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A STUDY TO EVALUATE THE EFFICACY OF *SARVAGRAHA DHOOPANA* IN OT STERILIZATION

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ABSTRACT

Introduction - Surgical site infections (SSIs) account to about 38% of nosocomial infections in surgical patients. They remain one of the most common causes of serious surgical complications till date. *Dhoopana* is an integral part of *Rakshavidhi*, which ensures the protection against microbes.[7] *Sarvagraha Dhoopana* is a *Rakshogna Yoga* mentioned by *Acharya Vagbhata* in *Ashtanga Sangraha Uttarasthana* which includes drugs like *Sarshapa, Nimba, Vaca, Girikarnika, Bhurjapatra* and *Ghrita.* This study is intended to prove the efficacy of *Sarvagraha Dhoopana* in operation theater sterilization. **Methodology** – Pilot OT of size 50 cm in length, breadth, and height is constructed and fumigation of *Sarvagraha dhoopana* was carried out on common microbes of OT. BF and AF results were then compared with results of standard formaldehyde. **Result** - 3 g *Sarvagraha Dhoopana* + 3 g ghee was taken as the least effective dose of *Dhoopana* drug to fumigate 50 cu cm of space. *Sarvagraha dhoopana* showed statistically insignificant result with that of standard formaldehyde, which conveys that is has similar effect as that of formaldehyde on *S aureus, E coli* and *P aeruginosa*. **Discussion** – The effect of the drug might be due to the anti-microbial effect of different phytochemical constituents in the drugs of *Sarvagraha Dhoopana*. *Sarvagraha dhoopana* yoga consist of drugs having mainly *Katu Thiktha* and *Kashaya* properties. All the drugs exhibit *Krimighna* properties. Hence the anti-microbial

activity of the *Sarvagraha Dhoopana* can be comprehended as the combined effect of specific phytoconstituents having anti-microbial property present in each drug.

INTRODUCTION

Surgical site infections (SSIs) account to about 38% of nosocomial infections in surgical patients.[1,2] They remain one of the most common causes of serious surgical complications till date.[3] Microbial contamination of OT leads to post-operative infections which will have serious implications on patients.[4] In modern science for sterilization and disinfection of OT, various chemical fumigants and disinfectants are used.

Ayurveda emphasizes the importance of human health and the surrounding environment.[5] In *Samhitas*, procedures of water purification, *Homa, Yajna*, *Dhoopana* have been mentioned, in which *Dhoopana* is an integral part of *Rakshavidhi*, which ensures the protection against microbes.[6] Concepts of sterilization and disinfection in all surgical and para surgical procedures are mentioned in the *Samhitas* by giving at most priority.

Dhoopana is the method by which drugs of herbo-mineral and animal origin are used for fumigation so as to sterilize the *Vrana* and to disinfect *Bheshajaagara* and *Vranaagara.*[7] *Sarvagraha Dhoopana* is a *Rakshogna Yoga* mentioned by *Acharya Vagbhata* in *Ashtanga Sangraha Uttarasthana* which includes drugs like *Sarshapa, Nimba, Vaca, Girikarnika, Bhurjapatra* and *Ghrita.*[8] These *Dhoopana dravyas* can be considered to be safer and cost effective as compared to the chemical fumigants.

Scientific evaluation of each and every *Ayurvedic* principles can be considered as need of the hour. Such research works will definitely be able to show new path to the human society in the direction of positive health. Thus, this study is intended to prove the efficacy of *Sarvagraha Dhoopana* in operation theater sterilization.

MATERIAL AND METHODOLOGY

Source of Data

- Drug source - Raw materials of *Sarvagraha Dhoopana* were identified by expert of the *dravyaguna* Department and was collected from Anamaya raw drug pharmacy from Udupi. All the drugs were taken in equal quantity of 500 gram each and mixed thoroughly in grinding machine into fine powder in Alva's Pharmacy, Mijar.

- Experimental source- Ready-made MacCankey agar and blood agar was collected from Alva MLT college, Moodubidri.

Method of Collection of Data

a) Sampling of microbes from the OT - Sterilized swabs were used to collect sample from the floor, wall, light source, boyles apparutus, Oxygen mask, suction apparatus, diathermy machine and table. These swabs were then sealed, labelled, and taken to the MLT lab for culture. Swabs were then applied over the prepared MacConkey and blood agar plates and it was incubated at 37^{o}C for 24 hours under aerobic condition. The plates were labelled with Sample number, site within theatre, time and date of sample collection. After incubation the colonies were identified and counted.

b) Preparation of pilot OT - Pilot and control OT is made up of acrylic material and having the dimensions of 50×50×50 cm with the thickness of 3 mm. One surface of the pilot OT was made removable. 5cm circular vent was made on one of lateral wall to facilitate entry of *Dhoopana* fumes from *Dhoopana* apparatus into pilot OT. *Dhoopana* Apparatus: The *Dhoopana* apparatus was prepared out of clay. This container had a 5cm circular vent for connecting pipe in its upper part for *Dhoopana* fumes.

c) *Dhoopana karma* done in the pilot OT - After the colonies were formed in the culture plate a single colony was taken and inoculated into a test tube containing 10ML of sterile water. A sterile cotton swab was then dipped in the solution and applied over the different surfaces of pilot OT and control OT. Another sterile cotton swab was then used to take a sample from the applied area in order to check for the number of Microbial colonies which is present in that area. Fumigation was then carried out in both the pilot OT and control OT. In the pilot OT the *Dhoopana* was carried out by using different dosage of *Sarvagraha Dhoopa* and in the control OT formalin fumigation was done by using 2ML of formaldehyde and 0.7 gram of potassium permanganate. Both the OT were then sealed and kept undisturbed for 24 hours. After 24 hours swabs were collected from all the surfaces of both and checked for microbial count. All the process was done with utmost precaution and aseptic conditions.

OBSERVATION AND RESULT

Observation on Samples Collected

The result of samples collected from 10 different procedures is as follows

Procedures	S1	S2	S3	S4	S5	S6	S7	S8
P1 – Hernioplasty			✓					✓
P2 – Open Appendicectomy			✓			✓		
P3 – Haemorrhoidectomy	✓			✓				
P4 – Amputation	✓		✓					
P5 – Peri-anal abscess	✓		✓					
P6 – circumcision								
P7 – ORIF								
P8 – wound debridement	✓		✓					
P9 – fistulectomy			✓	✓				
P10 – herniotomy								

SAMPLES SOURCE

Samples	Collected form
S1	Floor
S2	Wall
S3	Operation table
S4	Diathermy machine
S5	Boyles apparatus
S6	Suction apparatus
S7	Light source
S8	Oxygen mask

Samples collected were incubated for 24 hours at 37^0 C. The microbes observed is as follows for each procedure

P1 – *Staphylococcus aureus* and Pseudomonas were observed from the swabs collected from the table and mask respectively.

P2 – *Escherichia coli* and *Staphylococcus aureus* was observed from the swabs collected from suction pump and table respectively.

P3 – *Staphylococcus aureus* and *Escherichia coli* was observed from the swabs collected from the floor and diathermy machine respectively.

P4 – *Staphylococcus aureus* was observed from the swabs collected from the floor and table.

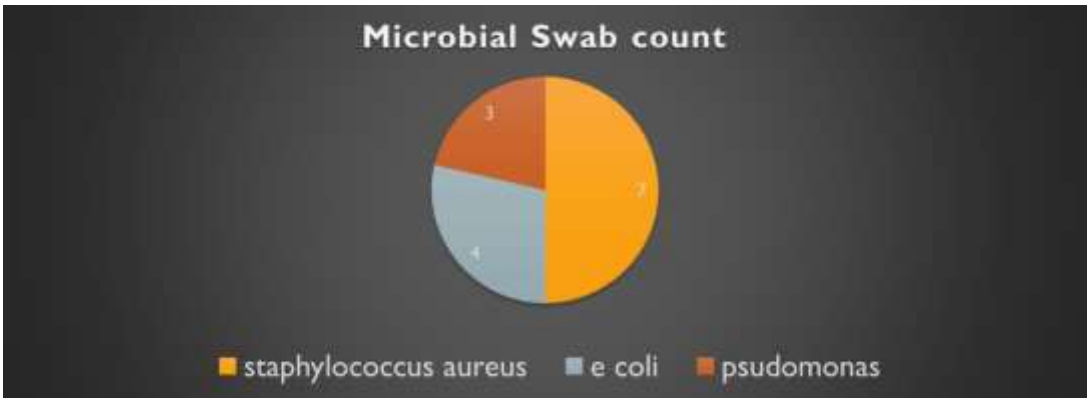
P5 – *Staphylococcus aureus* was observed from the swabs collected from floor and table.

P8 – pseudomonas was observed from the swabs of table and floor.

P9 – *Escherichia coli* was observed from the swabs collected form diathermy machine and table.

Based on the observation on table 1 out of the 80 swabs collected 14 came positive for microbes. In these 7 samples were of *Staphylococcus aureus* 4 samples were of E coli and 3 sample was of Pseudomonas.

Highest concentration of microbes was identified from the swabs collected from the table. (GRAPH 1)



OBSERVATION OF STANDERDIZATION OF *SAVARVAGRAHA DHOOPANA*

To calculate the amount of *Sarvagraha Dhoopana* required to fumigate 50 cm^3 of pilot OT different dosage of *Sarvagraha Dhoopana* drug was used in a trial-and-error method and sterility of the pilot OT was checked in each case to observe the least quantity of *Sarvagraha Dhoopana* required to fumigate the pilot OT.

The trail was started with a base quantity of 10 g *Sarvagraha Dhoopana* and 10 g of *Goghrita*. The procedure was repeated with 5g, 3g, 2g of *Sarvagraha Dhoopana* and in each case sterility of the OT was checked.

Standardization of *Sarvagraha Dhoopana*

QUANTITY OF *SARVAGRAHA DHOOPANA* TAKEN	QUANTITY OF GHEE TAKEN	STERILITY AFTER FUMIGATION
10 g	10 g	Sterile
5 g	5 g	Sterile
3 g	3 g	Sterile
2 g	2 g	Not sterile

The result came sterile in *Sarvagraha* dosage of 10 g, 5 g, and 3 g. But when the dosage was reduced to 2 g of *Dhoopana Dravya* and 2 g of *Goghritha* the result came non sterile. Thus, the minimum quantity of *Sarvagraha Dhoopana* required to sterilize 50 cm^3 of space = 3 g *Sarvagraha Dhoopana* + 3 g ghee.

OBSERVATION ON FORMALIN FUMIGATION

The procedure of fumigation with formaldehyde was conducted for 10 trials for different quantity of microbes of *Staphylococcus aureus*, *Escherichia coli*, and *Psudomonas aeruginosa*. Before and after fumigation result of formaldehyde fumigation with 2 ml of formaldehyde and 0.7 gm of potassium permanganate in all 3 microbes is as follows.

1. Formalin fumigation on *Staphylococcus aureus*

Trails	1	2	3	4	5	6	7	8	9	10
BF	73	75	51	43	37	24	31	17	14	9
AF	0	0	0	0	0	0	0	0	0	0

2. Formalin fumigation on *Escherichia coli*

Trails	1	2	3	4	5	6	7	8	9	10
BF	97	58	40	31	25	18	11	77	69	9
AF	0	0	0	0	0	0	0	0	0	0

3. Formalin fumigation of *Pseudomonas aeruginosa*

Trails	1	2	3	4	5	6	7	8	9	10
BF	157	140	138	111	102	86	74	77	40	37
AF	0	0	0	0	0	0	0	0	0	0

OBSERVATION ON *SARVAGRAHA DHOOPANA* FUMIGATION

The procedure of fumigation with *Sarvagraha Dhoopana* was conducted for 10 trials for different quantity of microbes of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Before and after fumigation result of formaldehyde fumigation of *Sarvagraha Dhoopana* with 3 g of *Dhoopana Dravya* and 3 g of *goghrita* is as follows.

4. *Sarvagraha Dhoopana* of *Staphylococcus aureus*

Trails	1	2	3	4	5	6	7	8	9	10
BF	121	110	103	85	74	54	34	37	24	19
AF	0	0	0	0	0	0	0	0	0	0

5. *Sarvagraha Dhoopana* of *Escherichia coli*

Trails	1	2	3	4	5	6	7	8	9	10
BF	110	117	94	86	72	61	57	46	34	21
AF	0	0	0	0	0	0	0	0	0	0

6. *Sarvagraha Dhoopana* of *Pseudomonas aeruginosa*

Trails	1	2	3	4	5	6	7	8	9	10
BF	151	120	109	85	77	64	57	37	21	17
AF	0	0	0	0	0	0	0	0	0	0

RESULTS

Statistical analysis of the given data for both formalin and *Sarvagraha Dhoopana* was done using paired t test, unpaired t test and one way ANOVA.

WITHIN GROUP ANALYSIS

Paired T test was done on within the group analysis for both formaldehyde and *Sarvagraha Dhoopana* fumigation for all microbes.

Formaldehyde Fumigation on *Staphylococcus aureus*

Treatment Name	Mean	Std Dev	SEM
BF	66.100	37.619	11.896
AF	0.000	0.000	0.000
Difference	66.100	37.619	11.896

The above table shows that there is statistically significant difference between before and after fumigation on *Staphylococcus aureus* with p>=0.001.

Formaldehyde Fumigation on *Escherichia coli*

Treatment Name	Mean	Std Dev	SEM
BF	43.500	30.281	9.576
AF	0.000	0.000	0.000
Difference	43.500	30.281	9.576

The above table shows that there is statistically significant difference between before and after fumigation on *Escherichia coli* with p>=0.001.

Formaldehyde Fumigation on *Pseudomonas aeruginosa*

Treatment Name	Mean	Std Dev	SEM
BF	96.200	41.155	13.014
AF	0.000	0.000	0.000
Difference	96.200	41.155	13.014

The above table shows that there is statistically significant difference between before and after fumigation on *Pseudomonas aeruginosa* with p>=0.001.

Sarvagraha Dhoopana* fumigation on *Staphylococcus aureus

Treatment Name	Mean	Std Dev	SEM
BF	66.100	37.619	11.896
AF	0.000	0.000	0.000
Difference	66.100	37.619	11.896

The above table shows that there is statistically significant difference between before and after fumigation on *Staphylococcus aureus* with p>=0.001.

Sarvagraha Dhoopana* fumigation on *Escherichia coli

Treatment Name	Mean	Std Dev	SEM
BF	69.800	31.916	10.093
AF	0.000	0.000	0.000
Difference	69.800	31.916	10.093

The above table shows that there is statistically significant difference between before and after fumigation on *Escherichia coli* with p>=0.001.

Sarvagraha Dhoopana* fumigation on *Pseudomonas aeruginosa

Treatment Name	Mean	Std Dev	SEM
BF	73.800	43.736	13.831
AF	0.000	0.000	0.000
Difference	73.800	43.736	13.831

The above table shows that there is statistically significant difference between before and after fumigation on *Pseudomonas aeruginosa* with p>=0.001.

BETWEEN THE GROUP ANALYSIS

One Way ANOVA was used for between the group analysis of before and after fumigation for both formaldehyde and *Sarvagraha Dhoopana* fumigation between all the microbes.

Formaldehyde fumigation

Before Fumigation

Group name	Mean	Std Dev	SEM	F	P
M1	37.400	23.306	7.370	9.943	<0.001
M2	43.500	30.281	9.576		
M3	96.200	41.155	13.014I		

The above table shows that there is statistically significant difference between the microbes in the before fumigation of microbes of formaldehyde group with p =< 0.001 and F = 9.943.

All Pairwise Multiple Comparison Procedures (Holm-Sidak method)

Comparison	Diff of Means	T	P	Significant?
M3 vs. M1	58.800	4.055	0.00038	Yes
M3 vs. M2	52.700	3.634	0.00115	Yes
M2 vs. M1	6.100	0.421	0.677	No

The above table shows that there is a statistically significant difference between the group M3 and M1 and between M3 and M2. This may be because there was slightly higher concentration of bacteria in M3 group as compared to M1 and M2.

After fumigation

Group name	Mean	Std Dev	SEM	F	P
M1	0.000	0.000	0.000	1.000	1.000
M2	0.000	0.000	0.000		
M3	0.000	0.000	0.000		

The above table shows that there is statistically insignificant result with P = 1.00 and F =1.00. This is because there was complete sterility attained after fumigation for all the microbes for formaldehyde fumigation.

***Sarvagraha Dhoopana* fumigation**

Before fumigation

Group name	Mean	Std Dev	SEM	F	P
M1	66.100	37.619	11.896	0.102	0.903
M2	69.800	31.916	10.093		
M3	73.800	43.736	13.831		

The above table shows that there is statistically insignificant difference between the microbes in the before fumigation of microbes of *sarvagraha dhoopana* group with P = 0.903 and F = 0.102. This implies there is no significant change in the number of microbes between the groups of M1, M2, and M3.

After fumigation

Group name	Mean	Std Dev	SEM	F	P
M1	0.000	0.000	0.000	1.000	1.000
M2	0.000	0.000	0.000		
M3	0.000	0.000	0.000		

The above table shows that there is statistically insignificant result with P = 1.00 and F =1.00. This is because there was complete sterility attained after fumigation for all the microbes for *Sarvagraha Dhoopana* fumigation.

Unpaired T test was used for between the group analysis of Before and After Fumigation of formaldehyde group with *Sarvagraha Dhoopana* group for all the microbes.

Before Fumigation

Staphylococcus aureus

Group Name	Mean	Std Dev	SEM
M1 FOR	37.400	23.306	7.370
M1 DH	66.100	37.619	11.896

The above table shows that there is a statistically insignificant result between before fumigation of M1 formaldehyde group and M1 *Sarvagraha Dhoopana* group with P = 0.055. This implies that there the no significant change in the microbial load of *Staphylococcus aureus* taken for both formaldehyde fumigation and *Sarvagraha Dhoopana* fumigation.

Escherichia coli

Group Name	Mean	Std Dev	SEM
M2 FOR	43.500	30.281	9.576
M2 DH	69.800	31.916	10.093

The above table shows that there is a statistically insignificant result between before fumigation of M2 formaldehyde group and M2 *Sarvagraha Dhoopana* group with P = 0.075. This implies that there the no significant change in the microbial load of *Escherichia coli* taken for both formaldehyde fumigation and *Sarvagraha Dhoopana* fumigation.

Pseudomonas aeruginosa

Group Name	Mean	Std Dev	SEM
M3 FOR	96.200	41.155	13.014
M3 DH	73.800	43.736	13.831

The above table shows that there is a statistically insignificant result between before fumigation of M3 formaldehyde group and M3 *Sarvagraha Dhoopana* group with P = 0.254. This implies that there the no significant change in the microbial load of *Pseudomonas aeruginosa* taken for both formaldehyde fumigation and *Sarvagraha Dhoopana* fumigation.

After Fumigation

Group Name	Mean	Std Dev	SEM
M1 FOR	0.000	0.000	0.000
M1 DH	0.000	0.000	0.000

Since the value came 0 for all groups of formaldehyde and sarvagraha dhoopana groups, there is a statistically insignificant result between after fumigation of M1, M2 and M3 offormaldehyde group and M1, M2 and M3 of *Sarvagraha Dhoopana* group with P = 1.000

respectively. This implies that complete sterility was attained after fumigation in both formaldehyde and *Sarvagraha Dhoopana* group for all the groups.

DISCUSSION

Out of the 80 samples collected from the OT from 10 different procedures, 14 samples came positive for microbial load. The microbes varied depending on the type of operation. Out of the 14 samples, 07 were of *Staphylococcus aureus*, 04 were of *E coli* and 03 were of *Pseudomonas*. The prevalence of *Staphylococcus aureus* may be because of its invasive nature and which is present in skin, armpits, and nose of human beings. Highest concentration of microbes was identified from the swabs collected from the OT table as it is in direct contact with the patient.

Both the fumigation process was repeated for 10 trails and had shown statistically significant result for all the microbes. In each trial the microbial load was increased to evaluate the effect of the drugs on higher concentration. During the process of fumigation, the oxygen concentration was lowered in the fumigation chamber as the chamber was filled with the fumes. Being facultative anaerobic bacteria, the microbes were able to withstand the reduce the oxygen concentration.

Sarvagraha Dhoopana is a *Rakshogna Yoga* mentioned by *Acharya Vagbhata* in *Ashtanga Sangraha Uttarasthana* which includes drugs like *Sarshapa, Nimba, Vacha, Girikarnika, Bhurjapatra* and *Ghrita.*[9] Based on the result of before and after fumigation, *Sarvagraha Dhoopana yoga* is having statistically insignificant result with formalin as it shows similar microbicidal effect as that of formaldehyde. This might be due to the anti-microbial effect of different phytochemical constituents in the drugs of *Sarvagraha Dhoopana.*

- *Azadiracta indica*[10]*– Nimba patra* has phytoconstituents like alkaloids, glycosides, flavonoids and saponins that has anti-bacterial effect for Bacillus, Pseudomonas and Staphylococcus variants of microbes.
- *Clitoria ternatea*[11]- The drug possesses major phytochemical constituents like phenols, tannins, flavonoids with high antimicrobial activity against E. coli, pneumoniae and pseudomonas variants.
- *Brassica campestris*[12] – The primary chemical constituent Ally iso thyocynate has anti-microbial and anti-fungal activity which help provide resistance against pathogens. The

Anti-microbial effect is thought to be due to the reaction of thiocyanate in the cytoplasm or cell membrane of microbes.

- *Betula utilis*[13] – It has shown high antimicrobial activity against pseudomonas and E coli due to the presence of phytoconstituents like glycosides and alkaloids in the drug.
- *Acorus calamus*[14]– Shows anti-microbial property against Pseudomonas, Staphylococcus and Aspergillus variants. This might be due to the three major components of the drug like Palmitic, Linoleic acid and Alkaloids that have microbial inhibition property.
- *Goghritha*[15]– Studies shown that clinical isolates of staphylococcus aureus and E coli were sensitive mostly at a higher concentration of cow ghee.

Sarvagraha dhoopana yoga consist of drugs having mainly *Katu Thiktha* and *Kashaya* properties. All the drugs exhibit *Krimighna* properties. Hence the anti-microbial activity of the *Sarvagraha Dhoopana* can be comprehended as the combined effect of specific phytoconstituents having anti-microbial property present in each drug.

CONCLUSION

In the present study *Sarvagraha Dhoopana* exhibited equal effect as that of Formalin on the different strains of microbes sampled. The probable cause for the death of colonies can be attributed to the antibacterial effect of the drug as the organism are facultative anaerobes and can live even without oxygen. While doing fumigation the oxygen content reduces but precautions were taken to nullify any change in pressure and temperature of the pilot OT.

Even though Formaldehyde has high biocidal effects it is a highly toxic, corrosive and is a severe irritant substance to skin, eyes and respiratory tract and is a potential carcinogen. It is explosive at 7.75% concentration in dry air.

Sarvagraha Dhoopana yoga consist of drugs having mainly *Katu, Thiktha, Kashaya* rasa properties and *Krimighna* properties. During *Dhoopana* procedure there was no irritant effect on skin and eyes apart from the odour. Moreover, the quantity of the drugs required for the procedure was minimal and cost effective. By all these factors *Sarvagraha Dhoopana* can be used for massive fumigation in ward and can be developed into other formulations to be used effectively in an OT.

REFERANCE

1. World Alliance for Patient Safety, author. WHO guidelines for safe surgery. Geneva: World Health Organization, 2008. [Google Scholar]
2. Weigelt JA, Lipsky BA, Tabak YP, et al. Surgical site infections: Causative pathogens and associated outcomes. Am J Infect Control, 2010; 38: 112–120. [PubMed] [Google Scholar]
3. Centers for Disease Control and Prevention, author. National Nosocomial Infections Surveillance (NNIS) System report, data summary from January 1992 through June 2004, issued October 2004. Am J Infect Control, 2004; 32: 470–485.
4. Spagnolo AM, Ottria G, Amicizia D, Perdelli F, Cristina ML. Operating theatre quality and prevention of surgical site infections. *J Prev Med Hyg*, 2013; 54(3): 131-137.
5. Shrestha, Sahara & Bedarkar, Prashant & Patgiri, B & Chaudhari, Swapnil. *DHOOPANA* KARMA: A REVIEW THROUGH BRIHATRAYI. IAMJ, 2017; 1.
6. Anantkumar V, Shekokar, Kanchan M Borkar. To Study the Efficacy of Ayurvedic *Dhoopana* for Operation Theater Sterilization. International Journal of Advanced Ayurveda, Yoga, Unani, Siddha and Homeopathy, 2013; 2(1): 143-147.
7. Shrestha, Sahara & Bedarkar, Prashant & Patgiri, B & Chaudhari, Swapnil. *DHOOPANA* KARMA: A REVIEW THROUGH BRIHATRAYI. IAMJ, 2017; 1.
8. Vagbhata, Astanga Sangraha, Uttarasthana 4/3, Translated by Prof.K.R. Srikantha Murthy, Chaukhamba Orientalia, Varanasi, Ninth Edition, 2005; 46.
9. Dr Shubha M and Dr Ravi Rao Sorake, Ashtanga Hridayam, Uttarasthana, 4th chapter, 3rd sloka, 2015 edition, by chaukamba Orientalia
10. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4054046/anti_microbial_effect_of_azadiracta_indica
11. https://www.researchgate.net/publication/282188601_Antimicrobial_activity_of_Clitoria_ternatea_L_extracts
12. https://www.researchgate.net/publication/281177907_Phytochemical_and_antimicrobial_activity_of_the_plant_extracts_of_Brassica_oleracea_against_selected_microbes
13. https://www.researchgate.net/publication/286490511_Pharmacological_and_phytochemical_properties_of_Betula_utilis_An_overview
14. https://www2.sci.u-szeged.hu/ABS/2009/Acta%20HP/5345.pdf_anti_microbial activity of acorus calamus
15. https://www.ajol.info/index.php/bjmls/article/view/221956