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EVALUATION OF DHOOPANA KARMA (FUMIGATION) USING CHARAKOKTHA DHOOPANA DRAVYAS W.S.R. TO OPERATION THEATRE STERILIZATION

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ABSTRACT

Background and Objectives: Surgical site infections (SSIs) are the second most common cause of hospital-acquired (nosocomial) infections. Ensuring a sterile environment in the operating theatre through effective sterilisation methods, such as fumigation (*Dhoopana Karma*) using herbal drugs, can proficiently disinfect the area. This study seeks to evaluate the antimicrobial effects of Dhoopana dravyas and to standardise their dosage for operating theatre sterilisation. **Methodology:** *Dhoopana Karma* was performed to assess its antimicrobial activity. In light of the insufficient references in classical textbooks regarding the dose and duration of the Dhoopana drug's effects, an initiative was undertaken to standardise the dosage for *Charakoktha Dhoopana Dravyas*. Air and surface samples were collected from operating theatres before and after fumigation at various intervals. A wooden cubicle was placed in the operating theatre to ascertain the appropriate dosage. Fumigation was carried out in the cubicle area using different weights, and the microbial load was evaluated based on microbial counts. The effectiveness of the antimicrobial action of *Dhoopana Karma* was determined by comparing the growth in Petri plates of NA, MSA, and PDA media before and after fumigation. With the established standard dose, the dosage required for larger areas was calculated, and these areas were subsequently fumigated. **Results:** For the *Charakoktha Dhoopana Dravyas,* 1 gram of *Varti* per eight cubic foot area was established as the standard dose,

demonstrating effectiveness from two hours post-fumigation and lasting up to 24 hours in the operating theatre. When larger areas were fumigated using the standard dose, they yielded significant results regarding the microbial load before and after fumigation. **Conclusion:** *Dhoopana* drugs exhibit antimicrobial properties against both bacterial and fungal species.

Keywords: Surgical site infections*, Dhoopana, Varti*, antimicrobial, fumigation, Sterilization.

INTRODUCTION

Ayurveda recommends fumigation (Dhoopana) as a method of sterilisation in various chambers, such as Shastragara, Kumaragara, and Sutikagara. For instruments like Yantra Shastras, sterilisation methods include Payana, Dhoopana, Parishekah, Agnitapana, and others. Numerous drugs are detailed within the procedures for various sterilisation methods, and their indications are addressed. The use of herbal fumigation has been a prevalent practice in Vedic rituals. According to the Vedas, "Surya and Agni are effective agents for destroying Krimi and Rakshasas (the visible and invisible organisms that cause disease). An organism with malignant characteristics that must be guarded against is referred to as Raakshaas, and the measures to expel the Raakshaas are known as "Rakshogna." "Rakshogna Karma," described in Ayurvedic literature, aligns with modern science's aseptic and disinfectant procedures. Many ancient cultures share a belief in the beneficial effects of exposure to certain types of carefully prepared smoke. Purposeful exposure to smoke serves various objectives, including medicinal use, religious obligation, recreation, psychedelic experience, and de-addiction. Other systems of traditional medicine, such as Chinese, Tibetan, and Korean, also widely utilised fumigation for different purposes. The concept of microorganisms is emphasised in Ayurveda through the contexts of Krimi, Bhuta, and Graha, mainly due to unhygienic practices. Management strategies closely align with modern microbiology, focusing on avoiding factors responsible for disease causation, removing microorganisms from the affected site (Apakarsanam), and effecting environmental changes, as Charaka suggested for managing infectious diseases.

Acharya Charaka, In Shaareera Sthana, Jaatisutreeya Shareera Adhyaya has mentioned that Dhoopana Karma to beds, seats, spreads, and apparels of the child with Yava, Sarshapa, Atasi, Hingu, Guggulu, Vacha, Choraka, Bramhi, Jatamamsi, Guggulu, Ashokarohini and slough of snake with ghee[3]. It shows that Dhoopana helps disinfect the environment of that particular area. With the same view, these Dhoopana drugs are adopted for fumigation of the operation theatre. The procedure of Dhoopana Karma on Shasthragara, Soothikagara, etc., is assumed to be effective because drugs like Guggulu[5], Sarshapa[5], etc have Krimighana,

Rakshogna and Grahanashaka's properties kill microorganisms when used as fumes. From the above references, we can opine that Ayurveda has placed greater emphasis on maintaining sterilisation. Here, an attempt was made to standardise, revalidate, and propagate this technique of Dhoopana Karma, which is cost-effective, eco-friendly, and easy to implement. Thus, the glory of Ayurveda can be restored.

METHODOLOGY

MATERIALS AND METHODS:

1. SOURCE OF DATA

(A) EXPERIMENTAL SOURCES:-

1. OT of JSS Ayurvedic Medical College & Hospital, Mysuru.
2. MICROBIOLOGY LAB of JSS College for women, Mysuru.

2. MATERIALS:

1. All dhoopana drugs in raw form,
2. Sterile swabs. Gloves, mask,
3. Corra clothes,
4. Marker pen,
5. Candle,
6. Wooden cubicle of 2×2×2 size,

7. Petri plates
8. Chemical agents for petridish preparation- 0.5% Peptone, 0.3% beef
extract/yeast extract, 0.5% NaCl, MRS media, Distilled water, Agar, Potato,
and Dextrose.

3. METHODOLOGY

1. Preparation of the OT before fumigation[6]:-

1) The fumigating area was cleaned thoroughly with soap water.
2) Close the fumigating area, and small openings were sealed with cellophane tape to
avoid the leakage of fumes.
3) Calculated the fumigating area (Operation theatre) in cubic feet (length × breadth ×
height) to check the required dose of fumigating agent.
4) Adequate care was taken by wearing a cap, mask, foot cover, spectacle, etc
5) For the dose and duration calculation 2×2×2 (length × breadth × height) feet cubicle
was prepared from the wooden plywood and was placed in the operating theatre.

2. Preparation of pellets from raw drugs:-

The following raw drugs (selected parts) were obtained from reliable sources and verified by the Dravyaguna department for their identity, quality, and purity.

1. Yava – Hordeum vulgare L (seeds), 2. Sarshapa – Sesamum indicum (seeds), 3. Atasi - Linum usitatissimum Linn (seeds). 4. Hingu - Ferula narthex Boiss (latex), 5. Guggulu – Commiphora mukul Engl (gum resin). 6. Vacha - Acorus calamus Linn (rhizome), 7. Coraka – Angelica glauca Edgw (root), 8. Brahmi – Bacopa monnieri Pennel (whole plant). 9. Jatamamsi – Nardostachys jatamansi (whole plant), 10. Ashoka Rohini (katuki) – Picrorhiza kurroa (seeds). 11. Slough of snake, 12. Ghee. A total of 500 g of Yava, Sarshapa, Atasi, Hingu, Vacha, Coraka, Brahmi, Jatamamsi, and Ashoka Rohini, along with 30 g of snake slough, were taken and pulverised into a fine powder. All were mixed homogeneously. 6000 g of Guggulu was taken, and the gross impurities were removed. Shodhana was performed in Dolayantra with jala (water) to obtain the semisolid form of Guggulu. After shodhana, approximately 4500 g of Guggulu was acquired in the semisolid form. This was added to the previously prepared powder mixture (4500 g). Moulds of various sizes, lubricated with ghee, were employed to prepare vartis, which were dried in sunlight for 2 days.

3. Swabbing for a culture study

Sterile swabs soaked in normal saline were used to collect the samples from the floor,
wall, light, and OT table. All the samples were sealed and labelled correctly and immediately.
sent to a laboratory for culture. This was done before and after (12hrs) fumigation.

4. Preparation of nutrient agar medium
5. Preparation of mannitol salt agar medium
6. Preparation of PDA(potato dextrose agar) medium
7. Isolating air microflora using the petriplate exposure method for bacterial and
fungal agents**.**

For isolating bacteria – NA (Nutrient agar medium), MSA (Mannitol salt agar medium),
For isolating fungi – PDA (Potato dextrose agar medium) was used.

8. a wooden cubicle measuring 2×2×2 ft (8 cubic feet) was taken to calculate the Drug dose. Soon after the procedures, it was exposed in the Operating theatre (it was contaminated). Then, the box's upper lid was closed with a glass sheet and kept aside.

2 Petri plates containing three different media were placed inside the wooden box for exposure.

Maintain a 45-degree angle for five minutes before returning the plates, closing their lids, and incubating them for approximately 24 hours at 35º-37º C. The interior of the wooden box was fumigated with varying doses starting from 0.5 g of varti. The procedure was repeated using 1 g, 1.5 g, and 2 g varti. At doses of 1 g, 1.5 g, and 2 g, varti showed virtually no count in the Petri dishes. This indicates no contamination; thus, 1 gram of varti was deemed the standard for calculations. The dose is increased for larger areas, such as Minor OT and Major OT.

9. To calculate the effective duration:-
Following fumigation, petri dishes were exposed at various intervals, including half an hour, two hours, and three hours.
Hours: 1, 5, and 7. This was done to determine the effective fumigation exposure duration with 1 gm varti.
Eight trials were repeated to confirm the effective dose and duration with 1 gram, and the effect was observed for up to 15 hours. After obtaining the standard duration and standard drug dose, fumigation was done to major OT and minor OT with an approximate standard dose.
Major OT with Minor OT volume= 18×9×10 cubic feet = 1620 cubic feet
Major OT volume = 22 × 21 × 10 cubic feet = 4620 cubic feet
Cubicle volume = 2×2×2 cubic feet = 8 cubic feet
The dose required to fumigate the cubicle was taken to be 1 gm.
8 cubic feet = 1
For Minor OT = 1620 cubic feet=
162018=202 gms
If 1.5 gms taken
For Minor OT = 1620 cubic feet=
16201.58=303 gms
So, with the dose of 200 gm, the study was carried out in minor OT with six trials and got a nil.
count on almost all plates.
For Major OT = cubic feet=4620
4620 ×1÷8= 577gms
If 1.5 gms taken
For Major OT = 4620 cubic feet=
46201.58=866gms
As there was less contamination in the Major OT, we started the fumigation with less.
dose of 50gms Varti, then with 250gms, 300gms, 350 gms, 400gms and 580gms Vartis.
But at 400 gms dose itself, we got negligible bacterial count. 3 trials were repeated with.
400gms confirm the dose for that area.

10. Settle plate method:-
Air sampling was performed using settled plate methods. Petri dishes containing Nutrient. Agar, Mannitol salt agar, and Potato dextrose agar media were transported to operation.
theatres in sealed plastic bags. The plates were labelled with sample numbers (A and B) and
the date of sample collection. The plates were placed at two chosen places in the wooden box.
and exposed for 15 minutes for inoculation.

11. Air sampling with active exposure method:-
The plates were exposed, holding plates in hands at 45⁰ angles at 4- 5 feet height while
moving in the operating theatre to isolate the microbes for 5 min. After this
exposure, the plates were covered with their lids and taken to the laboratory in sealed plastic.
bags and incubated NA, MSA plates at 37⁰ C for 24 hours, PDA plates at 25⁰C for 3
days. After incubation, colonies were counted manually and noted in the Excel sheets.

12. Method of fumigation:-
Fumigation method:- Ghee-smeared dhoopana Novartis were ignited with candle fire. Then,
These were placed at different places in the OT. Varti started emitting fumes when.
ignited and began to burn independently. The areas were closed until the entire varti was contained.
Completely burnt, and fumes spread throughout the area. Burnt ash was removed.

INCLUSION CRITERIA:-
Contaminated Operative theatre (OT), - Major OT, Minor OT,
Cubicles exposed inside OT.

EXCLUSION CRITERIA:-
Sterilised OT.

ASSESSMENT CRITERIA[7] :
Assessment was done on pre- and post-study of
1) Swab culture test.
2) Microbes in a closed room are assessed through the Petri plate Exposure Method of
1. NA
2. MSA
3. PDA medium.

ASSESSMENT PARAMETERS:
The swab culture, NA, MSA, and PDA plate exposures were done before and after
fumigation at different intervals with different concentrations, then the results were.
assessed.
STATISTICAL METHOD:-
Data collected were entered into MS Excel and analysed using SSPS; descriptive statistical. measures like mean, standard deviation and standard error were applied. TWO WAY
ANOVA, ONE-WAY ANOVA, AND PAIRED T TESTs were applied to find out.
The result. The association were interpreted as statistically significant at P<0.05. Data were.
represented as tables and graphs.

Observations and Results: The cubicle bacterial and fungal count values were placed at significant intervals with varied durations.
MAJOR OT- NA media Table 1:- Mean CFU counts of NA media with varied dosages at various durations.

Duration /dose	0.5gms		1.0gms		1.5 gms		2gms		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BF	13.00	4.24	15.00	4.24	19.00	7.07	24.00	14.14	17.75	7.81
AF – 1 hr	1.50	2.12	1.00	0.00	0.00	0.00	0.00	0.00	0.62	1.06
AF- 3 hr	2.50	0.71	0.00	0.00	0.00	0.00	0.00	0.00	0.62	1.19
AF- 6 hr	0.50	0.71	0.50	0.71	0.50	0.71	0.00	0.00	0.37	0.52
Total	4.37	5.68	4.12	6.92	4.80	9.13	6.00	12.32	4.84	8.47

Table 2:- Summary results of 2-way ANOVA for Mean CFU counts of NA media with varied dosages at various durations.

Source of variation	F value	P value
Dose	.303	.823
Duration	32.403	.000
Dose × duration	.839	.592

Between doses, a non-significant difference was observed in the mean CFU counts as the observed F value of 0.303 was found to be non-significant (p=.823). We discovered that BF there was a maximum CFU count and later decreased significantly over durations.

MAJOR OT- MSA media
Table 3:- Mean CFU counts of MSA media with varied dosages at various durations.

Duration /dose	0.5gms		1.0gms		1.5 gms		2gms		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BF	24.5	3.53	15	1.41	13	4.24	24.5	2.12	19.25	6.11
AF-1hr	1.5	0.70	0	0	0	0	0	0	0.37	0.74
AF- 3 hr	0.5	0.70	0	0	0	0	0	0	0.12	0.35
AF- 6 hr	1	1.41	0	0	0	0	0	0	0.25	0.70
Total	6.87	10.98	3.75	6.96	3.25	6.22	6.12	11.36	5	8.86

Table 4 - Summary results of 2-way ANOVA for Mean CFU counts of MSA media with varied dosages at various durations.

Source of variation	F VALUE	P VALUE
Dose	10.03	.001
Duration	288.83	.000
Dose × duration	6.86	.000

We found that BF there was a maximum CFU count and later decreased significantly over durations. Lastly, the interaction effect between doses and durations was significant (F=6.86; p=.000), indicating a pattern of similarity in the CFU in different durations, irrespective of their dosage.

MAJOR OT- PDA media

Table 5:- Mean CFU counts of PDA media with varied dosages at various durations.

Duration /dose	0.5gms		1.0gms		1.5 gms		2gms		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BF	10	2.82	13.5	2.12	9	4.24	13	7.07	11.37	3.96
AF-1hr	1	1.41	0.5	0.70	0	0	0	0	0.37	0.74
AF- 3 hr	1	1.41	0	0	0	0	0	0	0.25	0.70
AF- 6 hr	0	0	0.5	0.70	0	0	0.5	0.70	0.25	0.46
Total	3	4.53	3.62	6.16	2.25	4.46	3.37	6.52	3.06	5.25

Table 6:- Summary results of 2-way ANOVA for Mean CFU counts of MSA media with varied dosages at various durations.

Source of variation	F	Sig.
Dose	.53	.665
Duration	45.71	.000
Dose × duration	.49	.854

We found that BF there was a maximum CFU count and later decreased significantly over durations. Lastly, the interaction effect between doses and durations was found to be non-significant (F=.49; p=.854), indicating a pattern of similarity in the CFU in different durations, irrespective of their dosage.

VALUES OF BACTERIAL AND FUNGAL COUNTS OF CUBICLE PLACED AT MAJOR OT WITH VARIED DOSAGE AT VARIOUS DURATIONS.

MINOR OT- NA media

Table 7:- Mean CFU counts of NA media with varied dosages at various durations.

Duration /dose	0.5gms		1.0gms		1.5 gms		2gms		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BF	27.5	9.19	23.5	9.19	14.5	3.53	18.5	6.36	21	7.70
AF-1hr	0	0	1	0	1	0	0	0	0.5	0.53
AF- 3 hr	0.5	0.70	0.5	0.70	0	0	0.5	0.70	0.37	0.51
AF- 6 hr	2.5	2.12	1.5	2.12	0.5	0.70	0	0	1.12	1.55
Total	7.	12.81	6.62	11.01	4	6.63	4.75	8.82	5.75	9.70

MINOR OT- MSA media

Table 9:- Mean CFU counts of MSA media with varied dosages at various durations.

Duration /dose	0.5gms		1.0gms		1.5 gms		2gms		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BF	21.5	4.94	21.5	0.70	33	7.07	22	4.24	24.5	6.39
AF-1hr	0.5	0.70	1	1.41	0	0	0	0	0.37	0.74
AF- 3 hr	0	0	0	0	0	0	0	0	0	0
AF- 6 hr	1.5	0.70	0	0	0	0	0	0	0.37	0.74
Total	5.87	9.84	5.62	9.82	8.25	15.50	5.5	10.30	6.31	11.10

MINOR OT- PDA media

Table 10:- Mean CFU counts of PDA media with varied dosages at various durations.

Duration /dose	0.5gms		1.0gms		1.5 gms		2gms		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BF	10	2.82	13.5	4.94	21	1.41	7	2.82	12.87	6.10
AF-1hr	0	0	1	1.41	0	0	0	0	0.25	0.70
AF- 3 hr	1.5	0.70	0.5	0.70	0	0	0	0	0.5	0.75
AF- 6 hr	5	2.82	0	0	0.5	0.70	0.5	0.70	1.5	2.44
Total	4.12	4.38	3.75	6.34	5.37	9.66	1.87	3.35	3.78	6.22

WITHIN GROUPS COMPARISONS OVER DURATIONS (DOSE AT 0.5GMS)

Table 12:- Mean difference before fumigation to different durations after fumigation of different media.

	BF to AF-1HR		BF to AF-3HR		BF to AF-6HR	
MEDIUM	Mean difference between BF and AF – 1 hr	P Val-ue	Mean Difference between BF and AF – 3hr	P Val-ue	Mean Difference between BF and AF – 6 hr	P Val-ue
NA – Major OT	11.50	.076	10.50	.075	12.50	.054
MSA- Major OT	23.00	.012	24.00	.011	23.50	.013
PDA- Major OT	9.00	.057	9.00	.057	10.00	.038
NA – Minor OT	27.50	.052	27.00	.054	25.00	.064
MSA-Minor OT	21.00	.027	21.50	.025	20.00	.030
PDA- Minor OT	10.00	.038	8.50	.054	5.00	.219

We found a significant decrease from before fumigation to 6 hours after fumigation. However, for the rest of the press, t-tests revealed non-significant differences in their mean values.

WITH THE DOSE- 1GM

Table 13:- Mean difference before fumigation to different durations after fumigation of other media.

	BF to AF-1HR		BF to AF-3HR		BF to AF-6HR	
MEDIUM	Mean difference between BF and AF – 1 hr	P Val-ue	Mean Difference between BF and AF – 3hr	P Val-ue	Mean Difference between BF and AF – 6 hr	P Val-ue
NA – Major OT	14.00	.043	15.00	.038	14.50	.041

MSA- Major OT	15.00	.004	15.00	.004	15.00	.004
PDA- Major OT	13.00	.014	13.50	.012	13.00	.014
NA – Minor OT	22.50	.074	23.00	.072	22.00	.081
MSA-Minor OT	20.50	.003	21.50	.001	21.50	.001
PDA- Minor OT	12.50	.075	13.00	.067	13.50	.061

We find a significant decrease from before fumigation to 6 hours after fumigation. However, t-tests revealed non-significant differences in the mean values for the rest of the media.

WITH THE DOSE- 1.5 GM

Table 14:- Mean difference before fumigation to different durations after fumigation of other media.

	BF to AF-1HR		BF to AF-3HR		BF to AF-6HR	
MEDIUM	Mean difference between BF and AF – 1 hr	P Value	Mean Difference between BF and AF – 3hr	P Value	Mean Difference between BF and AF – 6 hr	P Value
NA – Major OT	19.00	.063	19.00	.063	18.50	.067
MSA- Major OT	13.00	.049	13.00	.049	13.00	.049
PDA- Major OT	9.00	.095	9.00	.095	9.00	.095
NA – Minor OT	13.50	.033	14.50	.028	14.00	.032
MSA-Minor OT	33.00	.022	33.00	.022	33.00	.022
PDA- Minor OT	21.00	.002	21.00	.002	20.50	.003

We found a significant decrease from before fumigation to 6 hours after fumigation. However, t-tests revealed non-significant differences in the mean values for the rest of the media.

WITH THE DOSE – 2GMS

Table 15:- Mean difference before fumigation to different durations after fumigation of other media.

	BF to AF-1HR		BF to AF-3HR		BF to AF-6HR	
MEDIUM	Mean difference between BF and AF – 1 hr	P Value	Mean Difference between BF and AF – 3hr	P Value	Mean Difference between BF and AF – 6 hr	P Value
NA – Major OT	24.00	.138	24.00	.138	24.00	.138
MSA- Major OT	24.50	.004	24.50	.004	24.50	.004
PDA- Major OT	13.00	.122	13.00	.122	12.50	.131
NA – Minor OT	18.50	.054	18.00	.058	18.50	.054
MSA-Minor OT	22.00	.018	22.00	.018	22.00	.018
PDA- Minor OT	7.00	.073	7.00	.073	6.50	.088

We found a significant decrease from before fumigation to 6 hours after fumigation. However, t-tests revealed non-significant differences in the mean values for the rest of the media.

Table 16:- Difference variants over various durations before fumigation mean CFU.

Difference between BF to	0.5hr		2 hrs		3 hrs		5 hrs		7hrs	
MEDIUM	MD	P	MD	P	MD	P	MD	P	MD	P

NA – Major OT	7.00	.111	8.50	.077	8.50	.077	8.50	.077	8.00	.088
MSA-Major OT	10.00	.059	10.50	.052	10.50	.052	10.50	.052	10.50	.052
PDA- Major OT	3.50	.089	6.50	.006	6.50	.006	6.50	.006	6.50	.006
NA – Minor OT	27.00	.056	38.50	.027	38.50	.027	37.50	.029	32.00	.039
MSA-Minor OT	13.00	.067	19.50	.031	19.50	.031	19.00	.033	16.00	.052
PDA- Minor OT	8.00	.037	9.50	.024	9.00	.030	9.50	.024	7.50	.053

We find a significant decrease from before fumigation to 7 hours after fumigation. However, t-tests revealed non-significant differences in the mean values for the rest of the media.

The cubicle was placed at Major and Minor OT, and fumigation was performed using a one-way ANOVA test and a 1 gm varti dose.

Table 17:- Mean CFU counts of NA, MSA, and PDA media with 1gms dosage at various durations in Cubicle.

Medium	NA			MSA			PDA		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
BF	14.12	6.93	1.73	19.5	12.01	3.00	12.06	7.37	1.84
AF-2hrs	1.06	0.92	0.23	1	1.26	0.31	0.81	1.27	0.31
AF-15hrs	0.56	0.72	0.18	0.43	0.62	0.15	0.37	0.71	0.17
TOTAL	5.25	7.48	1.08	6.97	11.26	1.62	4.41	6.92	0.99
F value	57.335			38.57			37.217		
P value	.000			.000			.000		

Comparisons over durations (Dose at 1gms)

Table 17:- Summary result of T-Test for Mean CFU counts of NA, MSA and PDA media difference with different durations.

Difference between BF to	2 hrs		15 hrs	
Medium	MD	P	MD	P
NA	13.06	0	13.56	0
MSA	18.5	0	19.06	0
PDA	11.25	0	11.68	0

We found a significant decrease from before fumigation to after 2 and 15 hours.

IN MAJOR OT- WITH NA MEDIUM

Table 18:- Mean CFU counts of NA media with varied dosages at various durations.

Duration /dose	50gms-n=2		250gms-n=2		300 gms-n=2		350gms-n=2		400gms – n =6		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BF	79	4.24	26.5	6.36	24.5	10.60	3.5	3.53	12.66	8.71	24.50	25.16
AF-2hrs	6.5	2.12	1	1.41	0.5	0.70	2.5	0.70	1	0.89	1.92	2.23
AF-15hrs	4.5	3.53	0	0	0.5	0.70	0.5	0.70	0.33	0.51	.92	1.85

AF-24hrs	4	5.65	0	0	0	0	0.5	0.70	0.83	1.32	1.00	2.21
Total=56	23.5	34.40	6.87	12.36	6.37	11.89	1.75	1.98	3.70	6.71	7.08	15.99

In major OT- with MSA medium

Table 19:- Mean CFU counts of MSA media with varied dosages at various durations.

Duration /dose	50gms-n=2		250gms-n=2		300 gms-n=2		350gms-n=2		400gms,n=6		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BF	79	4.24	26.5	6.36	24.5	10.60	3.5	3.53	12.66	8.71	24.5	25.1
AF-2hrs	6.5	2.12	1	1.41	0.5	0.70	2.5	0.70	1	0.89	1.92	2.23
AF-15hrs	4.5	3.53	0	0	0.5	0.70	0.5	0.70	0.33	0.51	.92	1.85
AF-24hrs	4	5.65	0	0	0	0	0.5	0.70	0.83	1.32	1.00	2.21
Total=56	23.5	34.4	6.87	12.36	6.37	11.89	1.75	1.98	3.70	6.71	7.08	15.9

In major OT- with PDA medium

Table 20:- Mean CFU counts of MSA media with varied dosages at various durations.

Duration /dose	50gms-n=2		250gms-n=2		300 gms-n=2		350gms-n=2		400gms, n =6		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BF	10	2.82	13	1.41	9	1.41	7	1.41	13.5	9.73	11.35	6.64
AF-2hrs	0	0	1	1.41	1	1.41	0.5	0.70	0.5	1.22	.57	1.01
AF-15hrs	0.5	0.70	1	0	0.5	0.70	0.5	0.70	0.5	0.54	.57	.51
AF-24hrs	0	0	0	0	0.5	0.70	0.5	0.70	1	1.26	.5714	.93
Total=56	2.62	4.68	3.75	5.77	2.75	3.95	2.12	3.09	3.87	7.32	3.26	5.75

Table 21: Mean difference in CFU counts of Major OT- NA, MSA, and PDA media at multiple durations.

Difference between BF to	2 hrs		15 hrs		24 hrs	
Medium	MD	P	MD	P	MD	P
NA	22.57	0.00	23.57	0.002	23.5	0.002
MSA	22.57	0.00	23.57	0.002	23.5	0.002
PDA	10.78	0.00	10.78	0.00	10.78	0.000

We found a significant decrease from before fumigation to after 2-, 15- and 24-hour fumigation.

Minor OT- Fumigated with 200 gms doses with five trials – one-way ANOVA test.

Table 22:-Mean CFU counts of NA, MSA, and PDA medium at various durations.

Medium	NA			MSA			PDA		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
BF	32.40	7.58	2.40	26.60	23.10	7.30	18.90	9.08	2.87
AF-2hrs	1.30	1.49	.47	3.60	3.09	.97	.60	1.07	.33
AF-15hrs	.80	.63	.20	1.40	1.34	.42	.80	.91	.29
TOTAL	11.5	15.64	2.85	10.5	17.42	3.18	6.7	10.11	1.84
F value	57.335			38.577			37.217		
P value	.000			.000			.000		

Graph:- 1

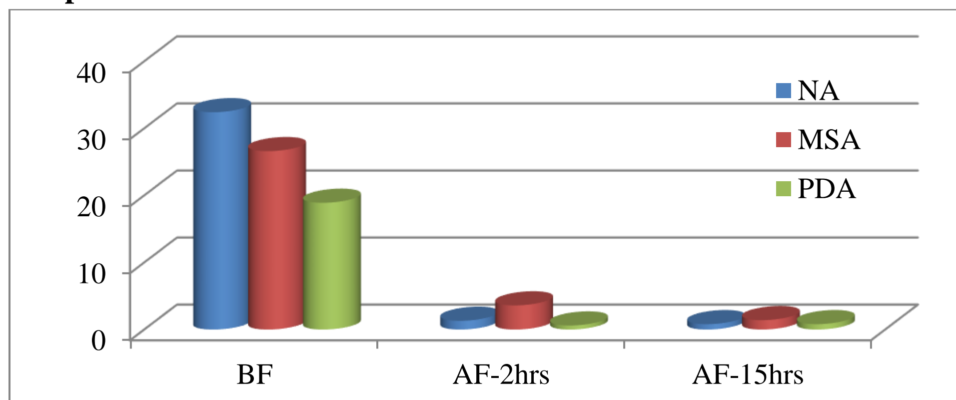

Table 41 presents the mean difference in CFU counts of Minor OT-NA, MSA, and PDA media at various durations.

CULTURE AND SENSITIVITY REPORT OF SURFACE SWAB REPORT MAJOR OT: At various intervals, swabs were taken for culture from locations such as the floor, wall, OT table, and OT light. Gram-negative bacilli and a few Gram-positive cocci were detected in samples from the OT table after surgical procedures were performed on contaminated cases. Subsequently, fumigation was conducted, and samples taken from the same sites showed no growth.

Commonly found bacteria and fungi on NA, MSA, and PDA plates include the following: In the MSA plate: 1. Staphylococcus aureus 2. Staphylococcus epidermidis. On the NA plate: Based on gram-staining properties, the bacteria are cocci and bacilli. In the PDA plate: The commonly found bacteria and fungi are Aspergillus brasiliensis, Aspergillus flavus, Aspergillus niger, Penicillium spp., Cladosporium spp., and Helminthosporium spp.

DISCUSSION

DISCUSSION ON OBSERVATION AND RESULTS: The pathogens isolated from infections vary primarily based on the type of surgical procedure. Staphylococcus aureus, derived from the patient's skin flora, is the usual cause of disease. When mucous membranes or skin are incised, the exposed tissues are at risk of contamination by endogenous flora. Approximately 20 to 30% of surgical site infections are caused by S. aureus, with over half originating from the endogenous flora[8]. In the present study, we utilised MSA high media to isolate Staph aureus, and following fumigation with the study drug, we demonstrated a highly favourable outcome against Staph aureus bacteria. This study revealed higher bacterial contamination was detected in air samples than in surface or article samples before fumigation. In both air and surface samples, minimal growth of contaminated bacteria was observed in the Major OT, indicating a reduced risk of secondary infections. Conversely, Minor OT exhibited more growth in the media before fumigation, likely due to being a fully ventilated area where personnel entry is unrestricted and continuous procedures are regularly conducted.

Discussion on the results of mean counts across various media and doses at different durations—When the study was conducted in a cubicle, Major OT with varying doses at different intervals revealed significant results for NA media, with an F value of 32.403 and a p-value of .001 indicating a difference between durations. The dose and dose-duration variants analyses showed non-significant results due to the different doses, such as 0.5, 1, 1.5, and 2 grams. Consequently, at lower doses, mean CFU counts remained similar to previous counts, with only minor differences observed.

The significance of MSA media was noted across all dose differences (F=10.03, p= .001), duration differences (F=288.83; p=.000), and both doses coupled

with duration differences (F=6.86; p=.000). Even at the lower doses, MSA plates showed no counts, indicating that this dhoopana varti is more effective against Staphylococci. A significant result for PDA media was found in the differences between durations (F=45.71; p=.000). We observed entirely nil CFU counts following two hours of fumigation. A study in a cubicle featuring a Minor OT with varying doses at different intervals revealed significant results for NA media, with an F value of 56.84 and a p-value of .000, reflecting differences between durations. The significance of the variables involving dose and dose with duration was evident, as there were varying doses, including 0.5, 1, 1.5, and 2 grams; thus, the mean CFU counts at the lower doses were comparable to those observed previously, with few differences noted.

MSA media significance was observed for duration difference (F=196.06; p=.000) and both doses with duration difference (F=2.91; p=.013), PDA media significance was observed for all dose differences (F=4.92; p= .013) duration difference (F=87.00; p=.000) and both dose with duration difference (F=6.70 p=.001).

Within groups, durations were compared with different dose trials. More changes were observed for MSA and PDA media than NA media counts, so significant results were found.

Discussion on time kinetic results with a standard dose of 1gm varti - Between duration differences, a significant difference was observed in the mean CFU counts of all mediums. In Major OT- NA with F value = 10.022 (P=.007), for MSA with F value = 16.677, (P=.002), for PDA with F value = 34.760, (P=.001), In Minor OT- NA with F value = 29.677 (P=.001), for MSA with F value = 22.800, (P=.001), for PDA with F value = 21.240, (P=.001) showed highly significant result. There was a substantial reduction in microbial counts over 2nd hour after fumigation and later on, but after 5 hours again, microbial counts were observed. This may be because the closed area was opened repeatedly for plate exposure, and outer environment contaminations might have entered that area. This happened more at Minor OT than at Major OT.

Discussion on the result of Cubicle fumigation with a Standard dose of 1 gms varti - The mean CFU counts at BF, AF-2 hr and AF-15 hrs of NA, MSA and PDA showed a significant result with the p-value of .000. And even in comparison to before to after 2 hours and after 15 hours of after fumigation showed a significant result. We found that BF there was a maximum CFU count and later decreased significantly over durations.

Discussion of the results of Major OT fumigation mean CFU counts for doses 50, 250, 300, 350, and 400 grams at different intervals: We observed significant results for NA and MSA with dose, duration, and dose-with-duration differences, but for PDA media, we found significant results only for the dose. Because we observed that the fungi die even after a bit of exposure to fumes, significant changes were seen over the duration and dose-with-duration.

Discussions on the results of Minor OT fumigation at a dose of 200 grams at two different intervals—after 2 hours and after 15 hours—revealed significant mean CFU counts at BF, AF-2 hr, and AF-15 hr for NA, MSA, and PDA, with a p-value of 0.000. Moreover, comparisons between the counts before fumigation and after 2 and 15 hours also showed significant results. We found that BF had the highest CFU count, which decreased significantly over time as we employed the standard dose for fumigation.

CONCLUSION

Dhoopana, or herbal fumigation using Charakoktha Dhoopana Dravyas, is effective against microorganisms. This study observed that Dhoopana drugs possess antimicrobial properties against bacterial and fungal species. The Dhoopana drugs, prepared in the form of Varti, were found to be readily accessible. The standard dose of 1 gram of Charakoktha Dhoopana Dravyas for an area of eight cubic feet was effective for up to 24 hours in the operating theatre. This study noted that no adverse reactions were affecting the eyes, skin, or respiratory system.

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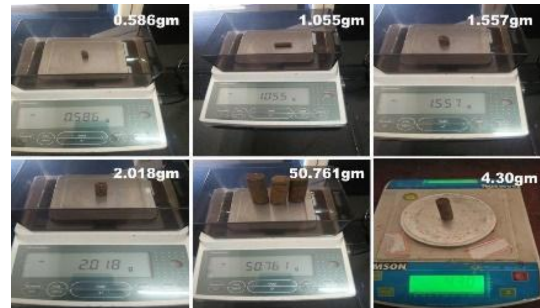
Preparation Guggulu Shodhana   Preparation of Varthi



Prepared Varthis with different weights (in grams)

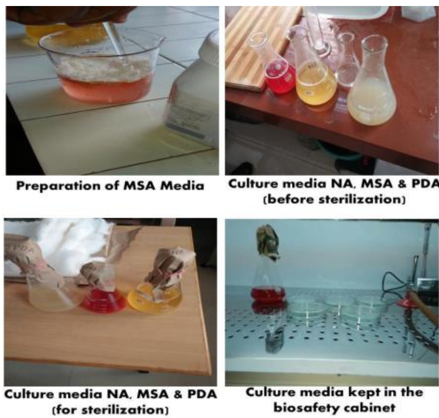
Prepared Varthis

Prepared Varthis

Slicing of Potato for media preparation

Preparation of Potato Infusion

Prepared media placed in Bio-safety chamber



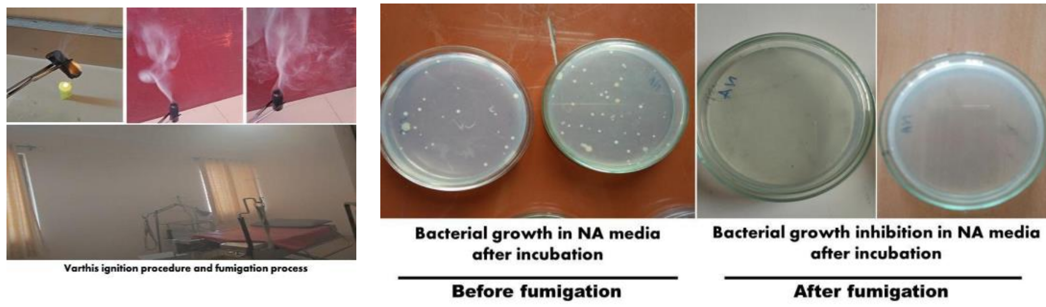
Preparation of MSA Media

Culture media NA, MSA & PDA (before sterilization)

Culture media NA, MSA & PDA (for sterilization)

Culture media kept in the biosafety cabinet

Fumigation Process inside the Cubicle



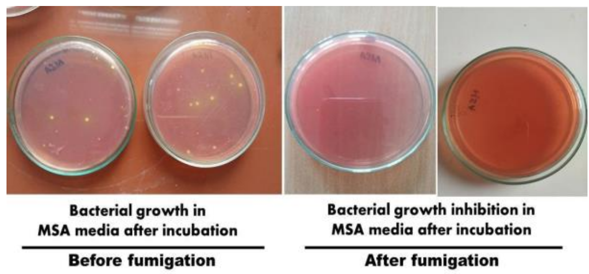
Varthis ignition procedure and fumigation process

Bacterial growth in NA media after incubation

Before fumigation

Bacterial growth inhibition in NA media after incubation

After fumigation



Bacterial growth in MSA media after incubation

Before fumigation

Bacterial growth inhibition in MSA media after incubation

After fumigation