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An In Vitro Study To Evaluate The Anti-Microbial Effect Of Dhoopana Karma With Nagakesaradi Dhoopana Yoga

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Abstract: **Introduction:** Air is the most important factor for all living being for their existence. It is the same air which is responsible for the spread of various infectious diseases. So purification of air and maintenance of air hygiene is very important to prevent the spread of infectious diseases. Fumigation is the first and foremost procedure which is followed for the disinfection of air as well as the maintenance of the air hygiene. Formaldehyde fumigation is the standard procedure which is followed for the disinfection of air. But Formaldehyde is the known carcinogen and had various health effects. Hence a better option is essential for the disinfective purpose. *Dhoopana* (fumigation) is the classical approach of fumigation, which is used to disinfect *Vranitagara* (post-operative care unit), *Sutikagara* (post-natal care unit), *Kumaragara* (paediatric care unit), *Bheshajagara* (drug preparation unit) etc. as well as for diseases like *Jwara* (Fever), *Vrana* (Wound/Ulcer), *Visha* (Toxin), *Graha* (Possession/ psychiatry) etc. *Nagakesaradi Dhoopana Yoga* which is explained in *Kriyakaumudi* in the context of *Jaladhi shudhikarana* indicated for *Vishavayu* (Polluted air) and against microorganisms. Majority of the drugs of the *Yoga* (Formulation) is *Vishaghna* (Anti-toxic) and *Krimighna* (Anti-helminthic) by *Karma* (Action). **Materials and methods:** This study was taken up to confirm the anti- microbial effect of *Nagakesaradi Dhoopana Yoga* against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Aspergillus niger*. All the drugs were collected, *Nagakesaradi Dhoopana Yoga* was prepared out and Experimental study was conducted. **Observations and Results:** The study proves that *Nagakesaradi Dhoopana Yoga* is having better anti-microbial activity than formaldehyde fumigation. It shows better activity towards *Pseudomonas aeruginosa* and *Aspergillus niger*. **Conclusion:** *Nagakesaradi Dhoopana Yoga* can be used an effective disinfectant.

Index Terms **-** *Nagakesaradi Dhoopana Yoga,* Anti-microbial activity, *Dhoopana, Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger*

I. INTRODUCTION

Air is one of the most important routes of transmission of disease. There are a lot of microorganisms in the exhaled air. These are saprophytic bacteria and may include pathogenic bacteria. When those microorganisms present in the air enter a host cause disease. These organisms are easily discharged into the atmosphere during the act of coughing, sneezing, conversation, and loud talking. [1] *Dhoopana* has an inevitable role in the therapeutics of the Ayurvedic system. The method in which drugs of herbal, herbo-mineral, or animal origin are used for fumigation is called dhoopana to heal various conditions such as *Vrana*(Wound/Ulcer), *Karnarogas*(Diseases of Ear), *Nasarogas* (Diseases of Nose), *Yonivyapath* (Gynaecological disorder), also to disinfect/sterilize *Vranitagara, Kumaragara, Bheshajagara, Sutikagara*, etc., multiple types of *Dhoopana yogas* are mentioned in *Brihatrayis* and other *Samhithas*.[2]

Formaldehyde is the standard chemical which is used for fumigation. But Formaldehyde is the known carcinogen and had various health effects.[3] Hence a better option is essential for the disinfective purpose. *Nagakesaradi Dhoopana Yoga* is explained in the Chapter of *Sthavara Visha Prakarana* in the context of *Jaladhi Shudhikarana*.[4] This *Yoga* is indicated for *Vishadushita Vayu* and against microorganisms. *Nagakesara, Daruharidra, Ela, Twak, Kushta, Priyangu, Laksha, Ativisha, Musta* and *Nirgundi* are the drugs explained in this *Yoga* (Table no.1). Among these *Nagakesara, Daruharidra, Twak, Kushta, Priyangu* and *Ativisha* are having *Vishaghna Karma; Twak, Laksha, Ativisha, Musta* and *Nirgundi* are having *Krimighna Karma*. [5]

II. OBJECTIVE

To evaluate the anti- microbial effect of *Dhoopana karma* with *Nagakesaradi Dhoopana Yoga* on cultured microorganisms.

III. MATERIALS AND METHODS

Preparation and Standardization of *Nagakesaradi Dhoopana Yoga*

All the drugs were collected in equal quantity and *Choorna* (Average Coarse Powder i.e.; 4 mm mesh size) were prepared as per the general method from G.M.P. certified S.D.M. Ayurveda Pharmacy, Kuthpady, Udupi, Karnataka, India. Ingredients of *Nagakesaradi Dhoopana Yoga* are tabulated in Table no.1.

All the standardization parameters including HPTLC were conducted from S.D.M. Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka, India, as a part of the thesis study undergoing in the Department of Agadatantra in S.D.M. college of Ayurveda and Hospital,. Kuthpady, Udupi, Karnataka under Rajiv Gandhi University of Health Sciences and drug was standardized.

Table no.1; Ingredients of *Nagakesaradi Dhoopana Yoga* [6- 8]

Sl. No.	Drugs	Botanical Name	Part Used
1.	***Nagakesara***	*Mesua ferrea* Linn.	Stamens
2.	***Daruharidra***	*Berberis aristata* DC.	Root
3.	***Ela***	*Elettaria cardamomoum* (Linn.) Maton	Fruits and seeds
4.	***Twak***	*Cinnamomum zeylanicum* Blume.	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 from S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi, with all the materials and specimens in the centre

Materials used for the study:

1. Closed glass chamber with a lid of size 11.5ft^3 was taken from S.D.M. Centre for Research in Ayurveda and Allied Sciences
2. *Mrit sharava* (earthern vessel) with lid, was obtained from local market
3. *Nagakesaradi Dhoopana Yoga*
4. The species of Organisms-
   Bacteria: *Pseudomonas aeruginosa* and *Staphylococcus aureus*
   Fungus: *Aspergillus niger*

Organisms were cultured in specially prepared media in S.D.M. Centre for Research in Ayurveda and Allied Sciences.
5. Formaldehyde solution (S.D.M. Centre for Research in Ayurveda and Allied Sciences)
6. Materials required for counting and assessing microorganisms were taken from S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi.

Preparation of microorganisms:

Nutrient Agar media was prepared for both *Staphylococcus aureus* and for *Pseudomonas aeruginosa;* Sabouraud's agar media was prepared for *Aspergillus niger*. *Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeruginosa* (MTCC 8077), *Aspergillus niger* (MTCC 10180) was procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh and inoculum was prepared.

Preparation of Petri dish:

- Prepared media and inoculum was kept in the laminar air flow chamber.
- 1ml of Inoculum with microorganism was pipetted, added to 10ml dilution and mixed properly.
- From above mixture again 1ml was pipetted and added to further consecutive dilution and mixed properly.
- Petri dish was taken, labeled and was poured with the suitable media as per the strain and further added with 1 ml solution from serially diluted samples respectively.
- Mixed uniformly for proper spreading of strains and kept aside and allowed to get solidify.
- One more set of petridish was prepared out by following same procedure as the duplicate one as the standard protocol

Preparation of *Dhoopa* in *Sharava:*

- *Mrit Sharava* was taken and added with sufficient quantity of charcoal
- Charcoal was ignited with the help of Ghee and made in to red hot
- Later 15gms of *Nagakesaradi Dhoopana Yoga* was added to the igniting charcoal

Preparation of Formaldehyde fumes

- 10 ml of Formaldehyde in 100ml of distilled water was taken
- Mixture was taken in a glass beaker
- Kept over the tripod and heated with Bunsen burner
- And fumes were generated

Experiment Procedure:

- Closed glass chamber with a lid of size 11.5ft^3 was taken.
- Experiment was conducted under 4 groups (Table no.2)
- Petri dish with cultured microorganism was kept inside the chamber.
- Later *Sharava* was introduced in to the chamber with fumes and the chamber was kept closed as per the mentioned time period (Table no. 2)
- Same procedure was followed in all 3 strains of microorganisms

Table no. 2; Groups of Experiment

Group A (Negative Group)	Group B (Test group 1)	Group C (Test group 2)	Group D (Standard group)
Only Inoculations of microorganisms was done without intervention.	After inoculation of Microorganisms *Dhoopana* was done with *Nagakesaradi Dhoopana yoga* for 15 mins	After inoculation of Microorganisms *Dhoopana* was done with *Nagakesaradi Dhoopana yoga* for 30 mins.	After inoculation of Microorganisms, 10 ml of Formaldehyde solution in 100ml of was fumigated for 30 mins.

Microbial load Analysis:

- After the procedure, Petri dish of *Staphylococcus aureus* and *Pseudomonas aeruginosa* was kept incubation in Incubator for 24 hours in 37° C and *Aspergillus niger* was 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 15 mins, 30 mins and without intervention.
- Comparison of microbial load was done with the Standard group

Figure no.1; Procedure followed for the experiment

Figure no.1 a; Strain *Staphylococcus aureus*

Figure no.1 b; Strain *Pseudomonas aeruginosa*

Figure no.1 c; Strain *Aspergillus niger*

Figure no.1 d; Collection of Strain

Figure no.1e; Mixing of Strain in dilution

Figure no.1 f; Pouring of medium in to petridish

Figure no.1g; Adding Strain in to medium

Figure no.1 h; Petridish with duplicates were kept for solidifying

Figure no.1i ; *Nagakesaradi Dhoopana Yoga* was weighed

Figure no.1j; Charcoal was ignited

Figure no.1k; *Sharava* with drug was kept inside the

Figure no.1l; Formaldehyde was kept inside the closed

	closed chamber	**chamber**

IV. OBSEVATIONS AND RESULTS

V.

Table no.3; Microbial load in 4 groups

Microorganisms	**Group A (Negative group)**		**Group B (Dhoopana for 15 mins)**		**Group C (Dhoopana for 30 mins)**		**Group D (Standard group)**	
Staphylococcus aureus	1345	1320	949	887	451	526	868	872
Pseudomonas aeruginosa	205	184	0	0	0	0	42	39
Aspergillus niger	51	44	23	21	2	0	44	43

The microbial load analysis of *Nagakesaradi Dhoopana Yoga* against *Staphylococcus aureus* shows that, number of colonies in *Dhoopana* for 15 minutes is almost equal to number of colonies found in fumigation with Formaldehyde for 30 minutes. Whereas colony count was reduced almost to half in case of *Dhoopana* with *Nagakesaradi Dhoopana Yoga* for 30mins as compared with formaldehyde fumigation for 30 mins.

The microbial load analysis of *Nagakesaradi Dhoopana Yoga* against *Pseudomonas aeruginosa* shows that, there was no bacterial growth after 15 and 30 minutes of Dhoopana with *Nagakesaradi Dhoopana Yoga*, whereas bacterial growth was found in formaldehyde fumigation in 30 minutes.

The microbial load analysis of *Nagakesaradi Dhoopana Yoga* against *Aspergillus niger* shows that, number of colonies in *Dhoopana* for 15 minutes is almost half to number of colonies found in fumigation with Formaldehyde for 30 minutes. Whereas colony count was reduced almost to zero in case of *Dhoopana* with *Nagakesaradi Dhoopana Yoga* for 30mins as compared with formaldehyde fumigation for 30 minutes.

Figure no.2; Microbial load analysis of *Nagakesaradi Dhoopana Yoga* against *Staphylococcus aureus*

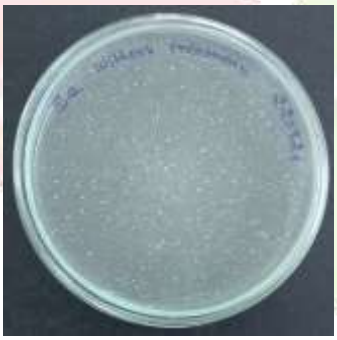

Figure no.2a; Without treatment

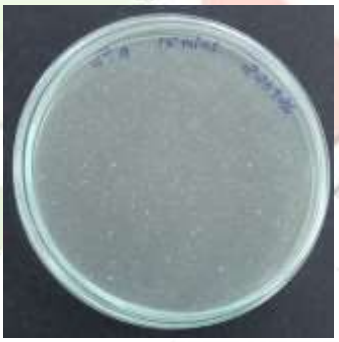

Figure no.2b; 15minutes

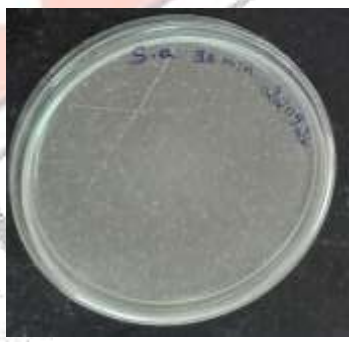

Figure no.2c; 30minutes

Figure no. 3; Microbial load analysis of Formaldehyde against *Staphylococcus aureus* for 30 minutes

Figure no. 4; Microbial load analysis of *Nagakesaradi Dhoopana Yoga* against *Pseudomonas aeruginosa*

Figure no.4a; Without treatment

Figure no.4b; 15minutes

Figure no.34c; 30minutes

Figure no.5 ; Microbial load analysis of Formaldehyde against *Pseudomonas aeruginosa* for 30 minutes

Figure no.6; Microbial load analysis of *Nagakesaradi Dhoopana Yoga* against *Aspergillus niger*

Figure no.6a; Without treatment

Figure no.6b; 15minutes

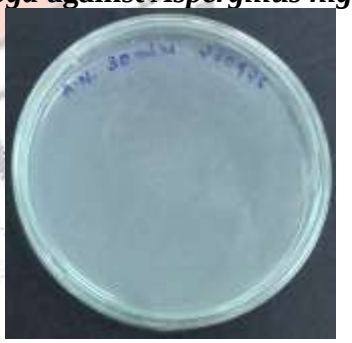

Figure no.6c; 30minutes

Figure no.7; Microbial load analysis of Formaldehyde against *Aspergillus niger* for 30 minutes

VI. DISCUSSION

Experimental study shows that *Nagakesaradi Dhoopana Yoga* showing very effective antibacterial activity against *Pseudomonas aeruginosa* even in 15 minutes of *Dhoopana*, as there was no bacterial growth even at 15 mins after Dhoopana against *Pseudomonas aeruginosa.* Also shows very effective antifungal activity against *Aspergillus niger* in 30 minutes compared to formaldehyde treatment, as there was no fungal growth at 30 mins. *Nagakesaradi Dhoopana Yoga* also showing good antibacterial activity against *Staphylococcus aureus* in 30 minutes compared to formaldehyde. There was considerable reduction of bacterial growth after *Dhoopana* with *Nagakesaradi Dhoopana Yoga*. And this is also evident through the figures no.2-7.

Experimental study shows that *Nagakesaradi Dhoopana Yoga* showing very effective antibacterial activity against *Pseudomonas aeruginosa* even in 15 minutes of *Dhoopana*, as there was no bacterial growth even at 15 mins after Dhoopana against *Pseudomonas aeruginosa.* Also shows very effective antifungal activity against *Aspergillus niger* in 30 minutes compared to formaldehyde treatment, as there was no fungal growth at 30 mins. *Nagakesaradi Dhoopana Yoga* also showing good antibacterial activity against *Staphylococcus aureus* in 30 minutes compared to formaldehyde. There was considerable reduction bacterial growth after *Dhoopana* with *Nagakesaradi Dhoopana Yoga*. As the part of standard protocol the entire study was conducted with a duplicate culture and microbial count from both the culture was taken. And the results of both were compared and that was effective and is depicted in Figure no: 8.

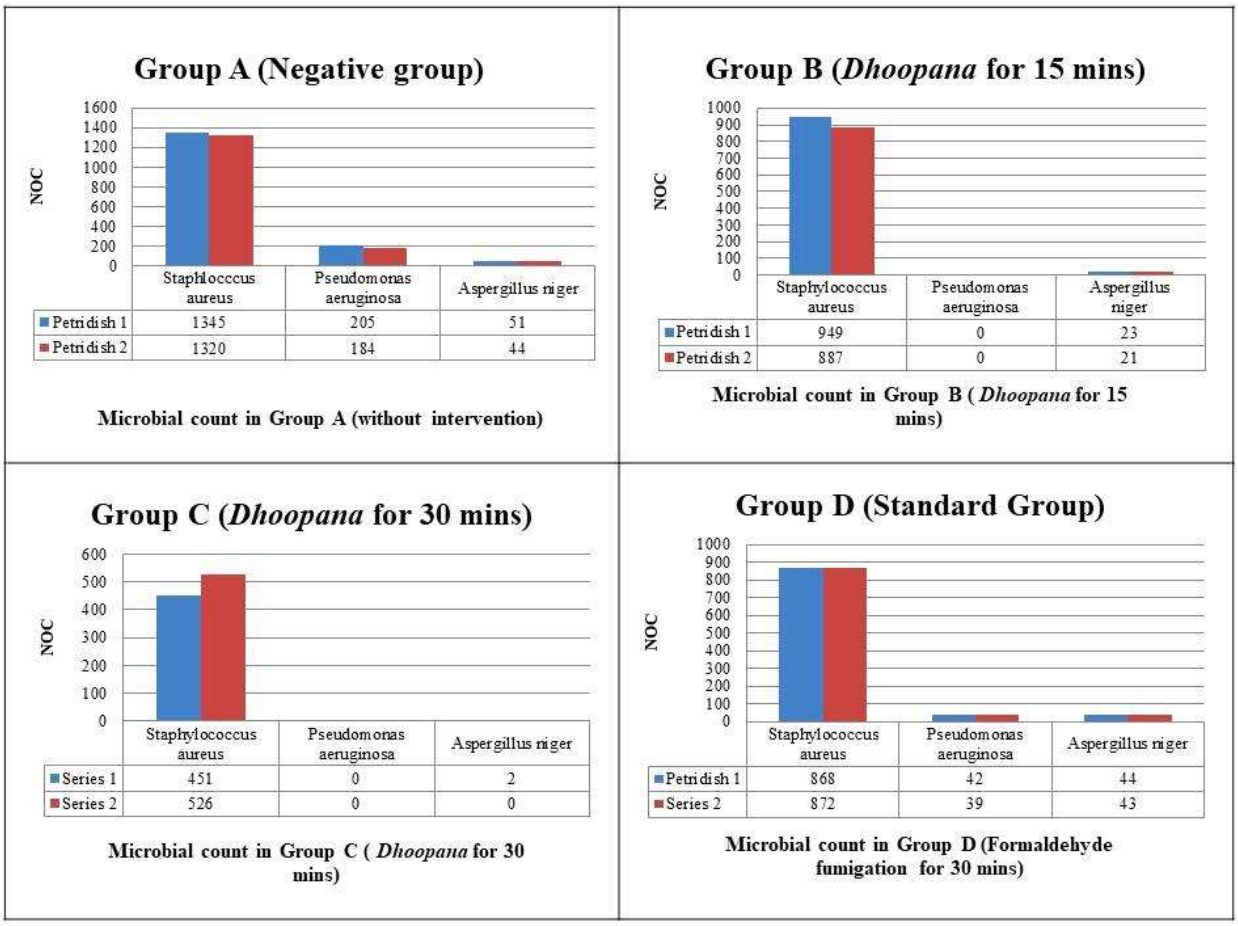

Figure no.8; Microbial analysis in 4 groups

The anti-microbial activity of *Nagakesaradi dhoopana Yoga* is may be due to the *Vishaghna* and *Krimighna* property of its drugs, 55% and 45% of the drugs are having *Vishaghna* and *Krimighna Karma* respectively. Nagakesaradi dhoopana yoga is explained in Kriyakaumudi in the context of *Jaladi shudhikarana* and the *Yoga* is indicated in *Vishavayu* as well as against microorganisms. The results of Pharmaceutico-analytical study of *Nagakesaradi Dhoopana Yoga* reveals the presence of Alkaloids, Tannin, Saponins, Coumarins, Carboxylic acid, which all have proven anti- bacterial and anti-fungal activity. And the Eugenol presence in the HPTLC indicates the strong antimicrobial activity. pH of the *Nagakesaradi Dhoopana Yoga* is 4.82, which is almost nearer to the pH of Formaldehyde, which is the standard drug for fumigation and these highlights its anti-microbial activity.[9]

VII. CONCLUSION

Dhoopana is the classical procedure meant for the disinfection of air, *Nagakesaradi Dhoopana Yoga* is one explained for Vishavayu as well as against microorganisms. Even majority of the drugs of the *Yoga* are *Vishaghna* and *Krimighna* by *Karma*. Hence the *Yoga* was taken up for the study and the experimental study proves that *Nagakesaradi Dhoopana Yoga* has a very good anti- bacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, that too more effective against *Pseudomonas aeruginosa* and better anti-fungal activity nagainst *Aspergillus niger*. Hence Nagakesaradi Dhoopana Yoga can be conidered as a good disinfectant for the purification of air.

Financial support and sponsorship

This was a self-funded study.

Conflicts of interest

There are no conflicts of interest.

VIII. REFERENCE

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