"An Experimental Study to Evaluate the *Krimighna* Effect of *Dhoopana* along with the Standardization of *Dhoopana* Formulations"

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ABSTRACT

The air around us contains a large number of microorganisms (Krimi) and it can get contaminated by these microorganisms. Thus it carries a lot of significance to disinfect the area where we reside, which in turn can result in the prevention of many infectious diseases. To get prevention from *Krimi* (microbes), *Rakshoghnavidhan* is indicated in our classics [1]. In this various medicinal plants were burnt on fire and the smoke generated from it was used for sterilization of different areas where chances of infections are more. In the present context *Dhoopana Yoga* was prepared out of drugs indicated for *Krimighna Karma* .Rooted in the holistic system of *Ayurveda,* the study explores the ancient healing practice of Dhoopana, focusing on its reputed ability to repel harmful entities, known as the Krimighna effect. Concurrently, the research endeavors to establish standardized formulations, addressing the challenge of variability within traditional practices. The study blends historical insights with modern scientific rigor, employing laboratory experiments and real-world observations to assess the efficacy of diverse *Dhoopana* blends. The paper also outlines a pathway for achieving standardization, harmonizing herb selection, ratios, and procedural consistency. This chapter serves as a bridge between ancient wisdom and contemporary scientific inquiry, inviting discourse and contributing to the preservation and evolution of traditional healing practices.

Keywords – *Dhoopana*, antimicrobial, *Krimi*, sterilization

I. INTRODUCTION

In the realm of traditional medicinal practices, the potential of age-old remedies to combat contemporary challenges continues to captivate researchers and practitioners alike. Within this context, the profound significance of *Dhoopana*, a practice rooted in the holistic system of Ayurveda, emerges as a subject of compelling intrigue. This chapter embarks on an exploratory journey into the heart of this ancient healing technique, delving deep into the realms of an experimental study aimed at unraveling the enigmatic *Krimighna* effect attributed *to Dhoopana*. As the aromatic tendrils of sacred herbs intertwine with the quest for scientific validation, we also embark upon the path of standardization, seeking to bring precision and consistency to the formulations that have traversed generations.

Guided by the wisdom of the sages and bolstered by the rigors of modern scientific inquiry, this chapter seeks to bridge the chasm between tradition and innovation. We traverse the corridors of history to resurrect the roots of *Dhoopana*, unveiling its evolution across cultures and epochs. Drawing from the annals of Ayurveda, we unearth the conceptual underpinnings of the *Krimighna* effect – the reputed ability of certain Dhoopana blends to ward off malevolent forces.

The heart of this chapter beats within the crucible of experimentation. With methodologies as diverse as the flora in the Ayurvedic apothecary, we embrace the principles of empirical inquiry to unravel the intricacies of *Dhoopana* effects. From controlled laboratory studies to real-world observations, we strive to paint a comprehensive picture that amalgamates the traditional narratives with modern scrutiny.

Yet, in our pursuit of validation, we do not tread alone. Standardization emerges as both a means and an end, an endeavor that seeks to codify the artistry of *Dhoopana* into reproducible science. As we grapple with the variability of herbs, ratios, and rituals, we confront the challenge of harmonizing tradition with contemporary expectations of consistency and quality.

As the fragrant plumes of *Dhoopana* ascend towards the heavens, carrying with them the aspirations of healers past and present, this chapter endeavors to honor the heritage that has bestowed upon us this aromatic legacy. It is an invitation to explore, question, and rejuvenate the ancient practice, while fostering a dialogue that transcends epochs and cultures, tradition and progress. Through the alchemy of rigorous research and profound respect for tradition, we embark upon a voyage to evaluate the *Krimighna* effect of *Dhoopana* and pave the way for its standardized resurgence in a world seeking both solace and science.

II. PLAN OF STUDY:

A. Conceptual Study

All the available authentic classical literature, their respective commentaries and texts written by eminent scholars of the modern era were referred to. Latest journals, research papers, periodicals, articles, symposia, lexical, medical literature, and available material on the internet were referred to keenly to throw light on the subject.

B. Experimental Study

Experimental study has been done in two parts one is performed in the Deptt. of Rog Nidan Evum Vikriti Vigyan, R.G.G.P.G. Ayurvedic College & Hospital and the other in Deptt. of Veterinary Microbiology, Dr. G.C. Negi College of Veterinary and Animal Sciences, C.S.K.H.P.K.V., Palampur (H.P.).

i. In an experimental (closed room)

Microbiological study of air including measuring the microbial load of various places with dimensions (passive monitoring technique was monitoring the microbiological population of air using sterile plates) was done.

ii. On Standard organism

Standard organisms were taken and it was cultured on different media.

By varying the amount of *Dhoopana* and time interval the effect of *Dhoopana* was analyzed.

iii. Standardization of *Dhoopana*

Standardization of *Dhoopana* formulations was done and the effectiveness of formulations was analyzed.

III. CONCEPTUAL STUDY (AYURVEDIC REVIEW)

A. *Krimi*

i. Etymology

Ayurveda has two aims i.e. promotion and preservation of health and cure of the disease. The concept of *Dhoopana* (fumigation) comes under promotion and preservation as it disinfects our air and prevents us from various harmful diseases by killing microbes *(Krimi).*

According to *Shabdhasagar*

1.A worm, an insect in general.
2.Lac, the red dye which is infact an insect.
3.An *Asur* or demon.

According to *Sidhantakaumudi*

The word *Krimi* is derived from dhatu ***'Kujna Himsayam'***which means to kill or to yield harmful effect.

It is also mentioned in different Veda, Samhita, Sangraha in detail. It described types of Krimi:-

1. Bahaya
2. Abhyantra

B. *Dhoopana*

i. Etymology:

According to Encyclopedic Dictionary of *Ayurveda*

Dhupa- incense, perfume, aromatic vapour or smoke proceeding from gum or resin. One of the 16th acts of homage or offerings in the *Panchyatra* ceremony.

Dhupana- incensing, fumigation, perfume, and incense.

Dhupana //kwiu /Fumigation:-Exposing the diseased part or the whole body to smoke of drugs. Smoke emanating from certain drugs can be applied to the *Vrana* after its rupture. *Dhupana* is essentially useful in *Vataja Vrana*with *Tivra Vedana* and *Dustasrava*. The drugs for *Dhoopana* are *Karpura, Sarjarasa, Guggulu, Srivestaka, Ghritam,* etc. (Ch. Sa. 1/87). (Encyclopedic Dictionary of *Ayurveda*).

Dhoopana is also described in *Veda*, *Samhita* and *Sangraha* in detail.

ii. Indications[2]

- For massive general sterilization (prevention) to create the aseptic environment as in *Homa, Havana, Yagya Vidhi* in holy places, pediatric wards *(Kumaragara Dhoopana),* postoperative wards (*Vranitagara Dhoopana),* labor room *(Sutikagara Dhoopana).*
- It includes specific fumigation from a treatment point of view for particular diseases. *Dhoopana Karma* is indicated for infectious and non-infectious diseases. In this context, we have *Dhoopana* in *Jwara, Arsha, and Kustha*, psychic ailments like *Unmada and Apasmara, Vrana* (wound), *Yoni Roga, Visha Vikara* etc.
- These are aimed at using specific drugs indicated for specific diseases.
- Other effects indications of *Dhoopana Karma* for bringing rain, for a good progeny, prosperity, and auspiciousness.
- Preventive- for prevention of diseases and healthy well-being.
- Therapeutic : for infectious and non- infectious disease
- Used in form of medicated fumes via oral or nasal route i.e *Dhoompana*
- *Raksoghana Dhoopana* : for protection from infections
- *Swastika Dhoopa*: for all diseases originating from *Bhuta* (microbes).
- *Punya Dhoopa*: for holy people & holy purpose.
- Act on the wound- *Kathinyakara Dhoopana*: fumigation for hardening of excessively soft tissues of the wound. When fumigated with aromatic drugs and heartwood of aromatic plants, the ulcerated tissue gets hardened. *Mardavakara Dhoopana*: fumigation for softening of excessively hard tissues of the wound. When fumigated with fumes of *Ghrita*, bone marrow and muscle fats (*Vasa*), softens the ulcerated tissue
- Used locally as *Karna Dhoopana, Vrana Dhoopana.*

- Systemic used in *Jvara* (generalized *Dhoopana), Kasa.*
- For masking offensive smell/air with fumigating fragrant drugs like *Chandana, Ela, Vacha, Gugglu* etc.

iii. Contraindications [3]

- In the case of asthmatic passage it must be avoided as it may start hypersensitivity reaction in them and worsen the symptoms.
- Smoke can irritate your eyes and respiratory system
- The biggest health threat from smoke is from fine particles. These microscopic particles can penetrate deep into your lungs. They can cause a range of problems ,from burning eyes and a runny nose to aggravated heart and lung diseases.
- Excessive exposure is harmful to pregnant women because there could be potential health effects for both woman and the developing foetus.

IV. MODERN REVIEW OF STERILIZATION [4]

As per the modern science, Hospital Associated Infections (HAI) or nosocomial infections are the infections which were neither present nor incubating at the time the patient was admitted to health care facility. The organisms come from many possible sources, such as patients' own resident flora, mouth, gastrointestinal tract, vagina or the skin, the resident microbial flora of health care workers and from other patients on the ward, patient to another one, Contaminated instruments, dressings, needles, etc. used for invasive procedures and in fusions.

The source of most of the hospital epidemics is an infected patient, i.e. patients contaminated with pathogenic microorganisms. These microorganisms are often released into the environment in very high numbers, exceeding the minimal infective dose and contaminate other patients who subsequently develop hospital-acquired infections.

Gas Fumigation is the process introduced in the modern science which can be correlated with *Ayurvedic Dhoopana Karma*. Fumigation is the process by which a lethal chemical is released into an enclosed area to eliminate an infestation of pests.

Correlation of *Ayurvedic Krimi Vigyana* with Modern Microbiology [5]

Krimi- *Drista*, *Adhrista*	Microbes- Visible (Fungi, Protozoa), Invisible (Bacteria, Virus)
Puti Aahara, Klinna Aahara Ashuchi, Aahara, Sandusta Aahara → *Krimi Roga*	Contaminated & deteriorated food material → Infectious diseases
Use of *Krimi –Anupahata* drug to make different *Ayurvedic* formulations as well as in single drug therapy, is recommended by classical text.	Use of uncontaminated material (raw drug) is the first cardinal rule for the pharmacy that is recommended by W.H.O.
Dhoopana Karma → To kill *Krimi*	Fumigation is successful measure → For Sterilization

Disinfection Methods in Modern Science and *Ayurvedic* Science [6]

Modern Disinfection Methods		***Ayurvedic* Disinfection Methods**
Physical Agents	***Chemical Agents***	
Sunlight Drying Dry Heat Moist Heat Filtration Radiation Ultrasonic and Sonic Vibrations	Alcohols Aldehydes Dyes Halogens Phenols Surface Active Agents Chlorine Metallic Salts Gases	• *Havana,Yagya, Homa, Agnihotra.* • Powdered herbs burnt in fire and smoke is used as *Dhoopana.* • *Taila, Ksara and Udaka, Payana* were methods of disinfection for instruments. • *Dhoopanvarti.* • *Bhautik Agni* • Muslin Cloth • *Bhasma*

V. BACTERIA MENTIONED IN THE STUDY [7]

A. Gram Negative

*Pseudomonas Spp.(*MTCC *No. 129)*
Escherichia Coli (MTCC No. 188)

B. Gram Positive

Staphylococcus Aureus (MTCC 87)
Bacillus Spp. (MTCC No 129)

Figure 1 (Bacterial Strains after Culturing)

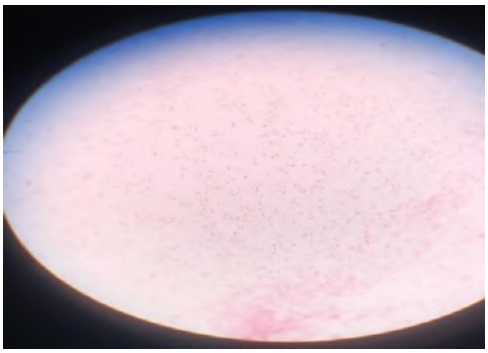


Figure 2 (*Pseudomonas spp.*(MTCC No. 129 *)

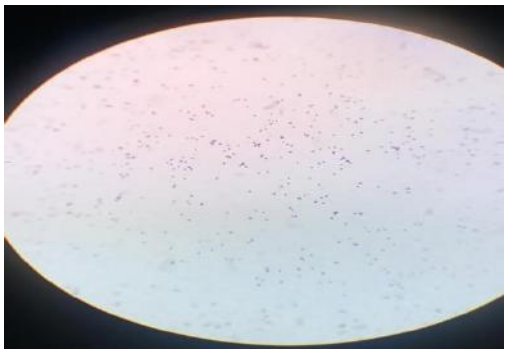
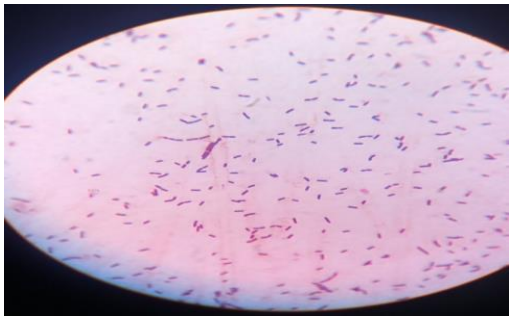


Figure 3 *(Staphylococcus aureus* (MTCC No.87)



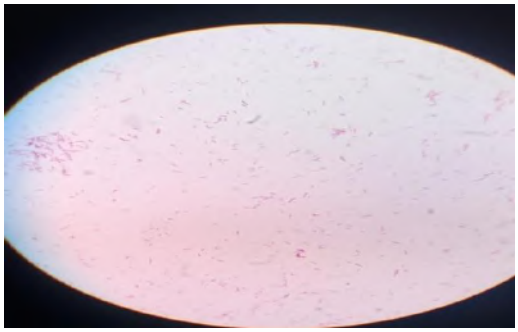


Figure 4 ***(Bacillus spp.*** **(MTCC No.297)** **Figure 5** ***(Esherchia coli*** **(MTCC No.118)**

VI. DRUGS USED IN STUDY

In the present study entitled **"An Experimental Study to Evaluate the *Krimighna* Effect of *Dhoopana* Along with Standardization of Formulations** "three *Dhoopana* Groups have been taken from *Sushruta Samhita Sutra Sthana* and *Kalpa Sthana.* Three *Dhoopa* has been used in this study exclusively for each group.These three Dhoopana are mentioned in the Ayurvedic Samhita i.e.Susruta Samhita.

A. *Dhoopana Dravayas* of Group 1[8]
If smoke and air are poisoned, birds fall to the ground exhausted; develop cough, nasal catarrh, headache, and severe eye diseases. Then *Laksha, Haridra, Ativisha, Abhya, Abda (Musta), Harenuka, Ela (Elaichi), Dala (Tejpatra), Vakra (Tagara), Kustha and Priyangu* should be put on fire and the resulting smoke purifies the air. (**Su. Kal 3/17)**

B. *Dhoopana Dravayas* of Group 2[9]
Dhoopana (Fumigation) should be done twice (to the room, coat, clothes, etc. being used by the patient) with the powder of *Guggulu, Aguru, Sarjarasa,* and *Gaur Sarsapa*, added with *Lavana* (*Saindhava*), *Nimbapatra* and *Ghrita*. The remains of *Ghrita* should be used for restoring his life. **(Su. Su 5/18).**

C. *Dhoopana Dravayas* of Group 3[10]
Dhoopana (fumigation) (of the chambers) should be done for ten days, twice a day without laziness using *Sarshapa* (mustard seeds), leaves of *Arishta*(*Nimba*) added with *Ghrita* (*Ghee*) and *Saidhav Lavana* (salt). (**Su. Su 19/28)**
The fuel used for burning was *Ashwatha* (Peepal) twigs which is mentioned in our *Samhitas.*

VII. EXPERIMENTAL STUDY

A. MATERIAL AND METHODS

i. **Aims and Objectives of the Study/ Research work:**

- To evaluate the antimicrobial effect of *Dhoopana.*
- To find the minimum time and amount for which *Dhoopana* shows antimicrobial effect.

ii. **Study Design**

- **Type of Study**

The present study was an experimental study.

- **Sources of Data**

The study collected data through experimentation using standard bacterial cultures and also by counting bacterial colony-forming units.

- **Place of Study**

1st part of the experiment- R.G.G.P.G. Ayurvedic College& Hospital Paprola, Kangra (H.P.)
2nd part of the experiment- Deptt. of Veterinary Microbiology C.S.K.H.P.K.V. Dr. G.C. Negi College of Veterinary and Animal Sciences, Palampur.

- **Duration of Study** – 4 months

iii. **Plan of Study**

- **The study consists mainly of two parts:**

Literary Review
Experimental Study

- **The materials used for the study:**

Materials for literary review
Materials for experimental study

- **Materials for literary review**

Various *Samhita* were used to collect material for literary review related to *Ayurveda*. *Samhita* consulted were *Veda, Manusamriti, Bhagvad Gita, Charaka Samhita, Sushruta Samhita, Ashtanga Sangraha, Ashtanga Hridya, Madhav Nidan, Kashyapa Samhita, Bhela Samhita, Harita Samhita, Sarangadhara Samhita.*

Modern Review – from books like Park's Textbook of Preventive and Social Medicine by K. Park 26th Ed.,Pharmaceutical Bacteriology w.s.r to Disinfection and Sterilization by Albert Schneider, Alpha Editions, Satish Gupta: Medical Microbiology; Tenth edition,Rajesh Bhatia, Lal Icchpunjai; Essential of Medical Microbiology; Tenth Edition etc. Understanding Pathogen Behaviour, 2005,

- **Materials for experimental study**

a) Collection of samples for the study

i. **Collection of raw drugs** Collection of raw drugs from Charaka Pharmacy Paprola, Herbal Garden Jogindernagar, and market at Palampur and Paprola as mentioned in *Sushruta Samhita* was done.

ii. **Making of *DhoopanaYantra (Havan Kund)***

iii. **Collection of fuel** (*Ashwatha Samidha* (twigs)) for burning

iv. **Growing Bacterial Cultures** (gram-positive and gram-negative) in the lab from CSIR-IMTECH Chandigarh.

B. METHODOLOGY

i. ***Dhoopana Yantra***

Firstly *Dhoopana Yantra* (*Havan Kund*) was made for the experiment. A copper *Havan Kund* was welded and fixed with a small metal horizontal plate. The fixing was done so that paste of yellow mud (*Chikni Mitti*) could easily get adhered to these plates. Then the *Havan Kund* covered with mud was dried in the sunlight. After applying the mud the *Havan Kund* was covered with cow dung. Then at last it was dried in sunlight so that everything gets adhered tightly.

Figure 6(Copper Havan Kund

Figure 7(Welding Done

Figure 8 Paste of mud and cow dung was applied and dried)

Figure 9 (Aswatha Samidha twigs piled up)

ii. ***Ashwatha Samidha* (Twigs) as Fuel**

The twigs, branches and dried bark of the *Ashwatha* (Peepal) tree were collected. They were cut into proper shape and length. The length of each twig was 4 inches with a diameter of 1 inch. The total weight used at one time was 150 g.The study was divided into two parts first part was performed at the college campus of Rajiv Gandhi Government Ayurvedic College Paprola i.e. Settle Plate /Sedimentation Plate Method **(For Colony Counting) a**nd the second part Surface Disinfection Method **(For Standard Bacterial Culture)** was performed at Department of Veterinary Microbiology C.S.K.H.P.K.V. Dr. G.C. Negi College of Veterinary & Animal Sciences Palampur.

iii. **Media for Bacterial Growth**

The media nutrient agar, nutrient broth were used for culturing and performing both the experiments i.e. sedimentation plate method and surface disinfection method.

iv. **Bacterial Cultures**

Four bacterial cultures from CSIR-IMTECH Microbial type culture collection and Gene bank, Institute of Microbial Technology, Chandigarh MTCC no. 297 (*Bacillus spp.*), MTCC no. 129 (*Pseudomonas spp.*), MTCC no. 87 (*Staphylococcus aureus*), and MTCC no. 188 *(Escherichia coli*) were used for this study.

v. **Controls used in the Study:**

- **Positive Control:**- ($KMnO_4$+ Liquid Formalin) (This is standard fumigation)
- **Negative Control:**- Without Intervention of *Dhoopana*
- **Intervention** (These were our research drugs):- *Dhoopana* 1, *Dhoopana* 2, and *Dhoopana* 3.

vi. **Room for Experimental Study**

The room was a closed room in which there was no air entry. Windows and doors were sealed tightly with a mixture made up of *Masha Dal (black gram) powder* and fevicol when *Dhoopana* was done. Then they were covered with a plastic sheet to prevent further air entry from outside and also escape of fumes of *Dhoopana outside*. The second part experiment was done in the lab for which a cubical glass chamber was made of equal dimensions.

a. Dimensions of Room

Dimensions of Room (Volume) where1st part of the experiment was done = L*B*H = 5.59 m^3

b. Dimensions of Cubical Glass Chamber

Dimensions of the cubical box (Volume) used for 2nd part of the experiment was = L*B*H= 16* 16*16 = 0.07 m^3

a) 1st part of the experiment:

i. **Settle Plate/ Sedimentation Plate Method (For Colony Counting)**

Settle plates (also known as plate sedimentation or settling plates) are used in the pharmaceutical industry for semi-quantitative determination of microbial contamination in the air. Settle plates are useful for qualitative analysis of airborne microorganisms and for revealing trends in airborne contamination. The use of settle plates (i.e., the sedimentation or depositional method) is not

recommended when air sampling for fungal spores, because single spores can remain suspended in the air indefinitely. [11] Settle plates have been used either in research studies or during epidemiologic investigations.

Results of sedimentation sampling are expressed as several viable particles or viable bacteria per unit area per the duration of sampling time (i.e., CFU/area/time or CFU/plate/time); this method cannot quantify the volume of air sampled. Because the survival of microorganisms during air sampling is inversely proportional to the velocity at which the air is taken into the sampler, one advantage of using a settling plate is its reliance on gravity to bring organisms and particles into contact with its surface, thus enhancing the potential for optimal survival of collected organisms.

On the college campus, the first part of the experiment was performed where bacterial colony counting through the plate sedimentation method was done. In this colony-forming units (CFUs) were counted per hour per mm of petridish (CFU/ plate /time).

1. Procedure:-

Initially, nutrient agar media was prepared by adding nutrient agar to the distilled water. It is generally made by suspending 28 g of nutrient agar powder in 1000 ml of distilled water by mixing and dissolving them completely. It was sterilized by autoclaving at 121 ℃ for 15 minutes. Then it was poured into the Petri dish and allowed to cool down. A closed room with a volume of 5.59 m^3 was selected for the experiment.

a. No Treatment CFUs

An opened petridish was placed in the selected room for 1 hour. After 1 hour the petridish was closed and placed in the incubator for 24 hours at 37 ℃. The next day the colony-forming units (CFUs/ plate/time) were counted which showed the microbial load of the room. This load was without any intervention from *Dhoopana*.

b. Standard Modern Fumigation ($KMnO_4$ and Liquid Formalin)

Standard fumigation was positive control and it was done for 24 hours. After checking the microbial load of the room it was performed. Then the CFUs were counted. It was very effective in lowering CFUs and showed the least CFUs every time it was used. It was used as a positive control for *Dhoopana* formulations (*Dhoopana* 1, *Dhoopana* 2, and *Dhoopana* 3). After doing standard fumigation room was left untreated for 1-2 week. The microbial load (CFUs) was analyzed after 1-2 weeks. After that the intervention of *Dhoopana* was done when both the CFUs in untreated rooms were roughly equal.

c. *Dhoopana* Intervention

i. Finding Effective Dose (Fixed Time-Varying Dose)

Then after checking the bacterial load, fumigation of the room with *Ayurvedic Dhoopana* Group 1 was done. The selected room was closed and sealed for 24 hours with the paste of fevicol and *Masha Dal* and then by a strong plastic sheet. Initially, the amount chosen for *Dhoopana* was 10 g. After 24 hours again petridish with the sterile nutrient agar was kept for 1 hour in that room and again incubated at 37 ℃.

The next day the colony-forming units (CFUs) were counted and again the room was fumigated with *Ayurvedic Dhoopana* increasing the dose by 10 g every time till the dose shows the least or no CFUs. The average effective was found to be100g (Effective dose).

After a gap of 1 week, the same procedure (process) was repeated with the other two groups Group 2 and 3 of *Dhoopana*. The average effective dose came to be 100 g for both formulations keeping that dose effective. The dose of 10 g was considered standard for the room and time was kept constant. The exposure time was 24 hours for which the plate was kept in that room.

The standard dose was found by checking the density of fumes in the room and 10 g showed dense fumes so we considered it standard. The effective dose that showed the highest effect on CFUs was an average of 100g for all three groups. This means the least number of CFUs (CFUs/1hour / 90mm petridish) were observed at a dose of 100g. The least CFUs are from 1 to 8.

ii. Fixed- Dose Varying Time

In the next procedure, the dose was fixed and the time was kept variable. The first 30g of *Dhoopana* 1 was taken and the procedure was performed with an exposure time of 8 hours. After eight hours petridish containing nutrient agar was kept open for 1 hour. Then it was incubated for 24 hours. After 24 hours, the plate was analyzed for colony-forming units (CFUs). Now 30g dose was given between two intervals of 8 hours i.e., exposure time of 16 hours, and *Dhoopana* was done twice in 16 hours. After 16 hours the nutrient agar media plate was again kept open for one hour and then incubated for 24 hours. After 24 hours plate was again analyzed for CFUs. Now 30g dose was given between three intervals of 8 hours i.e. exposure of 24 hours, *Dhoopana* was done thrice in 24 hours. After 24 hours the nutrient agar media plate was again kept open for one hour and then incubated for 24 hours. After 24 hours plate was again analyzed for CFUs.

- **With 60 g dose**

Now again same procedure was repeated with a dose of 60g of *Dhoopana* 1 respectively for three intervals (exposures) of 8 hours, 16 hours, and 24 hours and CFUs were analyzed.

- **With 90g dose**

Now again same procedure was repeated with a dose of 90g of *Dhoopana* 1 respectively for three intervals of 8 hours, 16 hours, and 24 hours and CFUs were analyzed.

The procedure was also repeated with *Dhoopana* 2 and *Dhoopana* 3 keeping a gap of 7 days in each group so that microbial load increases at an interval of 8 hours, 16 hours, and 24 hours and at a dose of 30 g, 60 g, and 90g respectively. Then CFUs were counted.

Figure 10

Figure11

Figure12

Figure 13

Figure 14

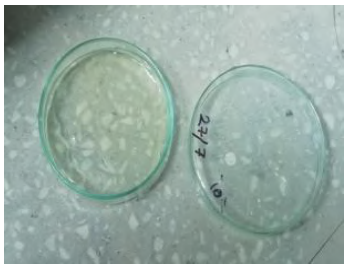
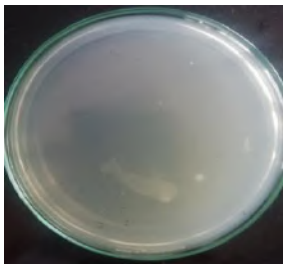





Figure 15 Figure 16 Figure 17 Figure 18

Figure 10,11,12,13,14,15,16,17,18 (1st Part Of The Experiment Sedimentation Plate Method For Colony Counting)

d. Standard Bacterial Cultures Used In The Study

The Following Standard bacterial cultures procured from CSIR-IMTECH Chandigarh were used in the trial:-
GRAM-POSITIVE:
1. *Bacillus spp.*
2. *Staphylococcus aureus*
GRAM-NEGATIVE:
1. *Escherichia coli*
2. *Pseudomonas spp.*

b) 2nd part of the experiment

i. Surface Disinfection Method (For Standard Bacterial Culture)

Surface disinfection is defined according to prEN 14885 (draft 2012) Chemical disinfectants and antiseptics- Application of European Standards for chemical disinfectants and antiseptics, as chemical disinfection of a solid surface, excluding those of certain medical and veterinary instruments, by the application of a product with or without mechanical action. Application methods include circulation, dipping, fumigating, immersion, spraying, wiping, etc. [12]

The second part of the trial was performed in the Department of Veterinary Microbiology C.S.K.H.P.V Dr. G.C. College of Veterinary Sciences and Animal Sciences Palampur. The experiment was done according to the guidance mentioned in the article. [13]

Environmental disinfection greatly reduces the occurrence of health-associated infections which are the major healthcare problems worldwide. In India, *Ayurvedic* traditional fumigation with natural plant products is used to disinfect the environment.
The traditional fumigation to disinfect inanimate groups was evaluated using the following bacterial strains two gram-positive and two gram-negative. The glass slide was artificially inoculated with the bacteria and fumigated whereas the non-fumigated slide served as control. The control and fumigated slides were analyzed for surviving bacteria.

- **Culturing of Standard Bacteria**

Freeze-dried bacterial cultures were procured from CSIR-IMTECH Microbial type culture collection and Gene bank, Institute of Microbial Technology, Chandigarh MTCC NO 297 *(Bacillus spp.)*, MTCC NO 129 *(Pseudomonas spp.*), MTCC 87 *(Staphylococcus aureus*), and MTCC 188 *(Escherichia coli*) and were grown by adding liquid media like nutrient broth. Then single colony was taken from each bacterial culture and was cultured on petridish containing nutrient agar. Then these plates were incubated for 48 hours at 37 ℃ except for *Bacillus* which was incubated for 24 hours.

- **Using McFarland Standard**

The next day one colony from every 4 plates was taken with the help of a sterile loop and added to the sterile test tubes containing 1ml of nutrient broth. Then these test tubes were again incubated for 24 hours. After 24 hours they were taken out and checked for McFarland standard by McFarland Densitometer. The standard in this densitometer is 0.5 reading. If the recording shows less than 0.5 then culture is added to the test tube, if it shows a reading of more than 0.5 then nutrient broth is added to it to dilute it.

- **Using Glass Slides Marked as Control and Test**

After that, 10 microlitres of 0.5, McFarland overnight was taken with the help of a micropipette and spotted on eight sterile slides marked as control and tested for 4 bacterial cultures for example *Pseudomonas spp*. Control, *Pseudomonas spp*. Test etc. for all. These were dried and placed in an empty sterile petridish. Now, these Petri dishes with their lid open were kept in 16 inches *16 inches *16 inches cubical glass chamber which is fumigated with 5 g *Dhoopana* group 1 for 1 hour.

- **Rubbing Swabs on Nutrient Agar**

Then after 1-hour exposure, the glass chamber was opened and petridishes were closed and kept in the incubator for 24 hours along with petridishes containing control glass slides. After 24 hours the petridishes were opened and glass slides were taken out. Then 100 microlitre of nutrient broth was added to each slide of test and control with the help of a micropipette. Then with the help of sterile swab sticks the slides were rubbed and these swabs were again rubbed on sterile petridishes containing nutrient agar.

- **Analyzing the petridishes**

After that, these eight petri dishes were marked as control and tested for each bacterial strain, and then kept in an incubator for 24 hours. Then after 24 hours, these were taken out and the bacterial colony growth was compared in each test and control group petridish. The slides were kept in a sterile petridish to prevent contamination.

The procedure was completed in 4 days with each bacterial culture and it was done with all the *Dhoopana* groups (1, 2, and 3). This was a surface disinfection method.

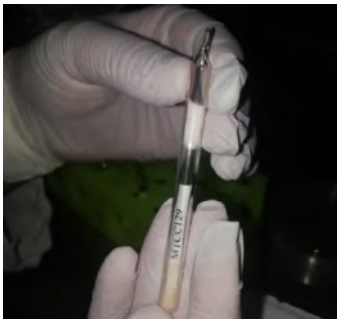


Figure19

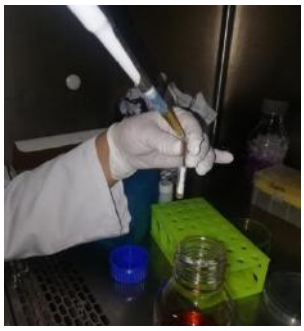
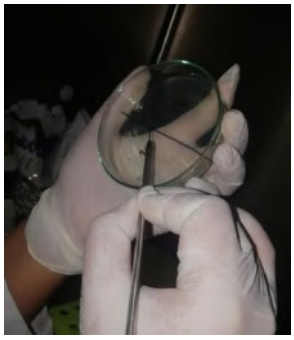


Figure20



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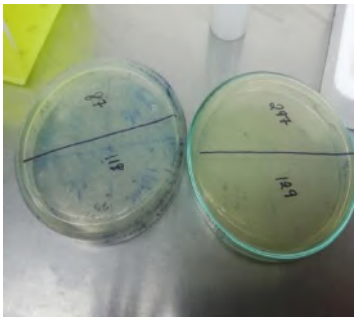

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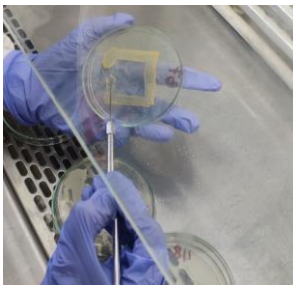


Figure23

Figure 24

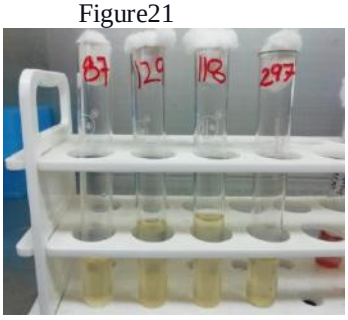
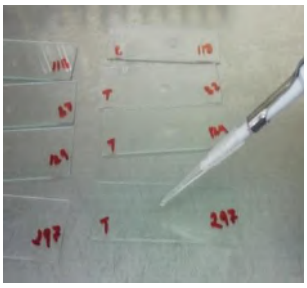


Figure 25



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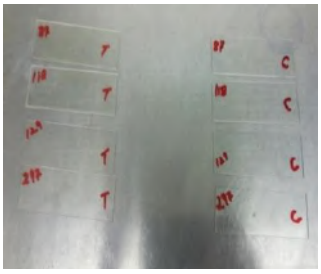


Figure 27

Figure 28

Figure 29

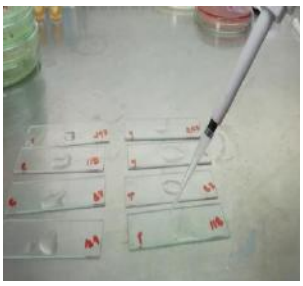


Figure 30

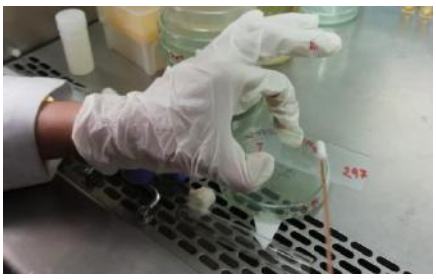


Figure 31

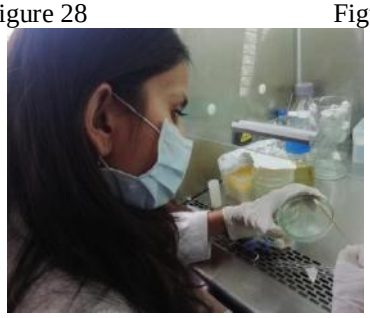


Figure 32

Figure 23,24,25,26,27,28,29,30,31,32 (2nd Part of the Experiment Surface Disinfection Method for Standard Bacteria)

c) Microscopic Studies

The sample from each culture was taken on the glass slide with the help of a sterile loop and allowed to dry. Then staining is done by adding four stains in a sequence from the Gram staining kit. Then these slides are dried and kept under a microscope for examination for bacteria (gram-positive and gram-negative), fungi, etc. Studies were done for both parts of the experiment done in Veterinary College Palampur and Govt. Ayurvedic College Paprola. The 1st part of the experiment showed gram-positive bacteria under the microscope as they stained purple color. 2nd part of the experiment showed purple color in gram-positive bacteria and pink color in gram-negative bacteria when stained.

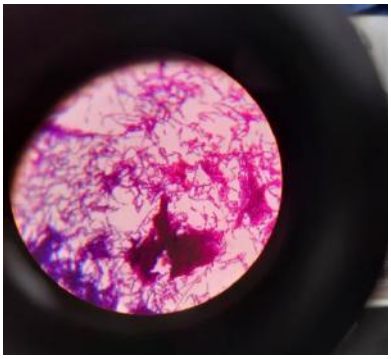
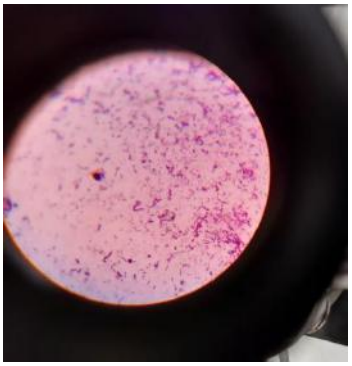
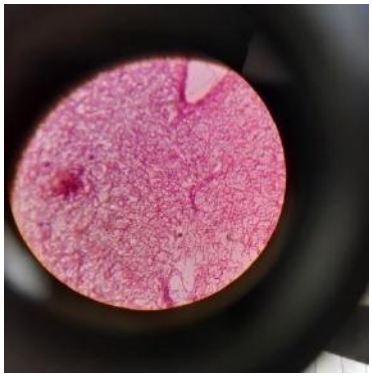




Figure 33 Figure 34 Figure 35

Figure33, 34, 35(Gram Positive Bacteria Found in Microscopic Examination After 1st Part of Experiment)

C. OBSERVATIONS AND RESULTS OF STUDY

a. Sedimentation Plate Method (1st part of the experiment)

- **Results of Negative control (No Intervention):-**

46 CFUs (microbial load) before *Dhoopana* 1,
31 CFUs (microbial load) before *Dhoopana* 2, and
35 CFUs (microbial load) before *Dhoopana* 3

- **Result of Positive Control:** - 1 CFUs after Standard Fumigation
- **Results of Test Samples (Intervention) (*Dhoopana*1, *Dhoopana* 2, and *Dhoopana* 3):-**

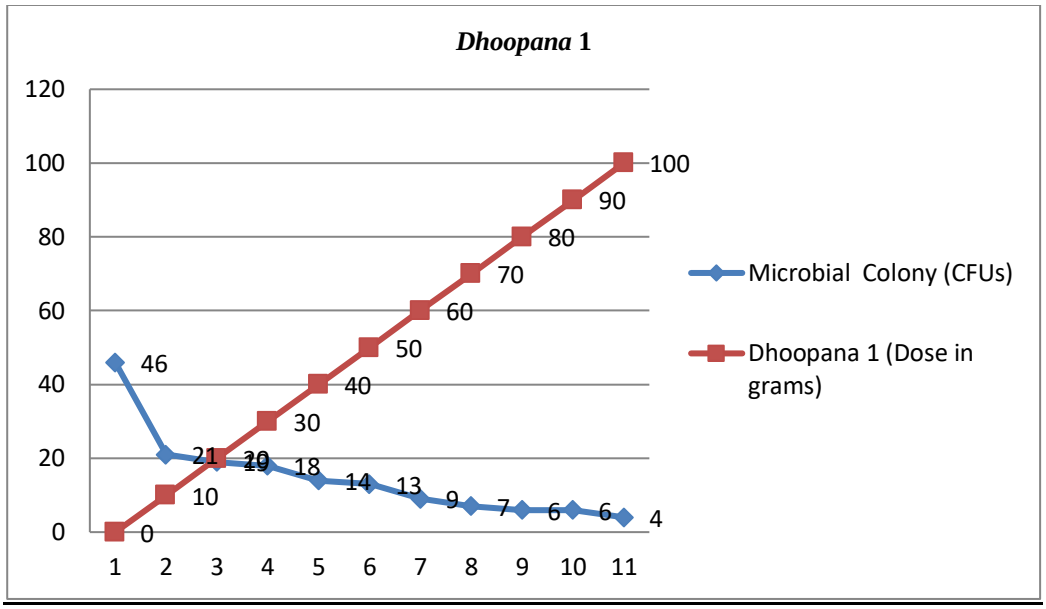
Percentage Change Formula = (Old Number – New Number) * 100 / Old Number

i. CHANGE IN CFUs AFTER *DHOOPANA* 1

Time interval = 24 hours exposure.
E.S. Table 1 *(Results after Dhoopana 1*

After *Dhoopana* 1 (Dose) in grams	**Microbial Colony in the room (CFUs) (CFUs/plate/hour)**	**Percentage Change(%)**
Without intervention= 46 CFUs /1hour/90mm of petridish		
10g	21 CFUs	54.35
20g	19 CFUs	58.69
30g	18 CFUs	60.86
40g	14 CFUs	69.56
50g	13 CFUs	71.74
60g	9 CFUs	80.43
70g	7 CFUs	84.78
80g	6 CFUs	86.96
90g	6 CFUs	86.96
100g	4 CFUs	91.30

Graph 1: Results after Dhoopana 1



Initially, there was no intervention of *Dhoopana* taking it as a negative control. The total colony-forming units (CFUs) came out to be 46. Then 10 g of *Dhoopana* 1/ *Dhoop* 1 was taken as the standard dose in which the room was covered by the fumes. 10g showed a decrease in CFUs i.e. from 46 to 21 (54.35%). Then the dose is increased to 10g daily till the effective dose is calculated. The effective dose came out to be 100g which shows a % change of 91.30 % and 4 CFUs.

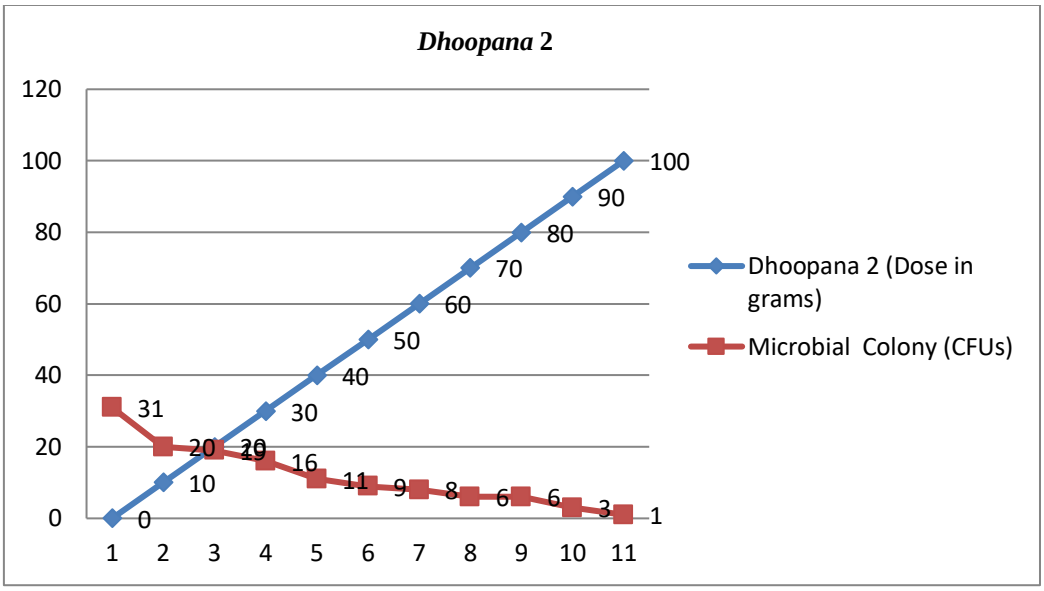
ii. CHANGE IN CFUs AFTER *DHOOPANA* 2

Time interval = 24 hours exposure

E.S. Table 2 (*Results after Dhoopana 2)*

After *Dhoopana* 2 (Dose) in grams	**Microbial Colony in the room(CFUs) (CFUs/plate/hour)**	**Percentage Change(%)**
Without intervention=31 CFUs/1hour/90mm of petridish		
10g	20 CFUs	35.48
20g	19 CFUs	38.71
30g	16 CFUs	48.39
40g	11 CFUs	64.52
50g	9 CFUs	70.97
60g	8 CFUs	74.19
70g	6 CFUs	80.65
80g	6 CFUs	80.65
90g	3 CFUs	90.32
100g	1 CFUs	96.77

Graph 2: Results after Dhoopana 2



Initially, there was no intervention of *Dhoopana* taking it as a negative control. The total colony-forming units (CFUs) came out to be 31. Then 10 g of *Dhoopana* 2/*Dhoop* 2 was taken as the standard dose in which the room was covered by the fumes. 10g showed a decrease in CFUs i.e. from 31 to 20(35.48%). Then the dose is increased to 10g daily till the effective dose is calculated. The effective dose came out to be 100g which shows a % change of 96.77% and 1 CFUs. This group showed the maximum results as it gave the best results among the three *Dhoopana / Dhoopa* Groups.

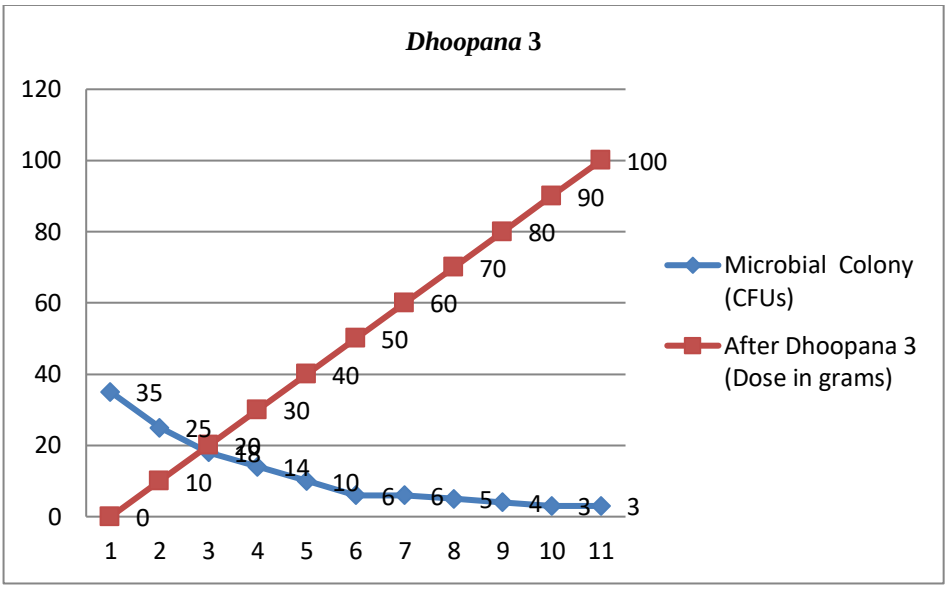
iii. CHANGE IN CFUs AFTER *DHOOPANA* 3

Time interval = 24 hours exposure

E.S. Table 3 (*Results after Dhoopana 3)*

After *Dhoopana* 3 (Dose) in grams	**Microbial Colony in the room (CFUs)(CFUs/plate/hour)**	**Percentage Change(%)**
Without intervention= 35 CFUs/1hour/90mm of petridish		
10g	25 CFUs	28.57
20g	18 CFUs	48.57
30g	14 CFUs	60.00
40g	10 CFUs	71.43
50g	6 CFUs	82.86
60g	6 CFUs	82.86
70g	5 CFUs	85.71
80g	4 CFUs	88.57
90g	3 CFUs	91.43
100g	3 CFUs	91.43

Graph 3: Results after Dhoopana 3

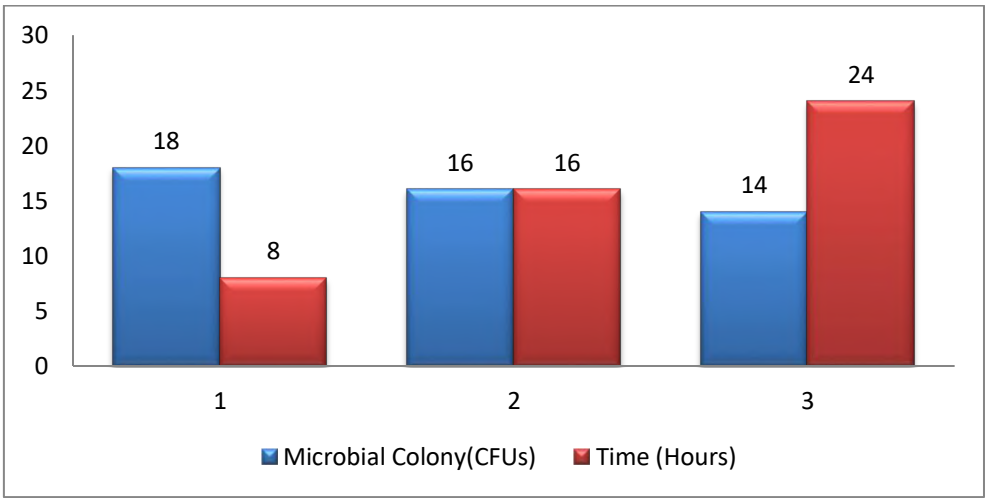


Initially, there was no intervention of *Dhoopana* taking it as a negative control. The total colony-forming units (CFUs) came out to be 35. Then 10 g of *Dhoopana* 3 / *Dhoop* 3 was taken as the standard dose in which the room was covered by the fumes. 10g showed a decrease in CFUs i.e., from 35 to 25(28.57%). Then the dose is increased to 10g daily till the effective dose is calculated. The effective dose came out to be 100g which shows a % change of 91.43 % and 3CFUs.

E.S. Table 4 *Results after Dhoopana* 1, Dose 30g for each exposure time (Dose 30g for each interval)
Microbial Load without intervention (CFUs =35/1 hour/ 90mm of petridish)

***Dhoopana* Formulations**	**Time (Hours)**	**Microbial Colony(CFUs)**
Dhoopana 1	8	18 CFUs
Dhoopana 1	16	16 CFUs
Dhoopana 1	24	14 CFUs

***Graph 4:* Results after *Dhoopana* 1 (Dose 30g for each interval)**

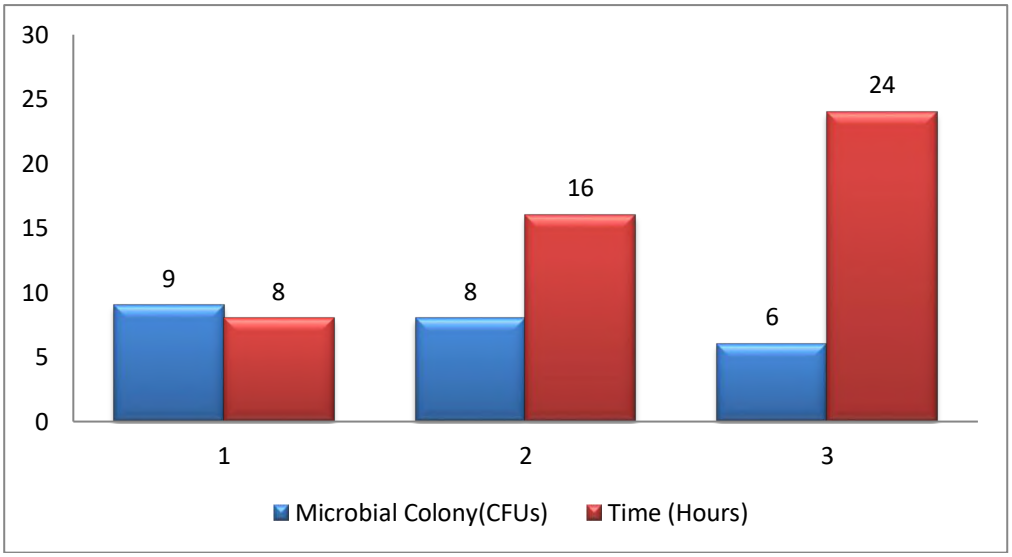


The time of 8 hours exposure (once a day) for dose 30g shows maximum result as the CFUs.

E.S. Table 5 Results of *Dhoopana* 1, Dose 60g for each interval (Dose 60g for each interval)

***Dhoopana* Formulations**	**Time (Hours)**	**Microbial Colony(CFUs)**
Dhoopana 1	8	9 CFUs
Dhoopana 1	16	8 CFUs
Dhoopana 1	24	6 CFUs

***Graph 5:* Results of *Dhoopana* 1 (Dose 60g for each interval)**

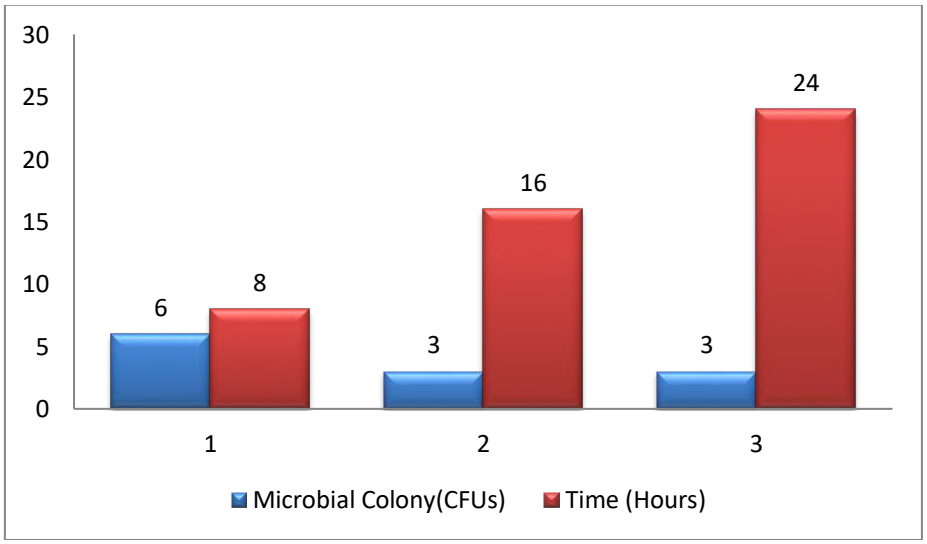


The time of 16 hours exposure (2 times each 8 hourly) shows maximum result as the CFUs were 6

E.S. Table 6 (Results after *Dhoopana* 1, Dose 90g for each exposure time (Dose 90g for each interval)

***Dhoopana* Formulations**	**Time (Hours)**	**Microbial Colony(CFUs)**
Dhoopana 1	8	6 CFUs
Dhoopana 1	16	3 CFUs
Dhoopana 1	24	3 CFUs

Graph 6: Results after *Dhoopana* 1 (Dose 90g for each interval)

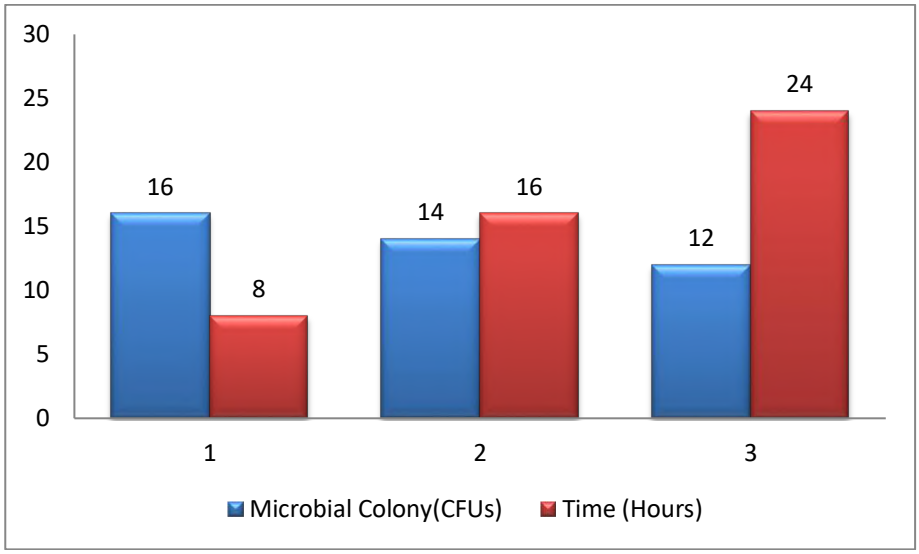


The time of 24 hours exposure (3 times each 8 hourly) shows maximum result as the CFUs were 3

E.S. Table 7 Results after *Dhoopana* 2, Dose 30g for each exposure time (Dose 30g for each interval)
Microbial Load without intervention (CFUs =33/1hour/90mm of petridish)

***Dhoopana* Formulations**	**Time (Hours)**	**Microbial Colony(CFUs)**
Dhoopana 2	8	16 CFUs
Dhoopana 2	16	14 CFUs
Dhoopana 2	24	12 CFUs

Graph 7: Results of *Dhoopana* 2, (Dose 30g for each interval)

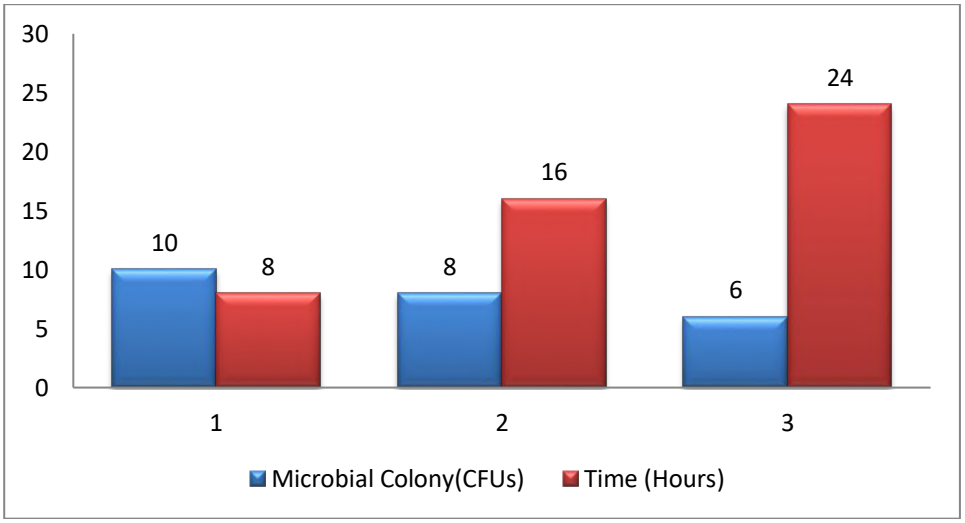


The time of 8 hours exposure (once a day) shows maximum result as the CFUs were 12.

E.S.Table 8 Results of *Dhoopana* 2, Dose 60g for each interval (Dose 60g for each interval)

***Dhoopana* Formulations**	**Time (Hours)**	**Microbial Colony(CFUs)**
Dhoopana 2	8	10 CFUs
Dhoopana 2	16	8 CFUs
Dhoopana 2	24	6 CFUs

Graph 8: Results of *Dhoopana* 2 (Dose 60g for each interval)

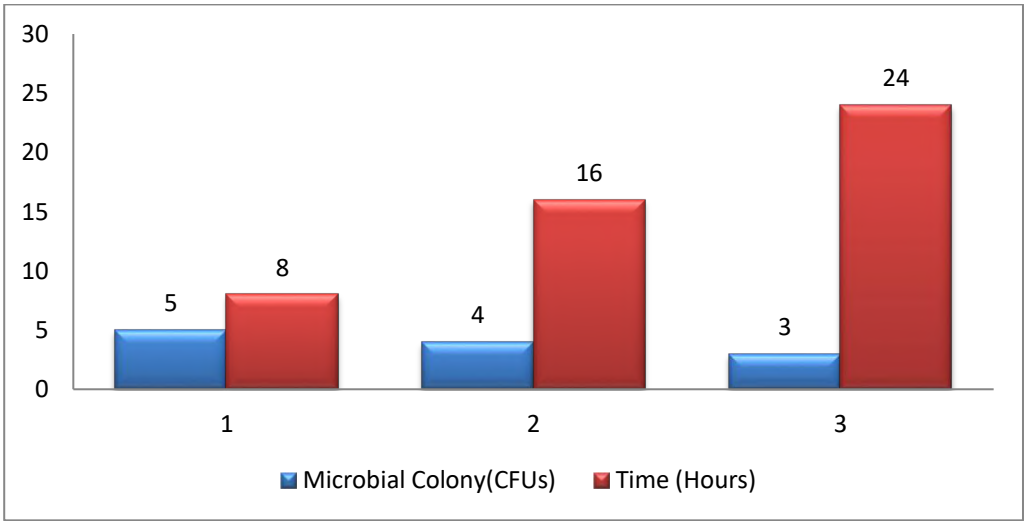


The time of 16 hours exposure (2 times each 8 hourly) shows maximum result as the CFUs were 6

E.S. Table 9 Results of *Dhoopana* 2, Dose 90g for each exposure time (Dose 90g for each interval)

***Dhoopana* Formulation**	**Time (Hours)**	**Microbial Colony(CFUs)**
Dhoopana 2	8	5 CFUs
Dhoopana 2	16	4 CFUs
Dhoopana 2	24	3 CFUs

Graph 9: Results of *Dhoopana* 2 (Dose 90g for each interval)

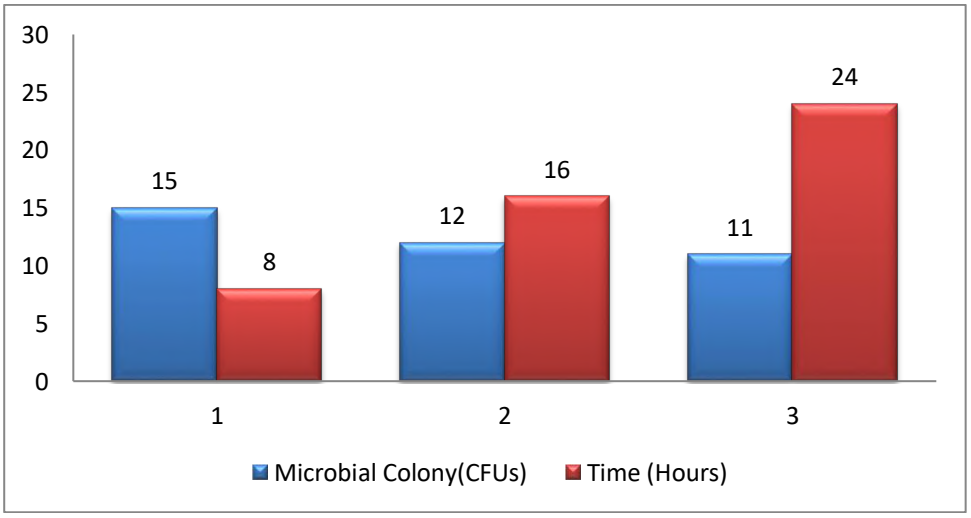


The time of 24 hours exposure (3 times each 8 hourly) for dose 90g shows maximum result as the CFUs were 3

E.S.Table 10 Results of *Dhoopana* 3, Dose 30g for each exposure time (Dose 30g for each interval)
Microbial Load without intervention (CFUs =31/1 hour/ 90mm of petridish)

***Dhoopana* Formulation**	**Time (Hours)**	**Microbial Colony(CFUs)**
Dhoopana 3	8	15 CFUs
Dhoopana 3	16	12 CFUs
Dhoopana 3	24	11 CFUs

Graph 10: Results of *Dhoopana* 3, Dose 30g for each interval

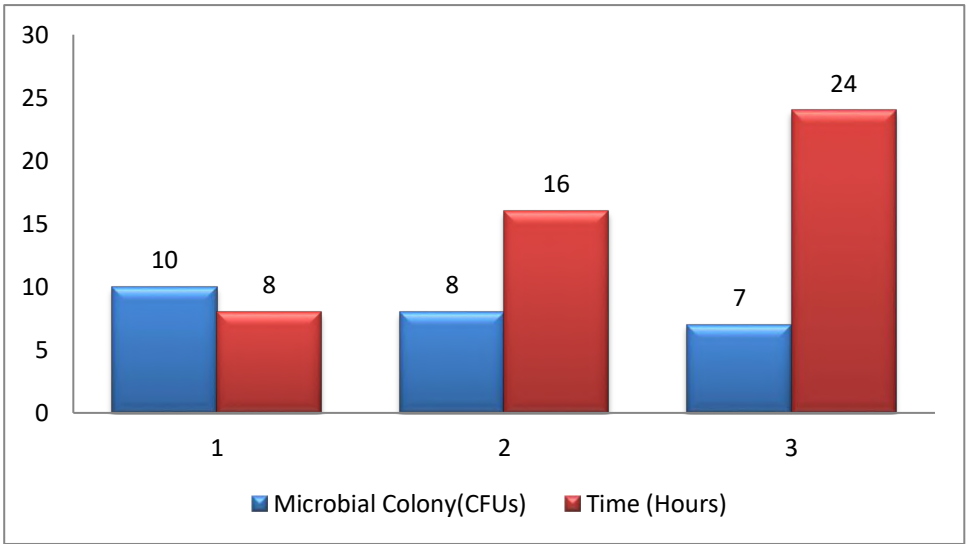


The time of 8 hours exposure (once a day) for dose 90g shows maximum result as the CFUs were 11

E.S.Table 11: Results of *Dhoopana* 3, Dose 60g for each exposure time (Dose 60g for each interval)

Dhoopana Formulation	Time (Hours)	Microbial Colony(CFUs)
Dhoopana 3	8	10 CFUs
Dhoopana 3	16	8 CFUs
Dhoopana 3	24	7 CFUs

Graph 11: Results of *Dhoopana* 3 (Dose 60g for each interval)

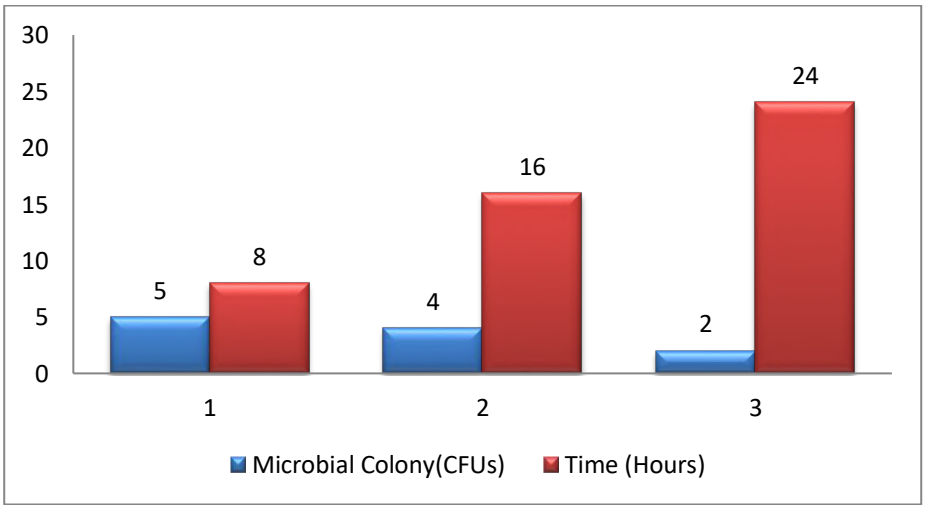


The time of 16 hours exposure (2 times each 8 hourly) for dose 60g shows maximum result as the CFUs were 7

E.S.Table 12 Results of *Dhoopana* 3, Dose 90g for each exposure time (Dose 90g for each interval)

Dhoopana Formulation	Time (Hours)	Microbial Colony (CFUs)
Dhoopana 3	8	5 CFUs
Dhoopana 3	16	4 CFUs
Dhoopana 3	24	2 CFUs

Graph 12: Results of *Dhoopana* Formulation 3 (Dose 90g for each interval)



The time of 24 hours exposure (3 times each 8 hourly) for dose 90g shows the maximum result as the CFUs were 2. Repeating the *Dhoopana* of all groups thrice a day (8 hourly each) and giving the maximum dose of 100 g shows the maximum result which is depicted by a decrease in CFUs.

- **Findings:-**

a. ***Krimighna* (antimicrobial effect) of the *Dhoopana* after 24-hour sterilization:-**

Krimighna effect increases with the increase in the dose of *Dhoopana* (*Ayurvedic* fumigation).

b. *Krimighna* (antimicrobial effect) of the *Dhoopana* after regular intervals:

Krimighna action is enhanced by increasing the interval i.e. *Dhoopana* shows maximum antimicrobial action when given thrice a day at an interval of 8 hours each.

Standard dose -10g
Effective dose-100g
Standard time - 8 hours (first interval)
Effective time - repeating 3 times in 24 hours after every 8 hours
According to the *Acharya Sushruta* also at least it must be given twice a day to reap maximum results (5/18 Su. Su.)

Comparison of Results of *Dhoopana* with Positive Control (Standard Fumigation) (E.S. Table13)

% cent decrease in CFUs with each *Dhoopana* Formulations	**Positive Control (Standard Fumigation)**	**Negative Control (When no *Dhoopana* was given**
Dhoopana 1- 91.30% (3 CFUs) *Dhoopana* 2- 96.77% (1CFUs) *Dhoopana* 3- 91.43% (1 CFUs) 24 hours exposure in a closed room	($KMnO_4$+Formaldehyde)Formalin- 95-99% For 24 hours exposure in a closed room	CFUs= 46 CFUs= 35 CFUs= 31

D. STATISTICAL CALCULATION

Statistical calculation was done manually and the Chi-Square test was used to test the association and significance of the experiment.

i. STATISTICAL SIGNIFICANCE OF EACH DOSE OF *DHOOPANA* 1

Group 1 (E.S.Table 14) (Result after *Dhoopana* 1)

Sr. No.	*Dhoopana* Doses/Groups	Survived CFUs	Died CFUs	Total CFUs	χ^2	p value
1	10g	A= 21	B= 25	A+B =46	34.3	<0.001
	Control	C= 46	D= 0	C+D=46		
2	20g	A= 19	B= 27	A+B =46	38.2	<0.001
	Control	C= 46	D= 0	C+D=46		
3	30g	A= 18	B= 28	A+B =46	40.3	<0.001
	Control	C= 46	D= 0	C+D=46		
4	40g	A= 14	B= 32	A+B =46	49.1	<0.001
	Control	C= 46	D= 0	C+D=46		
5	50g	A= 13	B= 33	A+B =46	51.5	<0.001
	Control	C= 46	D= 0	C+D=46		

6	60g	A= 9	B= 37	A+B =46	61.9	<0.001
	Control	C= 46	D= 0	C+D=46		
7	70g	A= 7	B= 39	A+B =46	67.7	<0.001
	Control	C= 46	D= 0	C+D=46		
8	80g	A= 6	B= 40	A+B =46	70.8	<0.001
	Control	C= 46	D= 0	C+D=46		
9	90g	A= 6	B= 40	A+B =46	70.8	<0.001
	Control	C= 46	D= 0	C+D=46		
10	100g	A= 4	B= 42	A+B =46	77.3	<0.001
	Control	C= 46	D= 0	C+D=46		

Every dose in the *Dhoopana* Formulations 1 /*Dhoopa* 1 is statistically highly significant ($p<0.001$) which rejects the null hypothesis (A *null hypothesis* is a type of statistical hypothesis that proposes that no statistical significance exists in a set of given observations) and shows that *Dhoopana* shows the *Krimighna* (antimicrobial effect) with each dose starting from 10g. It also shows the association between exposures to *Dhoopana* and decreases in bacterial colony-forming units (CFUs) when studied with the control group.

ii. STATISTICAL SIGNIFICANCE OF EACH DOSE OF*DHOOPANA* 2

Group 2 (E.S.Table 15) (Results after *Dhoopana* 2)

Sr. No.	*Dhoopana* Doses/Groups	Survived CFUs	Died CFUs	Total CFUs	χ^2	p value
1	10g	A= 20	B= 11	A+B=31	13.4	<0.001
	Control	C= 31	D= 0	C+D= 31		
2	20g	A= 19	B= 12	A+B= 31	14.9	<0.001
	Control	C= 31	D= 0	C+D= 31		
3	30g	A= 16	B= 15	A+B= 31	19.8	<0.001
	Control	C= 31	D= 0	C+D= 31		
4	40g	A= 11	B= 20	A+B= 31	29.5	<0.001
	Control	C= 31	D= 0	C+D= 31		
5	50g	A= 9	B= 22	A+B= 31	34.1	<0.001
	Control	C= 31	D= 0	C+D= 31		
6	60g	A= 8	B= 23	A+B= 31	36.6	<0.001
	Control	C= 31	D= 0	C+D= 31		
7	70g	A= 6	B= 25	A+B= 31	41.9	<0.001
	Control	C= 31	D= 0	C+D= 31		
8	80g	A= 6	B= 25	A+B= 31	41.9	<0.001
	Control	C= 31	D= 0	C+D= 31		
9	90g	A= 3	B= 28	A+B= 31	51.5	<0.001
	Control	C= 31	D= 0	C+D= 31		

10	100g	A= 1	B= 30	A+B= 31	58.1	<0.001
	Control	C= 31	D= 0	C+D= 31		

Every dose in the *Dhoopana* Formulations 2 / *Dhoopa* 2 is statistically highly significant (p<0.001) which rejects the null hypothesis and shows that *Dhoopana* shows the *Krimighna* (antimicrobial effect) with each dose starting from 10g. It also shows the association between exposures to *Dhoopana* and a decrease in bacterial colony-forming units (CFUs) when studied with the control group.

iii. STATISTICAL SIGNIFICANCE OF EACH DOSE OF*DHOOPANA* 3

Group 3 (E.S.Table16) (Results after *Dhoopana* 3)

Sr. No.	*Dhoopana* Doses/Groups	Survived CFUs	Died CFUs	Total CFUs	χ^2	p value
1	10g	A= 25	B= 10	A+B= 35	11.7	<0.001
	Control	C= 35	D= 0	C+D= 35		
2	20g	A= 18	B= 17	A+B= 35	22.04	<0.001
	Control	C= 35	D= 0	C+D= 35		
3	30g	A= 14	B= 21	A+B= 35	30	<0.001
	Control	C= 35	D= 0	C+D= 35		
4	40g	A= 10	B= 25	A+B= 35	38.9	<0.001
	Control	C= 35	D= 0	C+D= 35		
5	50g	A= 6	B= 29	A+B= 35	49.5	<0.001
	Control	C= 35	D= 0	C+D= 35		
6	60g	A= 6	B= 29	A+B= 35	49.5	<0.001
	Control	C= 35	D= 0	C+D= 35		
7	70g	A= 5	B= 30	A+B= 35	52.5	<0.001
	Control	C= 35	D= 0	C+D= 35		
8	80g	A= 4	B= 31	A+B= 35	55.6	<0.001
	Control	C= 35	D= 0	C+D= 35		
9	90g	A= 3	B= 32	A+B= 35	59	<0.001
	Control	C= 35	D= 0	C+D= 35		
10	100g	A= 3	B= 32	A+B= 35	59	<0.001
	Control	C= 35	D= 0	C+D= 35		

Every dose in the *Dhoopana* Formulations 3 / *Dhoopa* 3 is statistically highly significant (p<0.001) which rejects the null hypothesis and shows that *Dhoopana* shows the *Krimighna* (antimicrobial effect) with each dose starting from 10g. It also shows the association between exposures to *Dhoopana* and a decrease in bacterial colony-forming units (CFUs) when studied with the control group.

a. Surface Disinfection Method (2nd part of the experiment)

i. Effect of *Dhoopana* 1 (*Dhoopa* 1) on gram-positive and gram-negative bacteria

Following observations were draw with *Dhoopana* 1 (*Dhoopa*1) when the exposure time was 1 hour and the dose was 5 g also when 10 ml of broth showing McFarland (0.5) reading was taken.

Figure 36 (*E. coli* Control and Test)

Escherichia coli is a gram-negative bacteria, *Dhoopana* 1 (*Dhoop* 1) shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C).
Finding- *Dhoopana* 1 is effective against *E. coli* (90% inhibition).

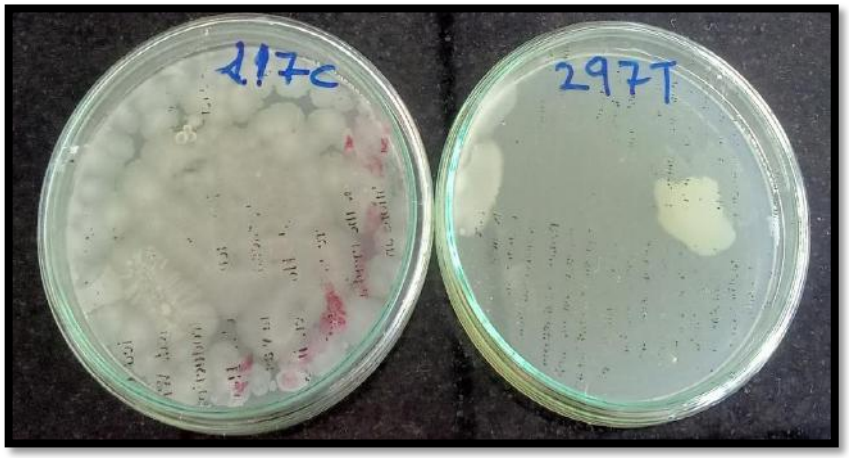


Figure 37 (*Bacillus spp.* Control and Test)

Bacillus spp. is gram-positive bacteria, *Dhoopana* 1 shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C).
Finding - *Dhoopana* 1 is effective against *Bacillus spp. (*90% inhibition)

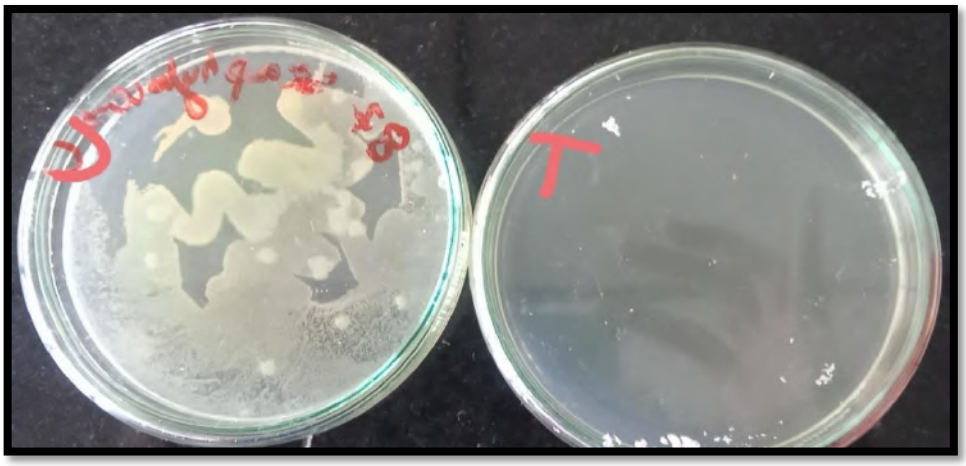


Figure 38 (*Staphylococcus aureus* Control and Test)

Staphylococcus aureus is gram-positive bacteria, *Dhoopana* 1 shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C)
Finding- *Dhoopana* 1 is effective against *Staphylococcus aureus* (100% inhibition).

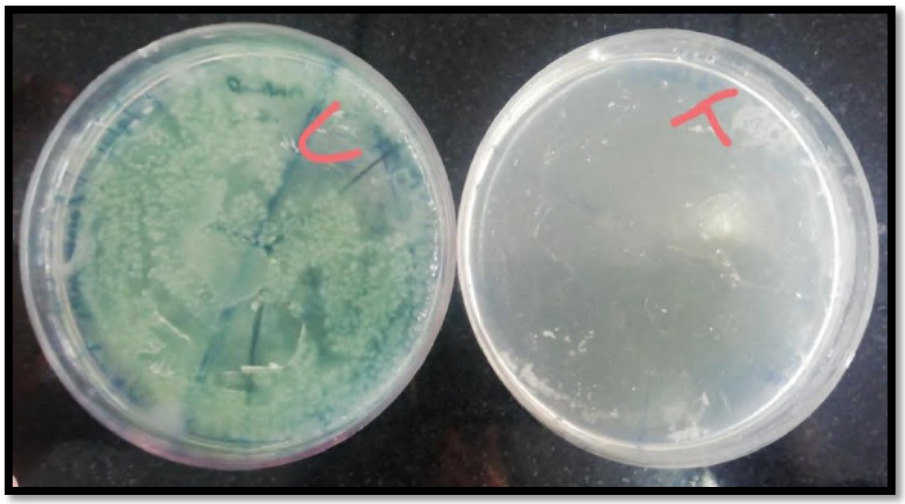


Figure 39 (*Pseudomonas spp.* Control and Test)

Pseudomonas spp. is a gram-negative bacterium, *Dhoopana* 1 (*Dhoop*a 1) shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C).
Finding-Which shows *Dhoopana* 1, is effective against *Pseudomonas spp.* (100% inhibition).

ii. Effect of *Dhoopana 2 (Dhoop2*) on gram-positive and gram-negative bacteria

Following observations were draw with *Dhoopana* 2 (*Dhoopa 2*) when the exposure time was 1 hour and the dose was 5 g also when 10 ml of broth showing McFarland (0.5) reading was taken

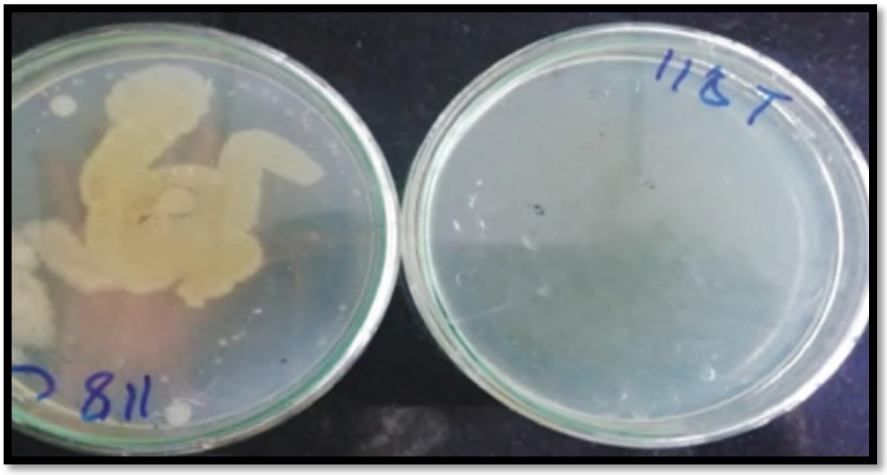


Figure 40 *E. coli* Control and Test)

Escherichia coli is a gram-negative bacteria, *Dhoopana* 2 (*Dhoopa* 2) shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C).
Finding- *Dhoopana* 2 is effective against *E. coli* (100% inhibition).

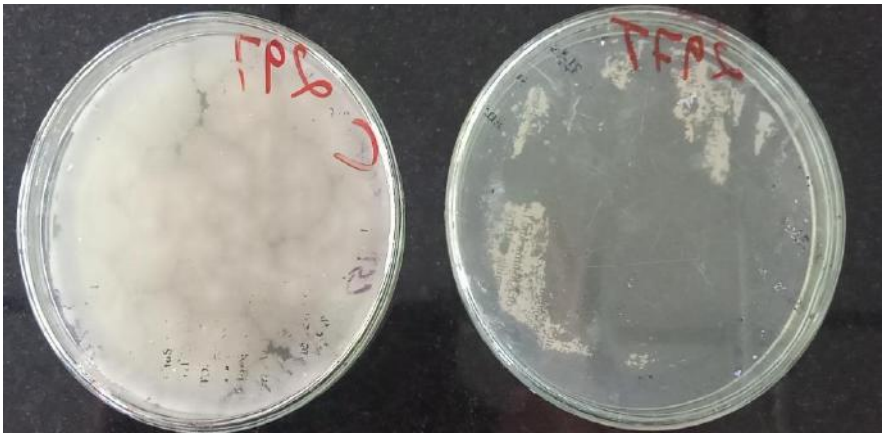


Figure 41 (*Bacillus spp.* Control and Test)

Bacillus spp is gram-positive bacteria, *Dhoopana* 2 shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C)
Finding- *Dhoopana* 2 is effective against *Bacillus spp.* (100% inhibition).

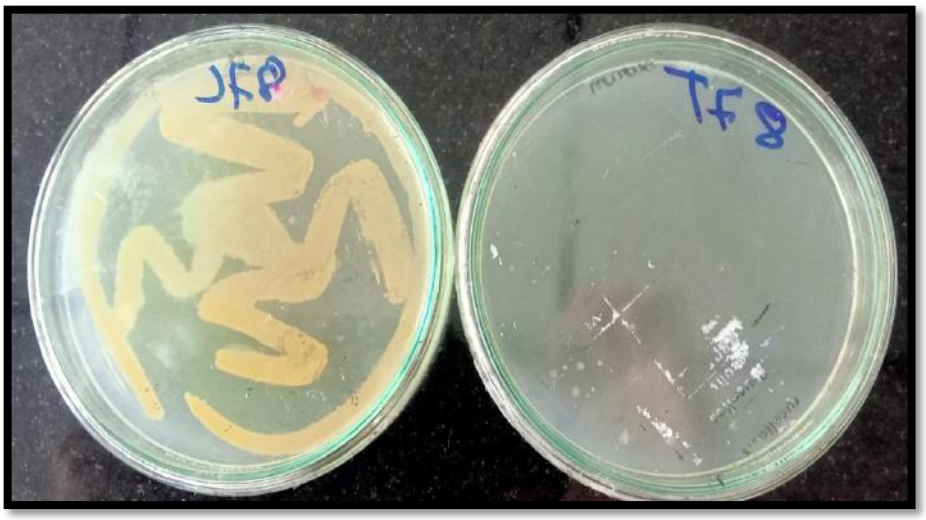


Figure 42 (*Staphylococcus aureus* Control and Test)

Staphylococcus aureus is gram-positive bacteria, *Dhoopana* 2
(*Dhoopa* 2) shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C)
Finding- *Dhoopana* 1 is effective against *Staphylococcus aureus*(100% inhibition).

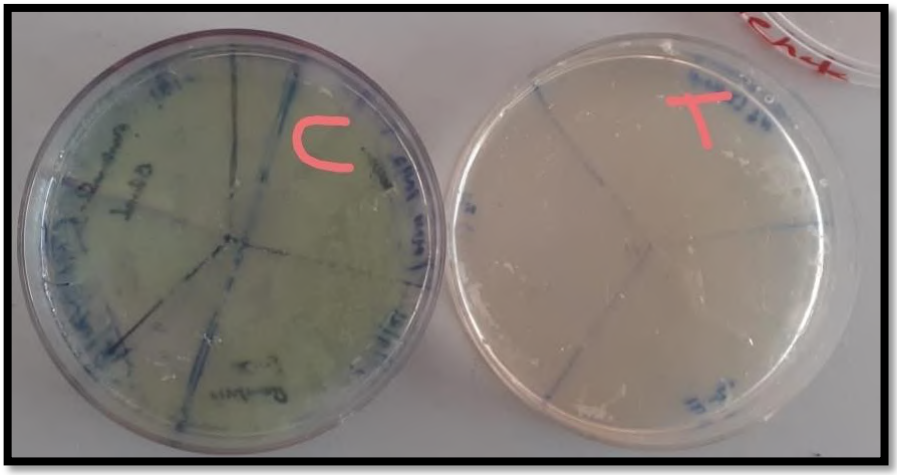


Figure 43 (*Pseudomonas spp.* Control and Test)

Pseudomonas spp. is a gram-negative bacterium, *Dhoopana* 2 (*Dhoop* 2) shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C).
Finding-*Dhoopana* 2 is effective against *Pseudomonas spp. (100% inhibition).*

iii. Effect of *Dhoopana* 3 (*Dhoopa* 3) on gram-positive and gram-negative bacteria

Following observations were draw with *Dhoopana* 3 (*Dhoopa* 3) when the exposure time was 1 hour and the dose was 5 g also when 10 ml of broth showing McFarland (0.5) reading was taken.

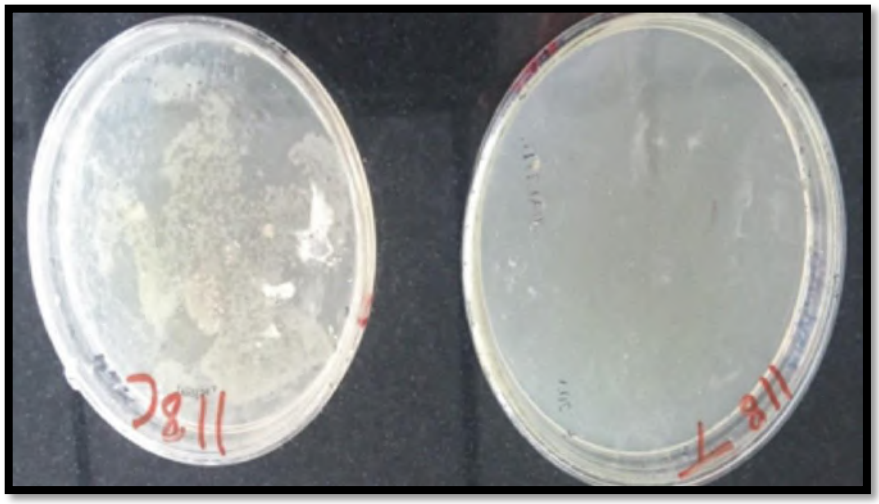


Figure 44 (*E. coli* Control and Test)

Escherichia coli is a gram-negative bacteria, *Dhoopana* 3 (*Dhoopa* 3) shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C).
Finding- *Dhoopana* 3 is effective against *E. coli* (100% inhibition).

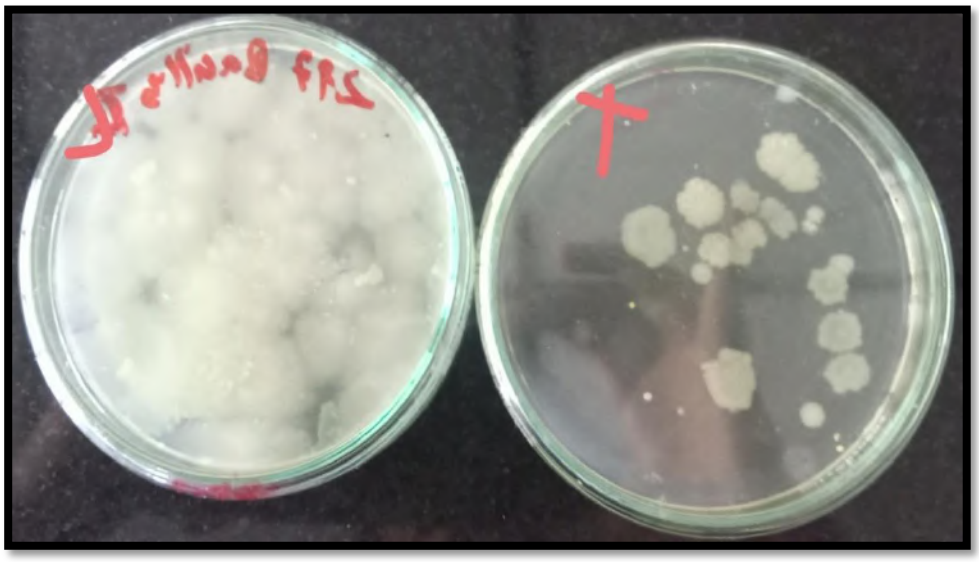


Figure 45 (*Bacillus spp*. Control and Test)

Bacillus spp. is gram-positive bacteria, *Dhoopana* 3 (*Dhoopa*3) shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C)
Finding- *Dhoopana* 3 is effective against *Bacillus spp.* (80% inhibition).

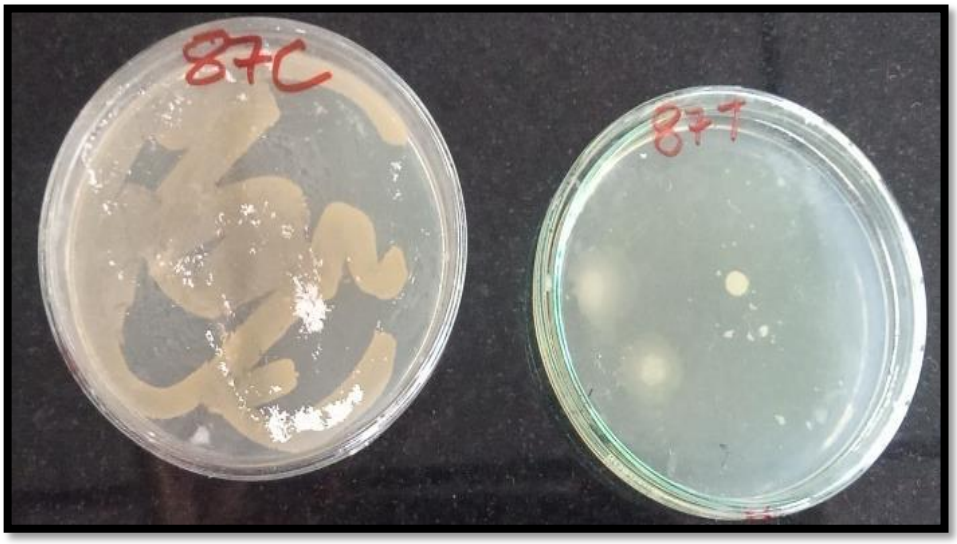


Figure 46 (*Staphylococcus aureus* Control and Test)

Staphylococcus aureus is gram-positive bacteria, *Dhoopana* 3 (*Dhoopa*3) shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C)
Finding- *Dhoopana* 3 is effective against *Staphylococcus aureus*(90% inhibition).

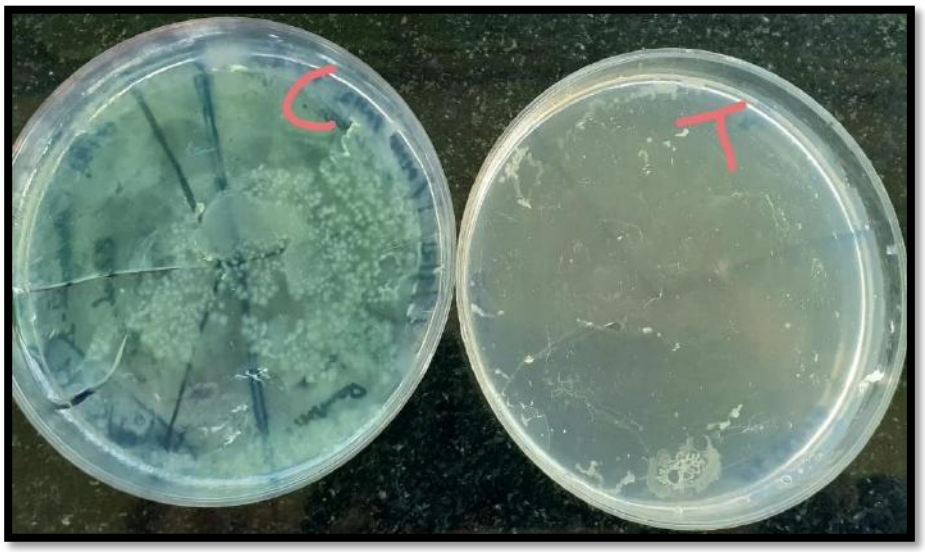


Figure 47(*Pseudomonas spp.* Control and Test)

Pseudomonas spp. is a gram-negative bacteria, *Dhoopana* 3 (*Dhoopa*3) shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C).
Finding- *Dhoopana* 3 is effective against *Pseudomonas spp.* (90% inhibition).
Findings:

Inhibition % of Bacterial Growth after Exposure of *Dhoopana* (E.S.Table17)

MTCC Bacterial Culture	Inhibition % after exposure of *Dhoopana* 1	Inhibition % after exposure of *Dhoopana* 2	Inhibition % after exposure of *Dhoopana* 3
Escherichia coli	90%	100%	100%
Bacillus spp.	90%	100%	80%
Staphylococcus aureus	100%	100%	90%
Pseudomonas spp.	100%	100%	100%

Dhoopana 1 (*Dhoopa* 1) shows an antimicrobial effect against gram-positive and gram-negative.
Dhoopana 2 (*Dhoopa* 2) shows an antimicrobial effect against gram-positive and gram-negative
Dhoopana 3 (*Dhoopa* 3) shows an antimicrobial effect against gram-positive and gram-negative
The maximum result (100% inhibition) was seen in *Dhoopana* 2 (*Dhoopa* 2) in growth inhibition (reduced growth) in case of *E coli, Pseudomonas spp., Bacillus spp., Staphylococcus aureus.*

VIII. DISCUSSION

The present research work entitled **"An Experimental Study to Evaluate the *Krimighna* Effect of *Dhoopana* along with the Standardization of Formulations"** was designed to evaluate the *Krimighna* (antimicrobial) effect of three different *Dhoopana* formulations by assessing colony forming units (CFUs) of bacteria, standardization of dose and time of these formulations, using formalin fumigation as positive control and also evaluate their effect on gram-positive and gram-negative bacteria cultures.
All the classical texts have described *Dhoopana* either for preventive or curative purposes. Some described it for purification in labor wards, OTs, and pediatric wards whereas some described it for protection from *Bhutagraha* (microbes), and some for treatment of disease of *Jvara, Arsha,* etc. This present study showed a decrease in number of Colony Forming Unit (CFUs) with an increase in dose in all three groups. Group 2nd (*Dhoopana* 2) showed maximum result and least CFUs count i.e. 1 and % cent change of 96%. The exposure of *Dhoopana* for 24 hours (thrice a day every 8 hourly) showed the maximum result in decreasing the colony-forming units (CFUs). The `1change in the growth of gram-positive and gram-negative bacteria on culture plates marked as Control and Test after exposure to three *Dhoopana* separately were analyzed after exposure time of 1 hour with dose 5 g each.

A. Conceptual Study

The *Vedas* also described *Dhoopana* in terms of *Havana, Homa,* and *Agnihotra* elaborately and also described its *Krimighna* (antimicrobial effect) to great extent.
Dhoopana has been mentioned in *Vimana Sthana, Sarir Sthana, and Chikitsa Sthana* for prevention, protection, and curative. Krimi is mentioned in *Vimana Sthana* where the cause, signs & symptoms, and treatment is mentioned. In *Charaka Samhita* also mentioned the *Krimighna* in *Krimighna Mahakshaya*. *Acharya Sushruta* is also described in post-operative procedures, management of wounds,

and purification of air. *Dhoopana reference* on which the study is based is taken from *Sushruta Samhita. Acharya Vagbhatta, Saranghdhara Samhita, Harita Samhita,* and *Bhela Samhita* also mentioned the *Krimi* and *Dhoopana* elaborately. *Kashyapa* dedicated a complete chapter to different *Dhoopana* formulations in the *Dhoop Kalpa* Chapter of *Kalpasthana*.

Dhoopana shows an antimicrobial effect. The antimicrobial effect in this present study is the bactericidal and bacteriostatic effect. A decrease in Colony Forming Units (CFUs) and culture growth satisfy the first aim of the study.

In the present study, the *Krimighna* (antimicrobial) effect of three *Dhoopana* formulations was assessed and standardization of dose and time was done in in-vitro studies.

B. Experimental Study:

i. Sedimentation Plate Method (1st part of the experiment)

a. ***Krimighna Karma* (antimicrobial effect) of the *Dhoopana* after 24 hours of sterilization (exposure):**

- ***Dhoopana*1:**

The antimicrobial activity increases with an increase in the dose of *Dhoopana* Group 1 (*Dhoop* 1) i.e. it shows 91.30% inhibition in the growth of Colony Forming Units (CFUs) with a dose of 100g (effective dose). At 10g (standard dose) % inhibition (change) in CFUs is 54.35%.

(E.S. Table No- 1 & Graph No- 1)

- ***Dhoopana* 2:**

The antimicrobial activity increases with an increase in the dose of *Dhoopana* Group 2 (*Dhoop* 2) i.e. it shows 96.77% inhibition in the growth of Colony Forming Units (CFUs) with a dose of 100g (effective dose). At 10g (standard dose) % inhibition (change) in CFUs is 35.48%. This group shows maximum *Krimighna* (antimicrobial) effect among all the three groups at 100 g (effective dose) and also in comparison to standard modern fumigation (97 to 99%). This was due to volatile oil, chemical constituents of drugs in the formulation. The drugs like *Guggulu, Nimba,*and *Vacha* showed proven anti microbial properties against various bacteria. This formulation also contains *Ghrita* (*Ghee*) which is inflammable and helps in the dispersion of the volatile oils.

(E.S. Table No- 2 & Graph No- 2)

- ***Dhoopana* 3:**

The antimicrobial activity increases with an increase in the dose of *Dhoopana*. *Dhoopana* Group 1 (*Dhoop*1) shows 91.43% inhibition in the growth of Colony Forming Units (CFUs) with a dose of 100g (effective dose). At 10g (standard dose) % inhibition (change) in CFUs is 28.57%.

(E.S. Table No- 3 & Graph No- 3)

b. ***Krimighna* (antimicrobial) effect of the *Dhoopana* after regular intervals (fixed exposure times):**

- ***Dhoopana* 1:**

When dose 30g is given for exposure time of 8 hours on 1st day and on 2nd day exposure time of 16 hours (i.e. twice with an exposure time of 8 hours each). On the 3rd day of 24 hours (i.e. thrice with an exposure time of 8 hours each), changes in CFUs are observed. Minimum CFUs (14) are observed when *Dhoopana* is given thrice in 24 hours with an exposure time of 8hours each. Maximum CFUs (18) are observed when *Dhoopana* (fumigation) is given for an exposure time of 8 hours.

(E.S. Table No- 4 & Graph No- 4)

- ***Dhoopana* 1:**

When dose 60g is given for exposure time of 8 hours on 1st day and on 2nd day exposure time of 16 hours (i.e. twice with an exposure time of 8 hours each), 3rd day of 24 hours (i.e. thrice with an exposure time of 8 hours each), changes in CFUs are observed. Minimum CFUs (6) are observed when *Dhoopana* is given thrice in 24 hours with an exposure time of 8 hours each. Maximum CFUs (9) are observed when *Dhoopana* (fumigation) is given for an exposure time of 8 hours.

(E.S. Table No- 5 & Graph No- 5)

- ***Dhoopana* 1:**

When dose 90g is given for exposure time of 8 hours on 1st day and on 2nd day exposure time of 16 hours (i.e. twice with an exposure time of 8 hours each), 3rd day of 24 hours (i.e. thrice with an exposure time of 8 hours each), changes in CFUs are observed. Minimum CFUs (3) are observed when *Dhoopana* is given thrice in 24 hours with an exposure time of 8 hours each. Maximum CFUs (6) are observed when *Dhoopana* (fumigation) is given for an exposure time of 8 hours. Colony Forming Units after (CFUs) 48 hours in the same room= 4, Colony Forming Units (CFUs) after 72 hours in the same room = 10.

(E.S. Table No- 6 & Graph No- 6)

- ***Dhoopana* 2:**

When dose 30g is given for exposure time of 8 hours on 1st day and on 2nd day exposure time of 16 hours (i.e. twice with an exposure time of 8 hours each), 3rd day of 24 hours (i.e. thrice with an exposure time of 8 hours each), changes in CFUs are observed. Minimum CFUs (12) are observed when *Dhoopana* is given thrice in 24 hours with an exposure time of 8 hours each. Maximum CFUs (16) are observed when *Dhoopana* (fumigation) is given for an exposure time of 8 hours.

(E.S. Table No- 7 & Graph No- 7)

- ***Dhoopana*2:**

When dose 60g is given for exposure time of 8 hours on 1st day and on 2nd day exposure time of 16 hours (i.e. twice with an exposure time of 8 hours each), 3rd day of 24 hours (i.e. thrice with an exposure time of 8 hours each), changes in CFUs are observed. Minimum CFUs (6) are observed when *Dhoopana* is given thrice in 24 hours with an exposure time of 8 hours each. Maximum CFUs (10) are observed when *Dhoopana* (fumigation) is given for an exposure time of 8 hours.

(E.S. Table No- 8 & Graph No- 8)

- ***Dhoopana* 2:**

When dose 90g is given for exposure time of 8 hours on 1st day and on 2nd day exposure time of 16 hours (i.e. twice with an exposure time of 8 hours each), 3rd day of 24 hours (i.e. thrice with an exposure time of 8 hours each), changes in CFUs are observed. Minimum CFUs (3) are observed when *Dhoopana* is given thrice in 24 hours with an exposure time of 8 hours each. Maximum CFUs (5) are observed when *Dhoopana* (fumigation) is given for an exposure time of 8 hours. Colony Forming Units after (CFUs) 48 hours in the same room = 3, Colony Forming Units (CFUs) after 72 hours in the same room = 5.

(E.S. Table No- 9 & Graph No- 9)

- ***Dhoopana* 3:**

When dose 30g is given for exposure time of 8 hours on 1st day and on 2nd day exposure time of 16 hours (i.e. twice with an exposure time of 8 hours each), 3rd day of 24 hours (i.e. thrice with an exposure time of 8 hours each), changes in CFUs are observed. Minimum CFUs (11) are observed when *Dhoopana* is given thrice in 24 hours with an exposure time of 8 hours each. Maximum CFUs (15) are observed when *Dhoopana* (fumigation) is given for an exposure time of 8 hours.

(E.S. Table No- 10 & Graph No-10)

- ***Dhoopana* 3:**

When dose 60g is given for exposure time of 8 hours on 1st day and on 2nd day exposure time of 16 hours (i.e. twice with an exposure time of 8 hours each), 3rd day of 24 hours (i.e. thrice with an exposure time of 8 hours each), changes in CFUs are observed. Minimum CFUs (7) are observed when *Dhoopana* is given thrice in 24 hours with an exposure time of 8 hours each. Maximum CFUs (10) are observed when *Dhoopana* (fumigation) is given for an exposure time of 8 hours.

(E.S. Table No- 11& Graph No- 11)

- ***Dhoopana* 3:**

When a dose of 90g is given for each interval on the 1st day of 8 hours and 2nd day of 16 hours (two intervals of 8 hours), 3rd day of 24 hours (three intervals of 8 hours) changes in CFUs are observed. Minimum CFUs (2) is observed within 24 hours (i.e. fumigation given thrice a day at an interval of 8 hours). Maximum CFUs (5) is observed when *Dhoopana* (fumigation) is given in 8 hours. Colony Forming Units after (CFUs) 48 hours in the same room = 4, Colony Forming Units (CFUs) after 72 hours in the same room= 8

(E.S. Table No- 12 & Graph No- 12)

Every dose in the *Dhoopana* Formulations 1/*Dhoopa* 1 starting from10 g to 100 g is statistically highly significant ($p<0.001$) as Chi-square ($\chi 2$) is 34.3 to 77.3 for 10 to 100 g which is more than 10. It means every dose shows antimicrobial which rejects the null hypothesis (A *null hypothesis* is a type of statistical hypothesis that proposes that no statistical significance exists in a set of given observations.) and shows that *Dhoopana* shows the *Krimighna*(antimicrobial)effect with each dose starting from 10g. It also shows the association between exposures to *Dhoopana* and a decrease in bacterial colony-forming units (CFUs) when studied with the control group. It also shows that even a small amount of 10g of *Dhoopana* 1 is effective as it is also statistically highly significant.

(E.S. Table 14)

Every dose in the *Dhoopana* Formulations 2 /*Dhoopana* 2 starting from10 g to 100 g is statistically highly significant ($p<0.001$) as Chi-square ($\chi 2$) is from 13.4 to 58.1 for 10 to 100 g which is more than 10. It means every dose shows antimicrobial which rejects the null hypothesis and shows that *Dhoopana* shows the *Krimighna* (antimicrobial) effect with each dose starting from 10g. It also shows the association between exposures to *Dhoopana* and a decrease in bacterial colony-forming units (CFUs) when studied with the control group. It also shows that even a small amount of 10g of *Dhoopana* 2 is effective as it is also statistically highly significant**.**

(E.S. Table 15)

Every dose in the *Dhoopana* Formulations 3/*Dhoopa*3 starting from10 g to 100 g is statistically highly significant ($p<0.001$) as Chi-square ($\chi 2$) is from 11.7 to 59 for 10 to 100 g which is more than 10. It means every dose shows antimicrobial which rejects the null hypothesis and shows that *Dhoopana* shows the *Krimighna* (antimicrobial) effect with each dose starting from 10g. It also shows the association between exposures to *Dhoopana* and a decrease in bacterial colony-forming units (CFUs) when studied with the control group. It also shows that even a small amount of 10g of *Dhoopana* 3 is effective as it is also statistically highly significant.

(E.S. Table 16)

Findings

- ***Krimighna* (antimicrobial effect) of the *Dhoopana* after 24-hour sterilization**: *Krimighna* effect increase with the increase in the dose of *Dhoopana* (*Ayurvedic* fumigation).
- ***Krimighna* (antimicrobial effect) after regular exposure time**:

Krimighna action is enhanced by increasing the interval i.e. *Dhoopana* shows maximum antimicrobial action when given thrice in 24 hours (with an exposure time of 8 hours each)

- Standard dose -10g
- Effective dose-100g
- Standard time - 8 hours (exposure time)
- Effective time - is repeating 3 times in 24 hours with an exposure time of 8 hours each
- Colony-forming units (CFUs) were increased when the room was checked after 36 hours and still further increased when checked after 48 hours of *Dhoopana* (*Ayurvedic* Fumigation).
- 91 % to 97% reduction in growth of bacterial colony forming units (CFUs) was seen which was comparable to standard modern fumigation which shows 95 to 99% reduction **(E.S. Table 13).**
- *Dhoopana* 2 was still effective after 48 hours.

According to the *Acharya Sushruta, Dhoopana* should be given at least twice a day to reap maximum results. (Su. Su. 5/18). This shows our ancient *Acharya* were aware of the importance of *Dhoopana* and used them for the prevention of diseases, and the purification of air.

The *Krimighna* (antimicrobial) effect is due to the chemical constituents of various drugs used in *Dhoopana* Formulations like *Guggulu, Nimba, Vacha,* etc.

ii. Surface Disinfection Method (2nd part of the experiment)

- **Effect of *Dhoopana* 1 (*Dhoopa* 1) on gram-positive and gram-negative bacteria**

Discussion of observations of *Dhoopana* 1 (*Dhoopa* 1) when the exposure time was 1 hour and the dose was 5 g alsowhen 10 ml of broth showing McFarland (0.5) reading was taken.

- ***Dhoopana* 1, 1 hour, 5gm, *Escherichia coli***

Escherichia coli is a gram-negative bacteria, *Dhoopana* 1 (*Dhoop* 1) shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C) when it was exposed to *Dhoopana* for 1hour.
Findings - *Dhoopana* 1 was effective against E. coli. It showed 90% inhibition in test petridish.

(Figure 36)

- ***Dhoopana* 1, 1 hour, 5gm, *Bacillus spp.***

Bacillus spp. is gram-positive bacteria, *Dhoopana* 1 shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C) when it was exposed to *Dhoopana* for 1hour.
Finding- *Dhoopana* 1 is effective against *Bacillus spp.* It showed 90% inhibition in test petridish.

(Figure 37)

- ***Dhoopana* 1, 1 hour, 5gm, *Staphylococcus aureus***

Staphylococcus aureus is gram-positive bacteria, *Dhoopana* 1 shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C) when it was exposed to *Dhoopana* for 1hour.
Finding- *Dhoopana* 1 is effective against *Staphylococcus aureus*. It showed 100 % inhibition in test petridish.

(Figure 38)

- ***Dhoopana* 1, 1 hour, 5gm, *Pseudomonas spp*.**

Pseudomonas spp. is a gram-negative bacteria, *Dhoopana* 1 (*Dhoopa* 1) shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C) when it was exposed to *Dhoopana* for 1hour.
Finding-*Dhoopana* 1 is effective against *Pseudomonas spp.* It showed 100% inhibition in test petridish.

(Figure 39)

- ***Dhoopana* 2, 1 hour, 5gm, *Escherichia coli***

Escherichia coli is a gram-negative bacteria, *Dhoopana* 2 (*Dhoopa* 2) shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C) when it was exposed to *Dhoopana* for 1hour.
Finding- *Dhoopana* 2 is effective against E. coli. It showed 100%inhibition in test petridish.

(Figure 40)

- ***Dhoopana* 2, 1 hour, 5gm, *Bacillus spp.***

Bacillus spp. is gram-positive bacteria, *Dhoopana* 2 (*Dhoopa* 2*)* shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C) when it was exposed to *Dhoopana* for 1hour.
Finding- *Dhoopana* 2 is effective against *Bacillus spp*.It showed 100% inhibition in test petridish.

(Figure 41)

- ***Dhoopana* 2, 1 hour, 5gm, *Staphylococcus aureus***

Staphylococcus aureus is gram-positive bacteria, *Dhoopana* 2 (*Dhoopa* 2) shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C) when it was exposed to *Dhoopana* for 1hour.
Finding- *Dhoopana* 1 is effective against *Staphylococcus aureus*.It showed 100% inhibition in test petridish.

(Figure 42)

- ***Dhoopana* 2, 1 hour, 5gm, *Pseudomonas spp*.**

Pseudomonas spp. is a gram-negative bacteria, *Dhoopana* 2 (*Dhoop* 2) shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C) when it was exposed to *Dhoopana* for 1hour.
Finding- *Dhoopana* 2 is effective against *Pseudomonas spp.*It showed 100% inhibition in test petridish.

(Figure 43)

- ***Dhoopana* 3, 1 hour, 5gm, *Escherichia coli***

Escherichia coli is a gram-negative bacteria, *Dhoopana* 3 (*Dhoopa* 3) shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C) when it was exposed to *Dhoopana* for 1 hour.
Finding- *Dhoopana* 3 is effective against E. coli.It showed 90% inhibition in test petridish.

(Figure 44)

- ***Dhoopana* 3, 1 hour, 5gm, *Bacillus spp.***

Bacillus spp. is Gram-positive bacteria, *Dhoopana* 3 (*Dhoopa*3) shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C) when it was exposed to *Dhoopana* for 1 hour.

Finding- *Dhoopana* 3 is effective against *Bacillus spp.* It showed 80% inhibition in test petridish.

(Figure 45)

- ***Dhoopana* 3, 1 hour, 5gm, *Staphylococcus aureus***

Staphylococcus aureus is gram-positive bacteria, *Dhoopana* 3 (*Dhoopa* 3) shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C) when it was exposed to *Dhoopana* for 1hour.
Finding- *Dhoopana* 3 is effective against *Staphylococcus aureus*. It showed 90% inhibition in test petridish.

(Figure 46)

- ***Dhoopana* 3, 1 hour, 5gm, *Pseudomonas spp*.**

Pseudomonas spp. is a gram-negative bacterium, *Dhoopana* 3 (*Dhoopa* 3) shows reduced growth in petridish marked as Test (T) as compared to petridish marked as Control (C) when it was exposed to *Dhoopana* for 1hour.
Finding- *Dhoopana* 3 is effective against *Pseudomonas spp.* It showed 100% inhibition in test petridish **(Figure 47)**
All *Dhoopana* Formulations showed 100 % inhibition in the case of *Pseudomonas spp. Dhoopana* 2 showed 100 % inhibition for all the bacteria used in the study and is the most effective **(E.S. Table 1**7**)**. This is due to the drugs like *Nimba, Vacha, and Guggulu* being present in the formulation which showed contained volatile oils and chemical constituents which showed an antimicrobial effect.

- **GENERAL MODE OF ACTION OF *DHOOPANA* (*AYURVEDIC* FUMIGATION)**[14]

Agni and *VayuMahabhoot Pradhan Dravays* used

↓

Guna Pradhanya- Laghu, Ushana,Ruksha and *Vishada*

↓

This helps in its rapid spreadability and quicker combustion.

↓

Dravya are volatile which is helpful.

↓

Volatile nature provides an advantage in lowering microbial contamination in the air and on difficult-to-reach surfaces.

- **Other Mode of Action** [15]
  - Dry animal excreta have also been used as a source of fuel. Animal excreta mainly contain combustible gases. Likewise, most of the *Dravya* have oleaginous substances like *Ghrita, Sarjarasa* and *Guggulu* to help in combustion.
  - When these inflammable drugs are burned, they release a large amount of energy making them useful as a fuel for sustaining the flame and may also help in dispersing the volatile components of the *Dhoopana* formulation. Almost all the *Dhoopana Dravya* have an inevitable source of combustible ingredient either as a potentiator or activator of the formulation.
  - Some other formulations have added fragrant substances like *Tejapatra, Ela,* etc. This may have been done to mask the obnoxious smell of some other pungent drugs and provide a soothing effect on the brain and help in restoring mental and spiritual balance.
  - Most of the formulations have been used for disinfection and removal of *Visha*. The ingredients of the formulations have been incorporated in such a way to induce antimicrobial properties of the raw materials which help in eliminating disease vectors.
  - The formulations generally consist of various drugs that show a synergistic effect and help in propagating the activity of the main antimicrobial drug.
  - *Guggulu* has been used in several *Dhoopana* formulations. The volatile oil of *Guggulu* was found to be highly effective against bacteria which suggested its role as a fumigant. An active compound,5(1-methyl,1-aminoethyl)-5-methyl-2- octa-none, of the methanolic extract of *Guggulu*gum,possessed significant antibacterial activity against gram-positive bacteria and moderate activity against gram-negative bacteria.
  - *Vacha* has been mentioned in the formulations. The alpha and beta-asarone of the Acorus calamus are mainly responsible for the antimicrobial activities further it has been established that beta asarone has high anti-microbial activity as compared to the alphaasarone. The leaf and rhizome part of Acor-uscalamusis were found to possess antibacterial activity. The methanolic extract of the *Acorus calamus* showed the inhibitory action against the bacterial strains of *Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoni, and Staphylococcus aureus.*
  - *Neem* also has been used quite often as one of the major ingredients in most formulations. Azadirachtin in the *Neem* seed oil is a proven chemical for its insecticidal properties. In a study, *Staphylococcus aureus* showed a high level of inhibition of100% in 10 min and 50% in 5 min to the fumes of *Azadirachta indica* showed a maximum of 90% inhibition.\

IX. SUMMARY

A. EXPERIMENTAL STUDY

i. Sedimentation Plate Method (1st part of the experiment)

The percentage inhibition of bacterial growth is high in all three *Dhoopana* formulations. *Dhoopa* 1 shows a % inhibition of 91.30, and *Dhoopa* 3 showed a % inhibition of 91.43. *Dhoopa*2 shows a % inhibition of 96.77 which is the highest among the three groups and comparable to modern fumigation/sterilization which shows 97% to 99%
Each dose starting from 10g to 100 g in three different *Dhoopa* Formulations is statistically **highly significant ($p<0.01$)**. This means each dose shows a significant *Krimighna* effect. It also shows the association between exposures to *Dhoopana* and a decrease in bacterial colony-forming units (CFUs) when studied with the control group.
The *Dhoopana* shows the maximum result when the *Dhoopana* is given thrice for 24 hours (with an exposure time of 8 hours each). The bacterial colony-forming units (CFUs) increase when evaluated after 48 hours and 72 hours respectively. This means the effectiveness of *Dhoopana* decrease after 48 hours as CFUs increase. *Dhoopa* 2 is still effective after 48 hours as colony-forming units remain the same after 24 hours as well as 48 hours i.e. 3 CFUs.

ii. Surface Disinfection Method (2nd part of the experiment)

All the *Dhoopana* Formulations were effective against gram-positive bacteria *Staphylococcus aureus* and *Bacillus spp.*and gram-negative bacteria *Escherichia coli* and *Pseudomonas spp.* when they were exposed to *Dhoopana* for 1 hour.
Group 2 *Dhoopana* formulations were most effective against gram-negative bacteria and gram-positive bacteria
Dhoopana is effective and shows *Krimighna* (antimicrobial) effect. The effectiveness of *Dhoopana* increases with an increase in dose and increase in the time interval.
The effectiveness of *Dhoopana* Formulations is between 91% to 97 % which is comparable to modern formalin fumigation/sterilization whose effectiveness is between 95% to 99%. However, more experiments with a larger number of samples and different environment settings are necessary.
This shows that *Dhoopana* (*Ayurvedic* Fumigation) is quite effective as compared to standard sterilization.

X. SUGGESTION

It is being suggested that the present study requires being conducted in vivo setup like in OTs, in surgical wounds, and various diseases like *Jvara, Arsha*, etc mentioned in *Samhita.*
Also, the effectiveness of each drug/ ingredient of *Dhoopana* formulations as an antimicrobial agent should be evaluated. So, it can be evaluated which ingredient or drug is responsible for the *Krimighna* (antimicrobial) effect of the whole formulation
Also, further study must be done on a minimum dose from where the *Dhoopana* starts showing significant results to avoid any unwanted effect in vivo.

XI. CONCLUSION

- **The following conclusion can be drawn based on the present study:**

Based on observations made during an experimental study the *Dhoopana* is effective and has comparable results to standard fumigation with Formaldehyde and $KMnO_4$. *Dhoopana* 2 (*Ayurvedic* fumigation) has shown significant (96.7%) results in decreasing bacterial colony-forming units with an increase in dose and exposure time. It was also effective against both gram-positive and gram-negative bacteria. However, more studies are necessary to arrive at a definite conclusion.

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