Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/20441
DOI URL: http://dx.doi.org/10.21474/IJAR01/20441

RESEARCH ARTICLE

AN IN VITRO STUDY TO EVALUATE THE ANTI-MICROBIAL EFFECT OF NAGAKESARADI DHOOPANA YOGAIN HOSPITAL ROOM

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Manuscript Info

Manuscript History
Received: 15 December 2024
Final Accepted: 17 January 2025
Published: February 2025

Key words:-
Dhoopana, Nagakesaradi Dhoopana Yoga, Microorganisms Anti-Bacterial Activity, Anti-Fungal Activity

Abstract

Introduction: Nosocomial diseases are the Hospital acquired disease which can be considered as the one of the main reason for contagious disease in hospital. Among the microorganismswhich are responsible for nosocomial disease Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger are the main strains. Hence maintaining the air hygiene is very essential in to prevent such spread. Formaldehyde fumigation is the standard procedure which is followed in hospital in order to fumigate OT as the part of sterilisation. But formaldehyde is a potent carcinogen and is also capable of causing various health hazards. Hence Dhoopana can be taken up as the measure to maintain the air hygiene without health effects. Nagakesaradi Dhoopana Yoga mentioned in the Malayalam textbook Kriyakoumudi in the aspect of Jaladhi shudhikarana is directly indicated in the Vishavayu and against microorganisms.

Materials and Methods: An in-vitro study was carried out withNagakesaradi Dhoopana Yoga.Multiple swabs were collected from different parts of in-patient rooms, cultured and Dhoopanaof the same rooms were done. Later swabs were collected from the same places and the results were compared.

Observations and Results: The study shows that Nagakesaradi Dhoopana Yogahasvery good action in preventing the microbial growth in hospital rooms.

Conclusion: The Nagakesaradi Dhoopana Yoga has better anti-microbial activity.

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Introduction:-

Indoor Air Quality (IAQ) refers to the air quality inside and around buildings and structures, affecting the health, comfort, and well-being of occupants. Poor IAQ has been associated with sick building syndrome, decreased productivity, and hindered learning in schools. [1] Additionally, microorganisms that thrive in home heating and cooling systems and humidifiers can contribute to these health issues. People who are particularly vulnerable to disease-causing biological agents in indoor air include children, the elderly, and individuals with respiratory conditions, allergies, or lung diseases. Airborne transmission is known to occur with diseases such as tuberculosis, measles, Staphylococcus infections, Legionella, and influenza. [2] Some of the most significant bacteria found in indoor air include Mycobacterium tuberculosis, Staphylococcus aureus, and Streptococcus pneumoniae.[1]

Nosocomial infections affect a significant number of patients worldwide, resulting in increased mortality and financial strain on healthcare systems. The primary mode of transmission is contact, where organisms are spread through direct or indirect contact. Droplet transmission occurs when microorganisms from the respiratory tract are carried by large droplets (greater than 5 microns) that travel less than 3 feet. Airborne transmission, on the other hand, involves the spread of organisms through small droplets (less than 5 microns) that can travel long distances.[3]Due to the challenges of thoroughly disinfecting rooms and equipment, chemical fumigation is now being utilized as a nosocomial infection control measure in healthcare settings. It is being considered because gases and vapours can penetrate areas that are difficult to reach. [4]

Nagakesaradi Dhoopana Yoga is described in Kriyakoumudi, a Malayalam Visha Chikitsa textbook authored by V.M. Kuttikrishna Menon in the aspect of JaladhiSudhikarana. The drugs mentionedin this Yoga include Nagakesara, Daruharidra, Ela, Twak, Kushta, Priyangu, Laksha, Ativisha, Musta, and Nirgundi

Objective:-

To evaluate the anti-microbial effect of Dhoopana Karma with Nagakesaradi Dhoopana Yoga in in-patient room

Material and Methods:-

Preparation and Standardization of Nagakesaradi Dhoopana Yoga

All the drugs were collected in equal quantity, authenticated and Choorna (Average Coarse Powder -4mm mesh size) were prepared as per the general method from G.M.P. certified S.D.M. Ayurveda Pharmacy, Kuthpady, Udupi, Karnataka, India. The ingredients of Nagakesaradi Dhoopana Yogaaretabulated in Table no.1.

All the Analytical tests including HPTLC were conducted from Shri Dharmasthala Manjunatheswara Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka, India.

Table no.1:-Ingredients of Nagakesaradi Dhoopana Yoga[5- 8]

Sl. No.	Drugs	Botanical Name	Part Used
1.	**Nagakesara**	Mesua ferreaLinn.	Stamens
2.	**Daruharidra**	Berberis aristata DC.	Root
3.	**Ela**	Elettaria cardamomoum(Linn.) Maton	Fruits and seeds
4.	**Twak**	Cinnamomum zeylanicumBlume.	Stem Bark
5.	**Kushta**	Saussurea lappa C.B. Clarke	Root
6.	**Priyangu**	Callicarpa macrophylla Vahl.	Seeds
7.	**Laksha**	Laccifer lacca (Kerr).	Resin
8.	**Ativisha**	Aconitum heterophyllum Wall. ex Royle	Tuberous root
9.	**Musta**	Cyperus rotundus Linn.	Tubers
10.	**Nirgundi**	Vitex negundo Linn.	Leaves

Experimental Source

Study was conducted in two non-sterile In-Patient rooms of size 168 sq. ft. (14'*12') of S.D.M. Ayurveda Hospital, Kuthpady, Udupi.

Materials used for the study:

1. Two non –sterile In-Patient rooms of size 168 sq. ft. (14'*12')
2. Two Mrit sharavas, was obtained from local market
3. Nagakesaradi Dhoopana Yoga

4. Materials required for collecting swabs, counting and assessing microbial load were taken from Shri Dharmasthala Manjunatheswara Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka, India.

In-Patient room Selection for Dhoopana:
- Two non- sterile In-Patient rooms of size 168 sq. ft. (14'*12') were selected from the Hospital.
- Rooms were named as Room A (Special room-325) and Room B (Special room- 310)
- Rooms were kept closed for 24 hours after the discharge of the patient without any disinfection procedures.
- And experiment was conducted after 24 hours.

Preparation of microorganisms:
Both Casein Soya bean Digest Agar Medium (CSDAM) and Sabaud's Dextrose Agar medium were prepared for bacteria and fungus respectively.

Preparation of swab:
- Both hands and the rod were sterilized priorly.
- Sterile absorbent cotton was taken and rolled over the rod.
- Prepared swabs were autoclaved at 121° C for 15 mins.

Preparation of Dhoopa in Sharava:
- Charcoal was ignited with the help of Ghee and made in to red hot
- 2 Mrit Sharavas were taken and added with sufficient quantity of charcoal
- Later 100gms of Nagakesaradi Dhoopana Yoga was divided and added to the igniting charcoal in 2 Sharavas.

Experiment procedure:
- Swabs were collected from 7 sites of selected room before Dhoopana
- Sites from where swabs are collected:-

a) Wall
b) Curtain
c) Chair
d) Switch
e) Bed
f) Bathroom door handle
g) Cupboard door handle

- These sites were marked with marker.
- 2 Mrit Sharavas with fumes were kept inside the room in center.
- All ventilators, windows, doors etc. were closed tightly in order to avoid the spillage of fumes out of the room.
- Dhoopana was done for 45 mins.
- After 45 mins both Sharavas were taken out and room was kept closed for further 2 hours by maintaining the fumes inside the room.
- After 2 hours room were opened and fumes was allowed to escape.
- Later multiple swabs were collected from the previously marked sites.
- Swabs were sent to the Research lab for further inoculation and microbial load analysis.

Inoculation of microorganism:
- The collected swabs were kept inside the laminar air flow chamber.
- Swabs were dipped in 10 ml saline and properly mixed and kept.
- Petri dish was taken and added with suitable media as per the strain, later added with 1 ml of solution from sample and mixed uniformly for proper spreading of strains.
- Kept aside and allowed to get solidify.

Microbial load Analysis:
- After the procedure, Petri dish with the bacterial mediumwas kept incubation in Incubator for 24 hours in 37° C and fungul culture mediawas kept incubation in BOD (Biological Oxygen Demand) for 4 days in 25° C.

- After incubation microbial load counting was done with the help of Digital Colony Counter.
- Comparison of microbial load was done with before and after Dhoopana.

Figure no. 1:- Procedure followed for the experiment.

Figure no.1a; Collecting swabs before Dhoopana

Figure no.1b; Nagakesaradi Dhoopana Yoga was weighed

Figure no.1c ; Sharavas was kept inside the Room

Figure no. 1d; Room was completely closed

Figure no.1e ; Swabs were collected after Dhoopana

Figure no.1f; Dipping swabs in saline

Figure no.1g; Pouring medium in to the petridish

Figure no.1h; Adding the sample to the medium

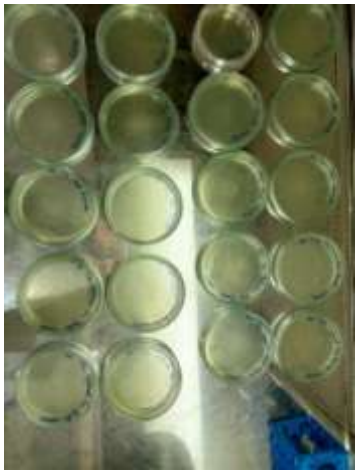


Figure no.1i ; All petridish were labeled (BT and AT) and kept for solidifying

Obsevations:-

Room A (Special room-325)

Table no. 1a:-Total Bacterial Count of swabs - Before treatment & after treatment in Room A.

Sl. No.	Name	Number of Colonies (NOC)	
		BT	AT
1	Wall	03	0
2	Curtain	01	0
3	Chair	10	02
4	Switch	12	0
5	Bed	19	0
6	Bathroom door handle	10	03
7	Cupboard door handle	03	0

Table no. 1b:-Total Fungal Count of swabs- Before and after treatment in Room A.

Sl. No.	Name	Number of Colonies (NOC)	
		BT	AT
1	Wall	02	0
2	Curtain	88	45
3	Chair	02	01
4	Switch	0	0
5	Bed	06	05
6	Bathroom door handle	0	0
7	Cupboard door handle	06	01

Observations:-

- **Wall:** Bacterial colony was reduced from 3 to nil and fungal colony was reduced from 2 to nil, showing remarkable reduction afterDhoopana.
- **Curtain:** Bacterial colony was reduced from 1 to nil and fungal colony was reduced from 88 to 45 after Dhoopana.
- **Chair:**Bacterial colony was reduced from 10 to 2 and fungal colony was reduced from 2 to 1, showing remarkable reduction afterDhoopana.
- **Switch:**Bacterial colony was reduced from 12 to nil, showing remarkable reduction afterDhoopana. No fungal colonies were found before and after treatment.
- **Bed:**Bacterial colony was reduced from 19 to nil, showing remarkable reduction and fungal colony was reduced from 6 to 5, afterDhoopana.
- **Bathroom door handle:** Bacterial colony was reduced from 10 to 3, showing remarkable reduction afterDhoopana.No fungal colonies were found
- **Cupboard door handle:** Bacterial colony was reduced from 3 to nil and fungal colony was reduced from 6 to 1, showing remarkable reduction after Dhoopana.

Room B (Special room-310)

Table no. 2a:- Total Bacterial Count of swabs - Before treatment & after treatment in Room B.

Sl. No.	Name	Number of Colonies (NOC)	
		BT	AT
1	Wall	0	0
2	Curtain	3	0
3	Chair	28	01
4	Switch	01	0
5	Bed	21	03
6	Bathroom door handle	04	01
7	Cupboard door handle	0	0

Table no. 2b:-Total Fungal Count of swabs- Before and after treatment in Room B.

Sl. No.	Name	Number of Colonies (NOC)	
		BT	AT
1	Wall	0	0
2	Curtain	54	15
3	Chair	09	01
4	Switch	01	0
5	Bed	04	07
6	Bathroom door handle	01	0
7	Cupboard door handle	01	0

Observation:-

- **Wall:** There were no bacterial and fungal colonies found before and after treatment.
- **Curtain:** Bacterial colony was reduced from 3 to nil and fungal colony was reduced from 54 to 15, showing remarkable reduction afterDhoopana.
- **Chair:**Bacterial colony was reduced from 28 to 1 and fungal colony was reduced from 9 to 1, showing remarkable reduction after Dhoopana.
- **Switch:**bacterial colony was reduced from 1 to 0 and fungal colony was reduced from 1 to 0.
- **Bed:**Bacterial colony was reduced from 21 to 3, and fungal colony showed slight increase from 4 to7.
- **Bathroom door handle:** Bacterial colony was reduced from 4 to 1, and fungal colony was reduced from 1 to 0, showing remarkable reduction after Dhoopana.
- **Cupboarddoor handle:** No bacterial colonies were found and fungal colony was reduced from 1 to 0.

Room A

Figure no.2:- Total Bacterial Count of swabs - Before treatment & after treatment.


Wall-BT


Wall-AT


Curtain-BT

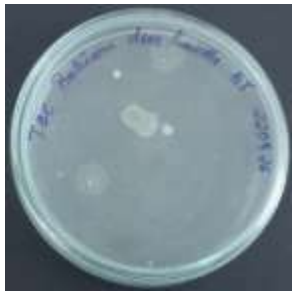

Curtain-AT

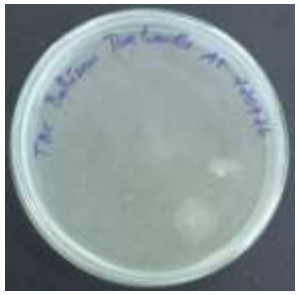

Chair-BT


Chair-AT


Switch-BT


Switch-AT

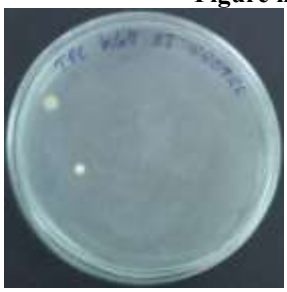


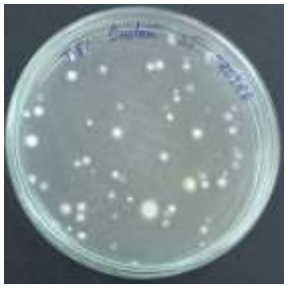


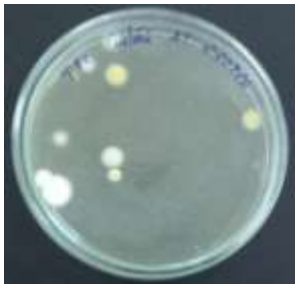
Bed-BT Bed-AT Bathroom door handle-BT Bathroom door handle-AT

Cupboard door handle-BT Cupboard door handle-AT

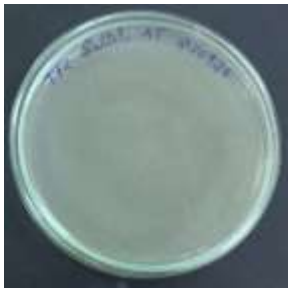
Figure no.3:- Total Fungal Count of swabs - Before treatment & after treatment.



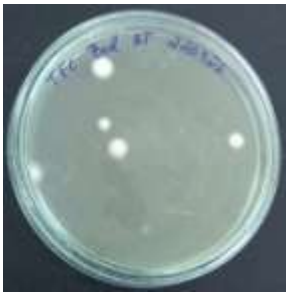


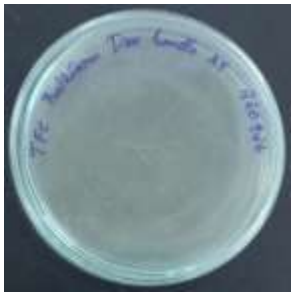


Wall-BT Wall-AT Curtain-BT Curtain-AT

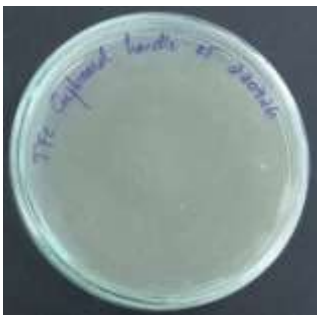


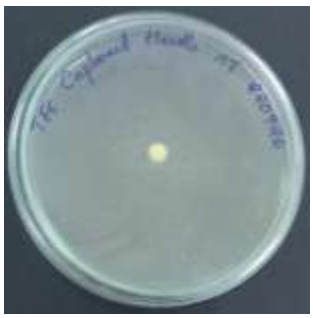
Chair-BT Chair-AT Switch-BT Switch-AT





Bed-BT Bed-AT Bathroom door handle-BT Bathroom door handle-AT





Cupboard door handle-BT Cupboard door handle-AT

Room B

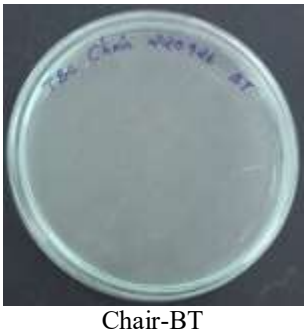
Figure no.4:- Total Bacterial Count of swabs - Before treatment & after treatment.


Wall-BT


Wall-AT


Curtain-BT


Curtain-AT


Chair-BT

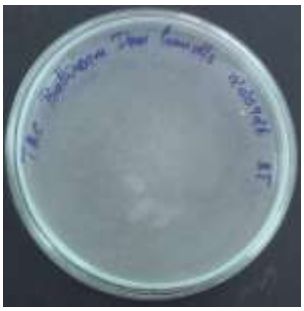

Chair-AT

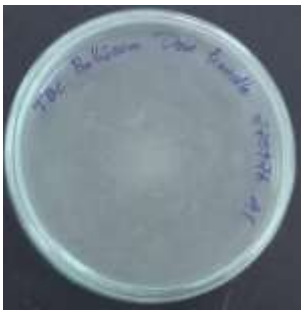

Switch-BT


Switch-AT


Bed-BT


Bed-AT

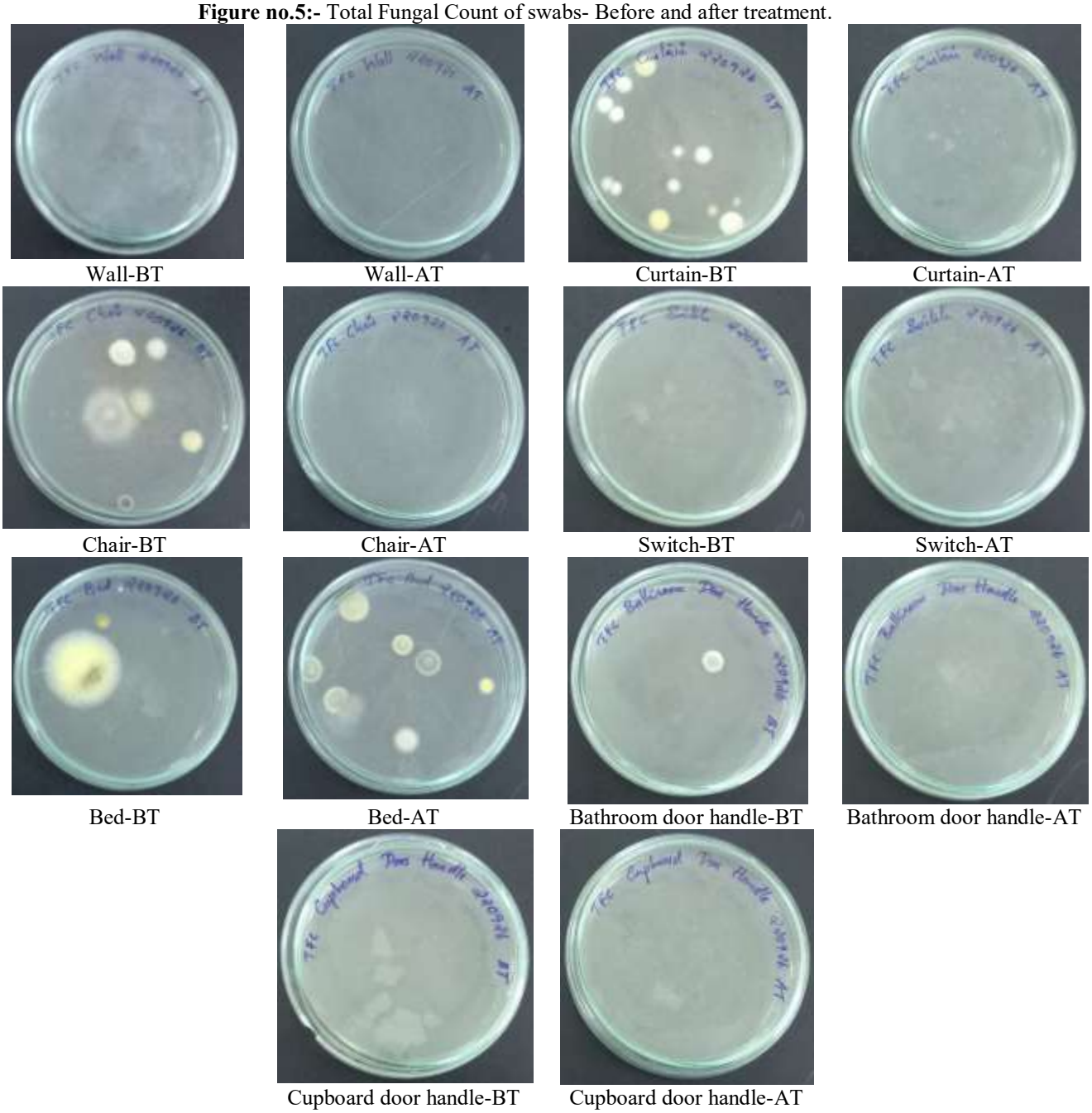

Bathroom door handle-BT


Bathroom door handle-AT


Cupboard door handle-BT


Cupboard door handle-AT

Figure no.5:- Total Fungal Count of swabs- Before and after treatment.



Results and Discussion:-

Room A (Special Room- 325)

Dhoopana with 100 gms of Nagakesaradi DhoopanaYoga showed better results as it reduced the microbial colony formation. It has shown both anti- bacterial and anti- fungal activity.

Room B (Special Room- 310)

Dhoopana with 100 gms of Nagakesaradi Dhoopana Yoga showed better results as it reduced the microbial colony formation. It has shown both anti- bacterial and anti- fungal activity. But a small amount fungal colony was found in Bed after Dhoopana, which may be due the inadequate exposure of fumes over that particular site or may be due to the activity of any particular strain of microorganism. Isolation of that particular strain and further study on that may help to know the reason for the growth.The results are depicted in Figure no:5

The presence Alkaloids, Carbohydrates, Tannins, Saponins, Coumarins and Carboxylic acid in the phytochemical analysis and Eugenol in HPTLC indicates the anti-microbial activity of the Yoga as these chemical constituents are proved for their anti-microbial activities. [8]

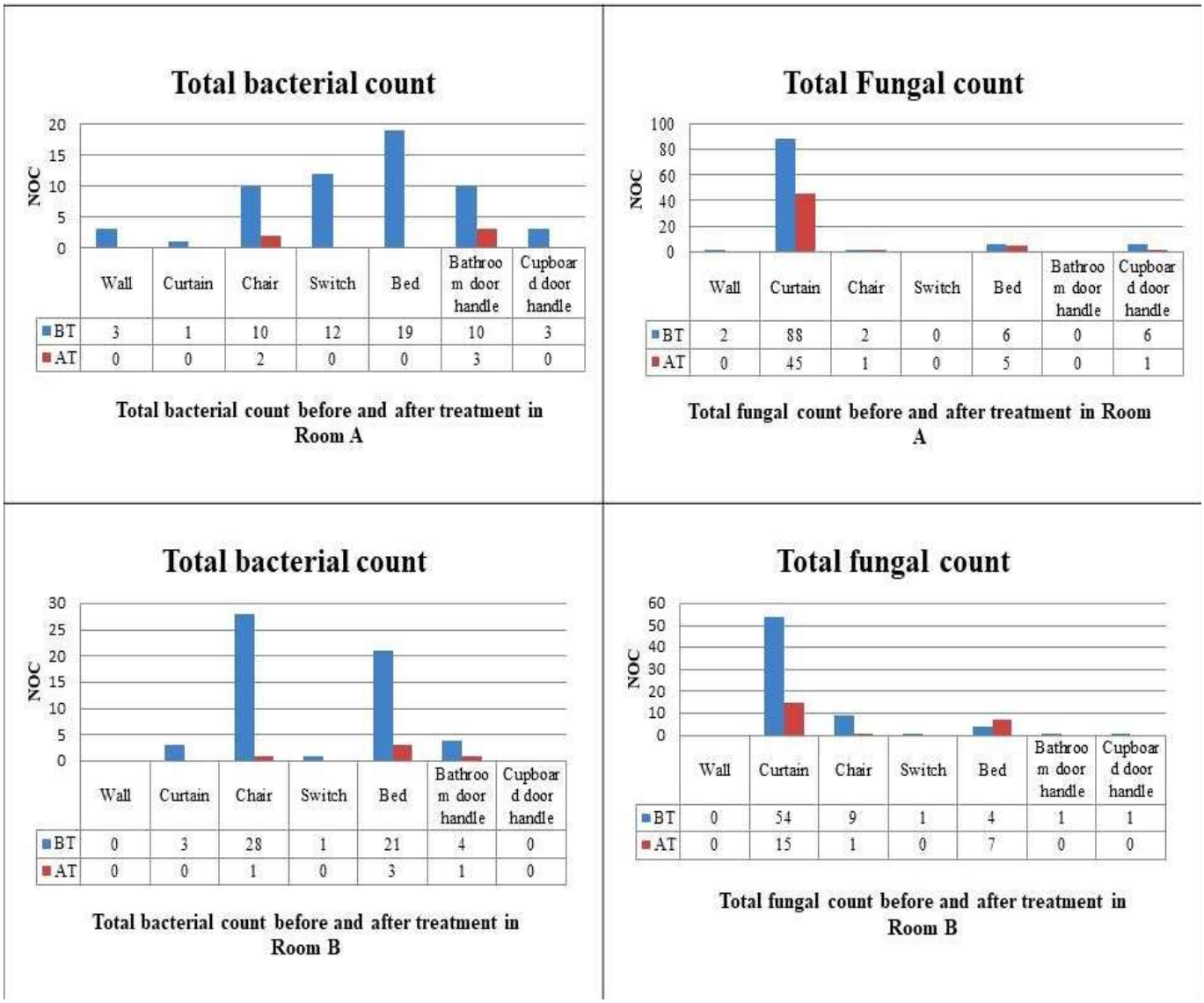


Figure no.6:- Microbial analysis in Room A and B.

Conclusion:-

Maintaining indoor air quality is equally importance as outdoor air quality, as it can help to prevent various contagious diseases. The Experimental study in Hospital rooms shows that Nagakesaradi Dhoopana Yogahas good anti-bacterial and anti-fungal activity.Dhoopana of 100gms of Nagakesaradi Dhoopana Yoga for 45 mins, keeping room closed for further 2 hours shows better results in reduction of bacterial and fungal growth.Dhoopanain hospital room can help to prevent the nosocomial diseases. Hence study concludes that Nagakesaradi Dhoopana Yoga shows better results on microorganisms in indoor environment and can be considered as an effective disinfectant.

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