Original Research Paper

Volume - 14 | Issue - 06 | June - 2024 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Ayurveda

IN-VITRO ANTI-MICROBIAL ACTIVITY OF LAKSHADI DHOOPA: A NATURAL HERBAL DISINFECTANT FOR AIR PURIFICATION

Dr. R B. Hosamani* — Professor and Head, Dept. of Swasthavritta, BVVS Ayurved Medical College & Hospital, Bagalkot, Karnataka, India. *Corresponding Author

Mr. Suraj S Gayakawad — 4th Year BAMS Student, BVVS Ayurved Medical College & Hospital, Bagalkot, Karnataka, India

ABSTRACT **Background:** Ayurveda described *Dhoopana* (fumigation) as a method of environmental disinfection. The various plants and their compound formulations are used for *Dhoopana* to purify the atmospheric air as well as to protect from poisonous animals and insects. Sushruta Samhita has highlighted the *Lākshādi Dhoopa* to purify air from the poison i.e., purify the polluted air from the atmosphere. The present study was intended to evaluate the preliminary phytochemical analysis and in-vitro Anti-microbial activity of *Lākshādi Dhoopa* as a natural herbal disinfectant for air purification. **Methods:** *Lākshādi Dhoopa* was analysed for antimicrobial activity against seven different standard microbial strains (bacteria and fungi) by using MIC & MBC methods. The antimicrobial effect was carried out in a sterilized chamber (i.e., LAF chamber) to assess the reduction in the microbial load (coliform counts) by exposing the nutrient agar plates to *Lākshādi Dhoopa* fumigation. **Results:** *Lākshādi Dhoopa* showed antimicrobial activity against seven tested standard microbial strains. A satisfactory degree of reduction of 53.65% in the microbial load (coliform counts) was found with the agar plates exposed to the outpatient department, 52.29% in the male general ward and 51.22% in the classroom. **Conclusion:** *Lākshādi Dhoopa* showed Antimicrobial activity tested against seven standard microbial strains. It acts as an efficient natural herbal antimicrobial agent for air purification. A satisfactory degree of reduction in the microbial load (coliform counts) was found with the nutrient agar plates exposed to *Lākshādi Dhoopa.*

KEYWORDS : *Lākshādi Dhoopa,* Antimicrobial activity, Herbal fumigation, Purification of environment.

INTRODUCTION:

The environment has been a major concern in the current scenario. The steady pollution thought of numerous populaces has gathered the attention of many people. A clean environment that includes clean air, water, land and energy is essential for human survival.[1] Air pollution is high on the global agenda and is widely recognised as a threat to both public health and economic progress. The World Health Organization estimates that 4.2 million deaths annually can be attributed to outdoor air pollution.[2] The microbial load of the air causes various airborne diseases that are caused by bacteria such as Staphylococcus, Streptococcus, Mycoplasma pneumonia, Chlamydia pneumonia, Bordetella pertussis, Pseudomonas aeruginosa, Pseudomonas mallei, Mycobacterium tuberculosis, Corynebacteria diphtheria etc. Various efforts have been taken to cleanse air to make free or fewer airborne pathogens such as chemical treatment but there are side effects of their applications.[3] Thus, in the search for a safe and effective alternative to the contemporary method, the concept of herbal fumigation is sought.

In the ancient era, different techniques like *Yajna*, *Homa*, *Havana*, *Dhoopana*, *Agnihotra* etc. have been performed to make the vicinity free of microbes from both indoor and outdoor environments. *Dhoopana* has also been mentioned for its antimicrobial and growth-promoting activities for the healthy production of plants in Vrikshayurveda. The usage of these purificatory methods emphasises its significant role in the reduction of microbial levels. The various plants and their compound formulations have been mentioned in Ayurvedic classical texts that are used for *Dhoopana* (fumigation) to purify the *Bheshajagara*, *Vranagara*, *Sutikagara*, *Shastrakarmaghruha*, *Kumaragara* etc. *Yantra* and *Shastra* as well as to protect from poisonous animals and insects etc. creatures. The *Krumighna* and *Vishaghna* (antimicrobial) properties of these drugs reduce the microbial load and prevent infection when viewed in light of the current micro-biological knowledge. Sushruta Samhita has highlighted the *Dhoopa* (fumigation) for the purification of the poisoned atmosphere. Drugs such as *Lākshā*, *Haridrā*, *Ativisha*, *Abhayā*, *Abda* (*Musta*), *Renukā*, *Elā*, *Vakra*, *Kushta* and *Priyangu* should be added to the fire and the *Dhuma* (smoke) that comes from it would purify *Anila* (air) form the poison i.e., purify the polluted air from the atmosphere.

Keeping these facts in mind and to understand the utility of herbs mentioned for Air purification in Sushrut Samhitha, the present study will be intended to evaluate the preliminary phytochemical analysis and in-vitro Anti-microbial activity of *Lākshādi Dhoopa* as a natural herbal disinfectant for air purification.

AIMS AND OBJECTIVES:

The present study aimed to analyze the phytochemical and Anti-microbial activity of *Lākshādi Dhoopa* as a natural herbal disinfectant for air purification.

The specific objectives of the study were

1. To screen the presence of phytochemical compounds in *Lākshādi Dhoopa*.
2. To evaluate the Anti-microbial activity of *Lākshādi Dhoopa.*

MATERIALS AND METHODS:

Lākshādi Dhoopa:

The ingredients of *Lākshādi Dhoopa* were procured from authorized drug suppliers i.e., Vaidya S.P. Kajarekara from Belagaum. They were dried under shade and used for the studies.

Table – 1 Ingredient of *Lākshādi Dhoopa*

SN	Name of the Plant	Parts Used	Qty. Used
1.	Lākshā (Laccifer lacca)	Resin	20 gm
2.	Haridrā (Curcuma longa)	Rhizome	20 gm
3.	Ativisha (Aconitum heterophyllum)	Roots	20 gm
4.	Abhayā (Terminalia chebula)	Fruit	20 gm
5.	Abdhā / Mustha (Cyperus rotundus)	Root	20 gm
6.	Harenuka (Vitex negundo)	Seeds	20 gm
7.	Elā (Elettaria cardamomum)	Seeds	20 gm
8.	Vakra (Cinnamomum tamal)	Bark	20 gm
9.	Kushta (Saussurea lapa)	Root	20 gm
10.	Priyangu (Callicarpa macrophylla)	Seeds	20 gm
		TOTAL:	200 gm

Preparation of Lākshādi Dhoopa:

The above-said ingredients were taken in the powdered form separately in an equal quantity (20 gm each). Under all aseptic precautions, these powders were sieved and mixed well and stored in a sterile container at room temperature in a dark place. Later conical-shaped *Dhoopabatti* was prepared by adding Ghrita, dried under shade and used for the study.

Preliminary phytochemical screening of Lākshādi Dhoopa:

A preliminary phytochemical study was screened for the presence of organic constituents such as – alkaloids, carbohydrates, anthraquinones, tannins, saponins, flavonoids, glycosides, terpenoids, phenols, steroids and proteins; and inorganic constituents such as – carbonates, sodium, potassium, chloride, sulphate, magnesium, calcium, phosphate, iron and iodine by using standard methods.

Analysis of Anti-microbial activity of Lākshādi Dhoopa:

The *Lākshādi Dhoopa* was evaluated for Anti-microbial activity. It

was tested against six standard bacterial strains of gram-positive micro-organisms i.e., *Staphylococcus aureus, Streptococcus mutans* as well as gram-negative micro-organisms i.e., *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and one standard fungus strain of *Candida albicans*. The study was carried out at the Maratha Mandal's Central Research Laboratory Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belgaum. The MIC and MBC methodology were used to assess the anti-microbial activity.

Minimum Inhibitory Concentration (MIC) Method:

The antimicrobial agent was dissolved in sterile normal saline water and dilutions were made in decreasing concentrations viz. 5.12 mg, 2.56 mg, 1.28 mg, 0.64 mg, 0.32 mg, 0.16 mg, 0.08 mg, 0.04 mg and 0.02 mg. To each dilution, an equal volume of 0.5 MacFarland turbidity (10^5 CFU/ml) standard of *S. aureus* (ATCC25923) was added in Mueller Hinton broth. All the respective tubes were incubated at 37^0C for 24 hours. The control tubes contained all the ingredients except the antimicrobial agent. The lowest concentration at which there was no visible turbidity was considered the MIC of the antimicrobial agent for that organism.

Minimum Bactericidal Concentration (MBC) Method:

The MBC test determines the lowest concentration at which an antibacterial agent will kill a particular bacterium. It is determined using a series of steps, undertaken after a broth dilution minimum inhibitory concentration (MIC) tests by subculturing to agar plates. A loopful of broth from each tube was sub-cultured on nutrient agar and incubated at 37^0C for 24 hours. The lowest concentration at which there is no visible bacterial growth was considered as the MBC of the antimicrobial agent for that organism.

Evaluation of Antimicrobial effect of Lākshādi Dhoopa:
Most Probable Number (MPN) method:

The evaluation of the polyherbal *Lākshādi Dhoopa* for their antimicrobial effect was carried out in a fumigation glass chamber. Two Nutrient Agar Plates were prepared and exposed to various environments such as - The Out Patient Department (OPD), the Male General ward (MGW) and the Classroom (CR) of BVVS Ayurved Medical College and Hospital, Bagalkot for one day, each for the control plate and test plate. The next day the control agar plates were then removed and incubated for 24 hours at 38^0C (pre-exposure plate) and the number of bacterial growth was observed respectively. The test agar plates were exposed to polyherbal *Lākshādi Dhoopa* (i.e., generated smoke,) by partial combustion for 30 minutes respectively in a sterilized chamber (i.e., LAF chamber) & then the apparatus was kept undisturbed for 3 hours. After fumigation (*dhoopana*), the test agar plates were incubated overnight at 38^0C (post-exposure plate), and the colonies (bacterial growth) were counted and expressed as Colony Forming Units (CFU)/mL which is calculated by the following formula.

CFU/mL = No. of colonies × Dilution factor, The dilution factor is the reciprocal of the dilution**.** The difference in the microbial counts of the plates indicated the antimicrobial activity of the polyherbal *Lākshādi Dhoopa*.

RESULTS AND DISCUSSIONS:

Preliminary phytochemicals of Lākshādi Dhoopa:

The organic compounds such as carbohydrates, proteins, phenols, glycosides, saponins, alkaloids, tannins, steroids and terpenoids were present in *Lākshādi Dhoopa*. However, anthraquinone and flavonoids were absent. The inorganic elements such as sodium, potassium, calcium, iron and iodine were present. The carbonates, chlorides, sulphates, magnesium, phosphate and nitrates were absent in the *Lākshādi Dhoopa.*

Anti-microbial activity of Lākshādi Dhoopa:

Table – 2 MIC & MBC values of *Lākshādi Dhoopa*

SN	Organisms	MIC Value (µg/ml)	MBC Value (µg/ml)
1.	Staphylococcus aureus	50	100
2.	Escherichia coli	25	25
3.	Streptococcus mutans	3.12	6.25
4.	Proteus mirabilis	12.5	100
5.	Klebsiella pneumoniae	25	50
6.	Pseudomonas aeruginosa	12.5	50
7.	Candida albicans	6.25	25

In the present study, the test drug polyherbal *Lākshādi Dhoopa* has shown its sensitivity with different dilution rates. The MIC value (sensitivity) tested against organisms *Streptococcus mutans* was 3.12 µg/ml, *Candida albicans was* 6.25 µg/ml, *Pseudomonas aeruginosa* and *Proteus mirabilis* was 12.5 µg/ml each, for *Escherichia coli* and *Klebsiella pneumoniae was* 25 µg/ml each and for *Staphylococcus aureus was* 50 µg/ml. The *Lākshādi Dhoopa* has shown its Antimicrobial activity (no growth) with different dilution rates. MBC value tested against *Staphylococcus aureus* was 100 µl/ml, for *Escherichia coli* was 25 µl/ml, for *Streptococcus mutans* was 6.25 µl/ml, for *Proteus mirabilis* was 100 µl/ml, for *Klebsiella pneumoniae* was 50 µl/ml, for *Pseudomonas aeruginosa was* 50 µl/ml and for *Candida albicans was* 25 µl/ml. It was revealed from Table 2 that the drug polyherbal *Lakshadi Dhoopa* has Anti-microbial activity.

Overall, the present study has suggested that the *Lākshādi Dhoopa* has shown a potential antimicrobial (Antibacterial and Antifungal) activity against above said all the seven tested standard microbial strains. It has an inhibitory effect, as well as bactericidal action on the growth of the above-mentioned seven tested micro-organisms.

Antimicrobial effect of Lākshādi Dhoopa (Fumigation)**:**

Table – 3 Antimicrobial effect of *Lākshādi Dhoopa*

Sl. No.	Plates Exposed to	Number of Bacterial colonies without exposure to LD Dhoop CFU (Pre-exposure)	Number of Bacterial colonies with exposure to LD Dhoop 30 mins CFU (Post-exposure)	% of reduction in Bacterial colonies
1	OPD	41	22	53.65%
2	MGW	34	18	52.29%
3	CR	29	15	51.22%

Assessments of the Antimicrobial effect of *Lākshādi Dhoopa* (fumigation) were done based on the reduction of the organism's growth count (CFU) of the organisms. From the above Table 3, it has been revealed that the polyherbal *Lākshādi Dhoopa* showed an Antibacterial effect. A satisfactory degree of reduction of 53.65% in the microbial load (coliform counts) was found with the agar plates exposed to the outpatient department, 52.29% in the male general ward and 51.22% in the classroom.

CONCLUSION:

The preliminary phytochemical analysis of *Lākshādi Dhoopa* revealed that organic compounds such as Carbohydrates, Proteins, Phenols, Glycosides, Saponins, Alkaloids, Tannins, Steroids and Terpenoids were present. However, Anthraquinone and Flavonoids were absent. The inorganic elements such as Sodium, Potassium, Calcium, Iron & Iodine were present. The Carbonates, Chlorides, Sulphates, Magnesium, Phosphate & Nitrates were absent. The different classes of phytochemicals such as alkaloids, glycosides and saponins (that are implicated in the defence mechanism against pathogens) show antimicrobial properties.

The polyherbal-formulated *Lākshādi Dhoopa* has shown a potential antimicrobial activity. It has antibacterial activity against all the six test gram-positive micro-organisms i.e., *Staphylococcus aureus, Streptococcus mutans* as well as gram-negative micro-organisms i.e., *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and antifungal activity against *Candida albicans*.

Hence, the overall findings of the present study indicate and confirm the claims of potential antimicrobial activity of the polyherbal-formulated *Lākshādi Dhoopa*. It showed a satisfactory degree of inhibition along with the advantages of these being non-toxic, economical and easy to prepare thus these can effectively replace the more hazardous methods of fumigation. Hence it may be an alternative form of fumigation strategy in hospital wards, houses, schools, and public places to prevent infections.

Scope for Future Work:

1. Effectiveness of different types of polyherbal fumigation.
2. Comparative efficacy of different types of polyherbal and chemical fumigation for purification of different indoor and outdoor environments.

Acknowledgement:

Authors are gratefully acknowledged to Rajiv Gandhi University of Health Sciences, Karnataka, Bengaluru for sanctioning and funding this project under the scheme RGUHS UG Research project for the FY:2021-22. We are also thankful to Maratha Mandal's Central Research Laboratory, Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belgaum for their kind help, support and analysis during this research project.

Conflicts of Interest:

The authors declare no conflicts of interest regarding the publication of this paper.

REFERENCES:

1. Clean Air Act. https://www.epa.gov/sites/production/files/201508/documents/peg.pdf. Assessed on April 2022
2. Ambient air pollution: Global assessment of exposure and BOD, update 2018. WHO (2020).
3. Cleaning and the Environment. https://www.ciriscience.org/a_269-Cleaning-andtheEnvironment. Assessed on April 2022
4. Sushruta: Sushruta Samhita of with the Nibhanhasangraha Commentary of Shri. Dalhanacharya; edited by Vaidya Yadavaji Trikamji Acharya; Kalpasthana 3/16-17, Choukambha Sanskrit Samsthana, Varanashi, Re-print (2010)