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EVALUATION OF ANTIMICROBIAL AND ANTIFUNGAL PROPERTY OF DHOOPANA KARMA (FUMIGATION) – BY "DHUP"AN AYURVEDIC DHOOPANA PRODUCT

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ABSTRACT: There are millions of micro-organisms around us, amongst 20-25% are non pathogenic. Pathogenic microbes present in the environment around us are responsible for various health related problems**.** Reducing microbial load to non-pathogenic levels may be the purpose of traditional fumigation techniques like Dhoopana, Homa, and Havana etc. This is also evident from Ayurvedic literature wherein fumigation is indicated for sterilisation of operation theatres as well as labour theatres and also in wound management. Taking all these into consideration, in present study an attempt is made to estimate anti microbial effect of *Dhoopana* of an indigenous fumigation formulation 'DHUP'. Two rooms of same size which are in routine usage in almost similar fashion were selected for the study. One room served as control without any intervention. In the second room *Dhoopana* was performed once daily for two days. In both the rooms agar gel plates were exposed at selected places and efficacy of DHUP *Dhoopana* was noted at selected intervals. The microbial load was estimated and subjected to statistical analysis. The study revealed that the effect of *Dhoopana* was significant in comparison to control both during study period and follow up period up to 6 days aganist bacteria and fungus.

INTRODUCTION: The air around us is filled with millions of micro-organisms such as Bacteria, fungi, protozoa, and viruses etc. Almost 37% of our house hold dusts are contributing by these micro organisms. The numbers of microbes in the air range from 10 to 10,000 per cubic meter. They live at temperatures less than -20 degrees Celsius to temperatures hotter than the boiling point. Where, there are microbes which need moisture to live and reproduce. Often, when people think about microbes, they think of disease also. But all the microbes are not pathogenic. Among the total microbes 20-25% are non pathogenic. Remaining microbes residing in air are responsible for various diseases.

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The prevalence shows that, air born diseases are more compare to vector born diseases. Even gram positive cocci (*Staphylococcus Aureus*) lives in nostrils of 30% diseased population. Air around us can get contaminated by such pathogens. Thus it carries a lot of significance to disinfect the area where we reside, which in turn result anticipation of many infectious diseases. Hence it is the right time to think about the control measures of infectious agents in terms of preventive as well as curative measures of diseases.

In Ayurveda, the term *krumi* has been used in broader senceie, it includes all pathogenic and non pathogenic organisms covering wide range of infection and infestation. They are explained under the tittle of *oupasargarogas*, which spread through different routes. To get prevention from *krumi* (microbes), *Rakshoghnavidhi* is indicated in our classics. In this various medicinal plants were burnt on fire and the smoke generated from it used to make starilization of different areas where chance

of infections are more. [1, 2] The references of *janapadodwansarogas*, Method of treating *vrana* (Wound management), *vranitaagara* (Casuality), *sutikagara* (Labour theatre) and *shastrakarmaghruha* (Operation theatre) by various *dhoopanadravyas* like *rakshoghnadhupa*, elaborately has been explained by acharyas.

All references comeunder the concept of *Dhoopana karma*.[3] These descriptions clearly indicate that Dhoopana is being used for disinfecting areas such as Operation theatre, Labour theatre etc by reducing microbial load around us as a daily routine and also as a curative measures for wound management.

In the present context Dhoopana yoga is prepared out of the drugs indicated for *Krimighna.* Though most of the selected drugs individually reported for antimicrobial property, it is proposed that in combination the yoga may exert maximum **Ingredients:** Antimicrobial property. With this hypothesis the study has been carried out.

Aims & objectives:
To assess the antimicrobial& antifungal property of 'DHUP' *Dhoopana*.

Study design:
Comparative study (Control verses Test drug)

MATERIAL AND METHODS:

Preparation of 'DHUP':
The formulation is prepared at GMP certified KLEU'S Ayurvedic pharmacy under experts supervision. The drugs which are indicated for Dhupana and Krumihara property classically are selected and taken in below mentioned proportion. All the drugs were authenticated in Central Research Facility of Shri. B M K Ayurveda Mahavidyalaya. The ingredients are coarse powdered (Mesh size 40-60)[4] separately and homogenous mixture is prepared by mixing all the ingredients.

TABLE 1: SHOWING COMPOSITION OF DHUP: [5]

Sr No.	Dravya	Latin Name	Family	Official part	Proportion
1	Guggulu	*Commiferamukul* Engl	Burseraceae	Niryasa	1.5 parts
2	Ushira	*Vetiveriazizanoids* Linn	Graminae	Root	1.5 parts
3	Vacha	*Acoruscalamus* Linn	Araceae	Rhizome	1 part
4	Rala	*Shorearobusta* Gaertn.	Dipterocarpaceae	Niryasa	1.5 parts
5	Nimba	*Azadirectaindica* Juss.	Meliaceae	Stem bark	1.5 parts
6	Arka	*Calotropisprocera* Linn	Asclepiadaceae	Moola	1.5 parts
7	Devadaru	*Cedrusdeodara* Loud.	Pinaceae	Bark	1.5 parts
8	Loban	*Boswelliacarterii* Roxb.	Bursuraceae	Niryasa	3 parts

TABLE 2: SHOWING PHARMACODYNAMICS OF DHUPDRAVYA: [6]

Sr No.	Dravya	Rasa	Guna	Virya	Vipaka	Karma
1	Guggulu	Tikta, Katu	Laghu, Ruksha	Ushna	Katu	Kushtaghna,Lekhana
2	Ushira	Tikta, Madhura	Ruksha , Laghu	Sita	Katu	Kushtaghna
3	Vacha	Katu, Tikta	Laghu, Tikshna	Ushna	Katu	Krimighna
4	Rala	Kashaya, Madhura	Ruksha, Ushna	Sita	Katu	Krimighna, Kandughna
5	Nimba	Tikta, Kashaya	Laghu, Ruksha	Sita	Katu	Krimighna
6	Arka	Katu, Tikta	Laghu, Ruksha	Ushna	Katu	Krimighna, Kushtaghna
7	Devadaru	Tikta, Kashaya	Ruksha, Laghu	Ushna	Katu	Krimighna, Kandughna
8	Loban	Kashaya, Tikta	Laghu, Ruksha	Sita	Katu	Vranahara

Methods:
Selection of area for *Dhoopana*: Two rooms of same size which are in routine usage in almost similar fashion were selected for the study. One room served as control without any intervention and labelled as 'C'. Second room labelled as 'T' where Dhoopana was performed once daily for two days. Prior consent was taken from the individuals who resides in the rooms after explaining the procedure at the same time it is confirmed that none of them have allergic manifestation towards smoke. Individually they are instructed to use the rooms routinely and to avoid extremes in keeping doors closed and opened.

***Dhoopana* procedure:**

200gms of dry cow dung cakes were burnt with the help of mentholated spirit in a sharava. The sharave kept at the centre of 'T' room and smoke is generated by subjecting dhup to fire. 25gm of Dhup is burnt little by little for about 10 minutes. Then the room was kept closed for 1hr. The procedure is performed between 10-11 am. The same procedure was repeated on second day of Dhoopana. Room 'C' also kept closed and opened in the same fashion without any intervention.

Assessment of microorganisms/ Sample collection:

Agar gel plates were exposed in both 'T' & 'C' rooms at intervals of before starting of Dhoopana procedure (B D), Immediately after 1st day of Dhoopana (D1), immediately after 2nd day of Dhoopana (D2) and later 3rd day onwards continuously for 6 days without Dhoopana procedure (AD1 to AD6) for 1 hr (11.00 – 12.00 am) in both the room during above mentioned timings at fixed places. ie, **RU**- Right upper, **RL**- Right lower, **LU**- Left upper, **LL** - left lower, and centre **C**- Central parts of the room. The exposed plates were incubated at 37°C for 24 hrs and were studied for microbial load.

RESULTS:

TABLE 3: SHOWING MEAN BACTERIAL COUNT OF TEST ROOM

Duration	RU	RL	LU	LL	C
B.D	51.66	42.66	15.33	37.33	24.33
D1	12.00	9.33	7.66	9.66	10.33
D2	10.00	11.66	12.66	6.66	7.00
AD1	12.66	15.33	8.33	11	13.33
AD2	12.66	13	9.33	10.33	11.33
AD3	15.33	11.33	16.33	13.33	16
AD4	9.00	10.00	11.00	17.33	9.66
AD5	14.33	13.33	15.33	17.00	9.66
AD6	12.66	19.66	20.66	27.66	15.33

TABLE 4: SHOWING MEAN BACTERIAL COUNT OF CONTROL ROOM

Duration	RU	RL	LU	LL	C
B.D	30.33	24.00	37.66	41.44	44.00
D1	36.00	23.00	28.76	49.00	39.88
D 2	46.66	29.66	48.66	37.66	47.00
AD1	40.66	38.33	26.00	36.66	39.33
AD2	34.33	24.66	41.33	39.33	30.66
AD3	50.66	43.33	28.66	39.44	37.33
AD4	32.33	26.00	38.33	38.66	44.00
AD5	48.66	45.33	46.33	38.00	38.33
AD6	41.00	42.33	25.66	48.33	37.00

TABLE 5: SHOWING MEAN FUNGAL COUNT OF TEST ROOM

Duration	RU	RL	LU	LL	C
B.D	5.33	7.6	4.33	6.33	6.33
D1	0.33	1.33	0.00	0.66	0.66
D2	0.00	0.33	0.00	0.00	0.00
AD1	0.00	0.66	0.00	0.00	0.00
AD2	0.33	0.33	1.00	0.33	0.00
AD3	2.33	0.33	1.66	1.00	1.00
AD4	0.33	0.33	0.66	0.33	0.00
AD5	0.66	0.33	0.66	0.33	1.66
AD6	1.33	1.33	1.66	0.33	2.00

TABLE 6: SHOWING MEAN FUNGAL COUNT OF CONTROL ROOM

Duration	RU	RL	LU	LL	C
B.D	5.00	6.00	4.33	3.66	3.66
D1	5.33	4.33	7.00	6.66	6.86
D2	5.00	7.33	6.66	6.33	5.00
AD1	5.33	4.66	3.44	5.33	7.00
AD2	5.00	4.66	6.00	7.00	4.33
AD3	7.00	4.00	5.66	6.33	5.33
AD4	5.00	3.44	6.33	6.00	7.33
AD5	6.33	5.00	6.00	4.66	6.33
AD6	6.00	4.33	6.66	3.44	5.33

TABLE 7: SHOWING SIGNIFICANCE OF EFFICACY ON BACTERIAL COUNT.

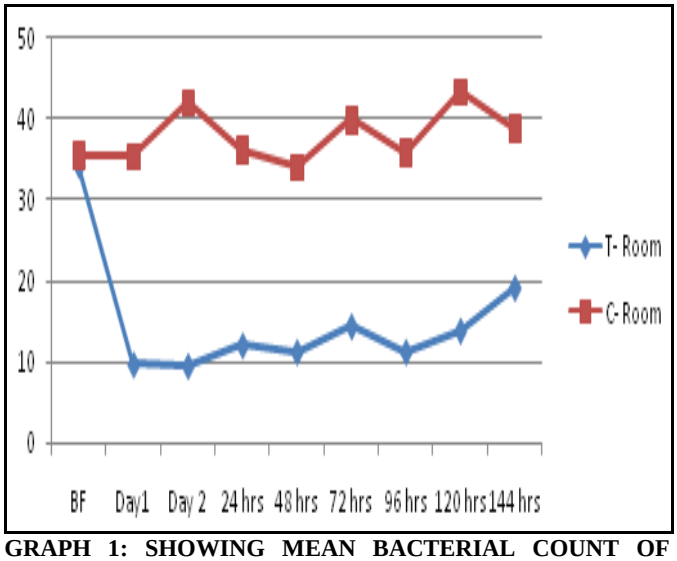
Duration	Mean± SEM Test room	Mean± SEM Control room	Pvalue& Summery	Significant? (p<0.05)
B.D	34.26±6.48	35.49±3.68	0.8736	No
D1	9.80±0.71	35.33±4.49	0.0005***	Yes
D2	9.60±1.21	41.93±3.62	0.0005***	Yes
AD1	12.13±1.18	36.20±2.63	<0.0001****	Yes
AD2	11.33±0.69	34.06±3.01	<0.0001****	Yes
AD3	14.46±0.94	39.88±3.61	0.0001***	Yes
AD4	11.40±1.52	35.86±3.08	<0.0001****	Yes
AD5	13.94±1.23	43.33±2.18	<0.0001****	Yes
AD6	19.19±2.56	38.86±3.77	0.002**	Yes

TABLE 8: SHOWING SIGNIFICANCE OF EFFICACY ON FUNGAL COUNT.

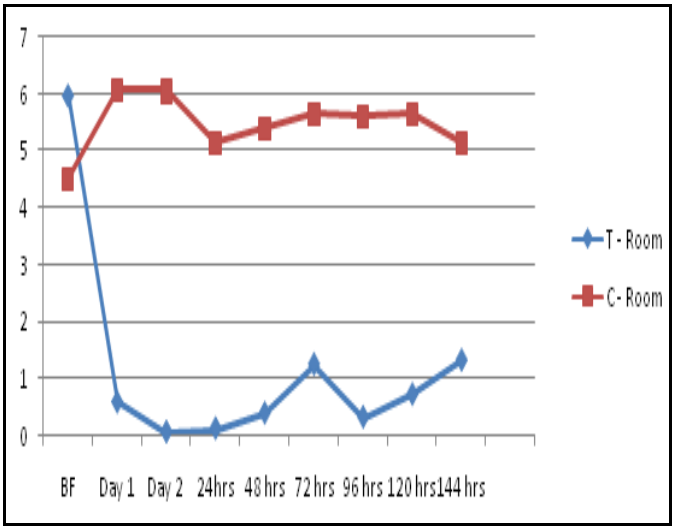
Duration	Mean± SEM Test room	Mean± SEM Control room	Pvalue& Summery	Significant? (p<0.05)
B.D	5.78±0.71	4.53±0.44	0.1731	No
D1	0.59±0.22	6.04±0.52	<0.0001****	Yes
D2	0.07±0.66	6.07±0.46	<0.0001****	Yes
AD1	0.13±0.13	5.15±0.58	<0.0001****	Yes
AD2	0.39±0.16	5.39±0.49	<0.0001****	Yes
AD3	1.26±0.33	5.66±0.51	<0.0001****	Yes
AD4	0.33±0.10	5.62±0.66	<0.0001****	Yes
AD5	0.73±0.24	5.66±0.35	<0.0001****	Yes
AD6	1.33±0.28	5.15±0.58	0.0003***	Yes

Mean values shows that 2days of fumigation was effective for 6 days

B.D – Before Dhupana, **D1**-1st Day of fumigation**, D2 –** 2nd day of fumigation**, AD1-** Day1After Dhoopana**, AD2-**Day 2 After Dhoopana **, AD3 –** Day3 After Dhoopana**, AD4-** Day 4After Dhoopana**, AD5 –** Day 5 After Dhoopana**, AD6 –**Day 6 After Dhoopana, **RU**- Right upper, **RL**- Right lower, **LU**- Left upper, **LL** - left lower, **C** centre of the room.



GRAPH 1: SHOWING MEAN BACTERIAL COUNT OF TEST ROOM & CONTROL ROOM



GRAPH 2: SHOWING MEAN FUNGAL COUNT OF TEST ROOM & CONTROL ROOM

DISCUSSION: *Dhoopana* with 'DHUP' shown significant antimicrobial and antifungal property, up to 1 week. This indicates significant (P Value < 0.05) antimicrobial effect of 'DHUP' *dhoopana.* Day 1 and day 2 fumigation had shown significant difference on bacterial (P Value 0.0063, 0.0069) and fungal (P Value 0.001, 0.0001) count with control. This shows that *Dhoopana karma* reduced the growth of microorganisms. Significant differences in bacterial and fungal count were seen after fumigation up to 6 days, when compare with control. This indicates that the effect of DHUP *Dhoopana* was retained up to 1 week.

On the basis of the result of the present study, it may be said that the microbial load may be under control by procedure of *Dhup Dhoopana*. M. Rajesh Harish chandra et al [7] *Nimba Dhoopana* has more sensitivity towards *Staphylococcus aureus* and *Streptococcus pyogenus.*

Rhata Saraf et al [8] Significant result of *Nimbadi Dhoopana* in converting *dushtavruna* to *shudhavrana* by antimicrobial activity of *nimba, rakshoghna* property of *vacha*etc.

Anantha kumar V et al [9] Ayurvedic *Dhoopana dravya* containing *vacha, guggulu, nimba, Agaru, Sarjarasa* etc. Shown the significant result in O.T fumigation there by concluded that it can be used as insect repellent, room purifier and air freshner. S B Dahikar et al [10] Ayurvedic herbal preparations have shown great potential as antimicrobial agents aganist most bacterial pathogens. SunitaAmrutesh[11] Enlisted the herbal drugs which process the Anti bacterial and Anti-fungal properties. Alam Sher [12] Natural products from medicinal plants serves as alternative source of combating infectious diseases in human being.

Scope for further study:

In the present study there may be a possibility that the dry cow dung also imparting anti bacterial property. So further research in this direction to compare the efficacy of both DHUP as well as cow dung Dhoopana should be under taken. Quantity of cow dung cakes required per area can be studied.

CONCLUSION: *Dhoopana karma* for 2 days with selected drugs under testing conditions showed statistically significant results in both Bacterial as well as fungal count with sustained effects for 6 days.

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