening – Nutrient Agar plates

Day	Before Dhupana – 4.10Pm	After Dhupana – 4.35pm & 4.45Pm	After ½ an hour after Dhupana – 4.50Pm & 5.00Pm	Micro organism
Day III – Entrance	Gram Positive Diplococci, Gram –ve bacteria	No growth	No growth	*Actinimyces, Tetrad, Pneumococci.sps*
Day III – Corridor	Gram Negative Bacilli, , Gram Positive Diplococci,	No growth	No growth	*Yersenia.sps, Actinomyces.sps, Pneumococci.sps*
Day III – Ward	Gram Negative Bacilli, Gram Positive, Diplococci,	No growth	No growth	*Pseudomonas.sps, Pneumococci.sps*

Table 13: Day III – Evening – Potato Dextrose Agar plates

Day	Before Dhupana– 4.10Pm	After Dhupana– 4.35pm & 4.45Pm	After ½ an hour after Dhupana– 4.50Pm & 5.00Pm	Micro organism
Day III – Entrance	Growth seen	No growth	No growth	*Aspergillus fumigatus, Cladosporium.sps Penicillum. spscurvularia.sps*
Day III – Corridor	Growth seen	No growth	No growth	*Penicillium.sps, Aspergillus nigar*
Day III – Ward	Growth seen	No growth	No growth	*Aspergillus fumigatus, Rhizopus.sps*

Table 14: Day IV – Morning – Nutrient Agar plates

Day	Before Dhupana – 9.10Am	After Dhupana – 9.45Am & 9.45Pm	Micro organism
Day IV – Entrance	Gram positive diplococcic, gram negative bacilli	No growth	*Aerobic spore forming bacteria, Pseudomonas.sps, Pneumococci.sps*
Day IV – Corridor	Gram positive cocci.gram positive diplococci	No growth	*Staphylococci.sps, Pneumococci.sps*
Day IV – Ward	Gram positive diplococci	No growth	*Pneumococci.sps*

Table 15: Day IV – Morning – Potato Dextrose plates

Day	Before Dhupana – 9.10Am	After Dhupana– 9.45Am & 9.45Pm	Micro organism
Day IV – Entrance	Growth seen	Growth seen	*Alternaria.sps, Cladosporium.sps Penicillium.sps, Aspergillus.sps*
Day IV – Corridor	Growth seen	Growth seen	*Aspergillus fumigatus, Penicillum. spscurvularia.sps*
Day IV – Ward	Growth seen	Growth seen	*Penicillium.sps, Aspergillus.nigar*

Table 16: Day IV – Evening – Nutrient Agar plates

Day	Before Dhupana – 4.10Pm	After Dhupana- 4.35Pm &4.45Pm	Micro organism
Day IV – Entrance	Gram negative bacilli, Gram positive diplocicci	No growth	*Actinomyces, Pneumococci.sps*
Day IV – Corridor	Gram positive diplococci,	No growth	*Pneumococci.sps*
Day IV – Ward	Gram negative bacteria	No growth	*Yersenia.sps, E.coli*

Table 17: Day IV – Evening – Potato Dextrose plates

Day	Before Dhupana– 4.10Pm	After Dhupana- 4.35Pm & 4.45Pm	Micro organism
Day IV – Entrance	Growth seen	No growth	*Aspergillus nigar, Rhizopus.sps, Mucor.sps*
Day IV – Corridor	Growth seen	No growth	*Rhizopus.sps, Mucor.sps Cladosporium.sps*
Day IV – Ward	Growth seen	No growth	*Penicillium.sps, Aspergillus.nigar*

Table 18: Day V – Morning – Nutrient Agar plates

Day	Before Dhupana – 9.30Am	After Dhupana- 10.00Am & 10.10Am	Micro organism
Day V – Entrance	Gram negative bacilli	Gram negative bacilli	*Aerobic spore forming bacteria*
Day V – Corridor	Gram Negative Bacilli, Gram Positive, Diplococci,	Gram positive Diplococci	*Pneumococci.sps, aerobic spore forming bacteria*
Day V – Ward	Gram Positive Diplococci, Gram –ve bacteria	Gram positive diplococci	*Yersenia.sps, Pneumococci.sps*

Table 19: Day V – Morning – Potato Dextrose plates

Day	Before Dhupana – 9.30Am	After Dhupana - 10.00Am & 10.10Am	Micro organism
Day V – Entrance	Growth seen	Growth seen	*Aspergillus.nigar, Aspergillus.fumigatus, Rhizopu.sps*
Day V – Corridor	Growth seen	Growth seen	*Aspergillus nigar, Rhizopus.sps, Mucor.sps*
Day V – Ward	Growth seen	No growth	*Penicillium.sps, Aspergillus.nigar*

Table 20: Day V – Evening – Nutrient Agar plates

Day	Before Dhupana – 4.20Pm	After Dhupana – 4.40Pm & 4.50Pm	Micro organism
Day V – Entrance	Gram positive diplococci, gram negative bacteria	No growth	*Pneumococci.sps, aerobic spore forming bacteria*
Day V – Corridor	Gram negative bacilli	No growth	*Aerobic spore forming bacteria*
Day V – Ward	Gram negative bacilli, gram positive cocci	No growth	*Yersenia.sps, Staphylococci.sps,*

Table 21: Day V – Evening – Potato Dextrose plates

Day	Before Dhupana – 4.20Pm	After Dhupana– 4.40Pm & 4.50Pm	Micro organism
Day V – Entrance	Growth seen	No growth	*Penicillium.sps Aspergillus.nigar,*
Day V– Corridor	Growth seen	No growth	*Aspergillusnigar, Rhizopus.sps, Mucor.sps Cladosporium.sps*
Day V – Ward	Growth seen	No growth	*Penicillum.sps, Alternaria.sps*

RESULT AND DISCUSSION

Before using dhupana, the air sample of the room contained fungi and Bacterial growth. The same room was treated with herbal fumigation that contain anti microbial essential oils possessing Flavonoids, Alkaloids, Steroids, Tannin, Coumarins, Phenol and Quinone. And second air sample was collected after fumigation. The second sample did not find growth after treated with dhupana except in 4th day morning sample, fifth day morning and evening sample. This shows herbal fumigation made of above mentioned drugs acts as germicidal for fungus, gram positive and gram negative bacteria. Regular use of this combination as fumigation in clinics, office, bedroom, living room, kitchen or any indoor working area may cause sterilization effect and disinfect the room. Fumes from above Herbal combinations have not reported irritation, respiratory distress, headache or any discomfort to Human during the study. Growth was found on one sample of fourth day and two samples of fifth day after fumigation. This may be due to contamination during the procedure therefore extreme care should be taken to avoid contamination. Growth may also be due to decreased fume concentration or improper burning of herbal chakrikas and therefore uniform fume concentration has to be maintained, chakrikas must be dry or the herbs must be dry, optimum fire so as to emit fumes has to be maintained otherwise all the active principles would burn before dhupana procedure.

CONCLUSION

Airborne particles are a major cause of respiratory ailments of humans, causing allergies, asthma, and pathogenic infections of the respiratory tract. Airborne fungal spores are also important agents of plant disease, and the means for dissemination of many common saprophytic fungi. Fumigation is not only essential in Operation Theater or Labour Theater but fumigation is required for entire hospital.

Dhupana or Herbal fumigation with above said Herbal combination are effective against air borne microorganisms. It has showed its antimicrobial property against Bacterial and fungal species. They have been non irritant and did not disturb any individual or any individual's routine activity. There were no adverse reaction to eye, skin, and respiratory system to any

individual during the procedure. The procedure can be adapted daily and regularly as a healthy practice in the hospital. Thus they can be effective germicidal and safe. The research has to be continued to observe the prolong effect of dhupana and a study has to be taken for number of fumigation needed for one single day.

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