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In Vitro Antimicrobial Evaluation of Herbal Dhoop Prepared from Cow Products and Herbs



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ABSTRACT

Several traditional techniques such as Dhoopana and Havana have been used in the fumigation process to minimize microbial load to nonpathogenic levels in the environment. As per Indian traditions, in various religious practices dhoop, diva and havans are used to remove microbes from the environment. In the present study herbal dhoop prepared from herbs and cow products such as cow dung, milk, and ghee was used and its efficacy was evaluated the removal of airborne microorganisms using Dhoopana practice as described in Ayurveda. In our study, we have observed good antimicrobial activity against airborne microorganisms. We observed a significant reduction in microbial load 66.12% in plates (environment), 55.38% (Main lab), and 60% (LAF room) after exposure of the plates to dhoop in laminar airflow. This herbal dhoop is prepared from easily available and cost-effective raw materials and has a pleasant smell. This dhoop can serve as an alternative to the use of chemical-based dhoop and incense sticks and other chemical-based products used as dhoop and disinfection of air in various areas such as households, hospitals, offices, etc.



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INTRODUCTION

Microorganisms are ubiquitous and present everywhere around us, amongst them only 20-25% are nonpathogenic. The disease-causing microorganisms are also present in our environment and may cause infections⁽¹⁾. Several traditional techniques such as Dhoopana and have been used in the fumigation process to minimize microbial load to a nonpathogenic level in the environment. It is also described in the literature that fumigation should be done for sterilization of operation theatres of hospitals and wound management⁽²⁾. The environment has been a major concern in today's era. The constant pollution all-around has gathered the attention of many people. A clean environment that includes clean air, water, land, and energy, is essential for human existence. The microbial load of the air causes various airborne diseases. Disease-causing pathogens are organisms that spread from an infected person to another through coughing, talking, and sneezing - even breathing and laughing⁽³⁾. Dhoopana is said to be extremely effective in preventing as well as curative aspects, yet, there remains an ample scope in this area as its practice is seldom in use. KashyapSamhita has mentioned several Dhoopana formulations and the present study is an effort to assess the efficacy of the same⁽⁴⁾. The patients suffering from swine flu, bronchial spasms, bronchitis, asthma, dyspnoea, rhinitis, bad smell of the nose, and mouth can be aided. The current work is focused on modifications in general dhoop formulations by incorporating cow products and several herbs⁽⁵⁾. Ayurveda is the ancient science of life that has always focused on the nurture and maintenance of good health in the people. The Ayurveda system works by maintaining a balance between the body, mind, soul, and spirit of an individual. Fumigation process Dhoopana as described in Ayurveda is a method of drug delivery through inhalation. This process has some advantages such as ease of drug administration, more bioavailability of the drug, and higher drug penetration power in the blood-brain barrier⁽⁶⁾. With the help of traditional knowledge, we tried to develop a method to prepare a herbal dhoop having antimicrobial activity against airborne microorganisms using various cow products and plant powders for cleansing the air. This herbal dhoop is prepared from easily available and cost-effective raw materials and has a pleasant smell. This dhoop can serve as an alternative to the use of chemical-based dhoop and incense sticks and other chemical-based products used as dhoop and disinfection of air in various areas such as households, hospitals, offices, etc.

MATERIALS AND METHODS

Materials

The present work was undertaken at Kehloor Biosciences and Research Centre laboratory located in Ghumarwin district Bilaspur, Himachal Pradesh. The plant materials were procured from the local market of Ghumarwin and were made to powder form in the laboratory. Cow dung, milk, and ghee were procured from a local dairy milk supplier of village Raghunathpura, Bilaspur (H.P).

Preparation of herbal dhoop

The plant powders and cow milk and ghee were taken in a clean mortar and pestle and macerated finely. The cow dung (semi-dried form) was added to this mixture and again macerated finely to obtain a fine paste (Table-1). A plastic syringe was cut from the apical side to open its mouth and the Dhoop sticks were made using this syringe. These dhoop sticks were dried for four days in an oven at 50 degrees Celsius temperature and stored in an airtight container. Dhoop sticks were further tested for their antimicrobial effects (Fig 1&2)⁽⁷⁾.

Evaluation of dhoop sticks for antimicrobial activity

The dhoop sticks were further evaluated for their antimicrobial efficacy as per the methodology described by Lad and Palekar (2016) with slight modifications. One set of Nutrient agar plates were exposed to various areas (Environment, Main lab and LAF room) for ten minutes by opening their lids. The second set of the plates were first exposed to air in the respective area for ten minutes and then to the prepared herbal dhoop for 30 minutes inside laminar airflow. All the plates were incubated at 37 degrees Celsius for 24-48 hrs and observed for microbial colonies. The colonies were counted and results were expressed as percent reduction of microbial load after exposure of media plates to dhoop⁽⁷⁾.

Table 1: The details of the ingredients of herbal dhoop.

Sr.No	Ingredient	Scientific Names	Plant part used	Quantity
1	Cows ghee (clarified butter)	-	-	15g
2	Cow dung (semi dried)	-	-	1 part
3	Cow milk	-	-	10ml
4	Guggul powder	<i>Commiphoramukul</i>	Resin	10g
5	Camphor powder	<i>Cinnamomumcamphora</i>	Resin	10g
6	Kapurkachri powder	<i>Hedychiumspicatum</i>	Rhizome	10g
7	Jatamansi powder	<i>Nardostachysjatamansi</i>	Rhizome	5g



(a) Guggul (b) Kapurkachri (c) Jatamansi (d) Camphor

Figure no-1 (a-d) Herbs used for the preparation of herbal dhoop.



(a) (b) (c)

Figure no 2: (a-c) Preparation of herbal dhoop.



Figure no 3: Exposure of agar plates to herbal dhoop in laminar airflow.

RESULTS AND DISCUSSION

Microorganisms are ubiquitous and present everywhere around us, amongst them only 20-25% are nonpathogenic. The disease-causing microorganisms are also present in our environment and may cause infections. Several traditional techniques such as Dhoopana and Havana are used in the fumigation process to minimize microbial load to nonpathogenic levels in the environment. It is also described in the literature that fumigation should be done for sterilization of operation theatres of hospitals and wound management ⁽²⁾. The use of traditional medicines in the treatment of many health issues is an integral part of the alternative health care system. The herbal formulations used traditionally have multi constituents in combinations. Medicinal plants are being used as a potential source of medicine for many years ⁽⁸⁾. Dhoop is one of the widely used products in rural and urban areas in India in religious rituals. Dhoopana is one of the important techniques described in the Indian ayurvedic system for decreasing microbial load in specific areas. There are various types of microorganisms present in our environment which may cause many health problems. The patients suffering from swine flu, bronchial spasms, bronchitis, asthma, dyspnoea, rhinitis, bad smell of the nose, and mouth can be aided. In the present study, herbal dhoop was prepared from herbs and cow products such as cow dung, milk, and ghee. The dhoop was further evaluated for antimicrobial activity against the airborne microorganisms. Sumitha and co-workers (2015), evaluated the antimicrobial effect of their formulation DHUP. These workers selected two rooms of the same size for their study; one room was kept as the control without any intervention and Dhoopana was performed in the second room once a day for two days period by exposing agar gel plates. The efficacy of DHUP Dhoopana was observed

at selected intervals. Their study demonstrated that there was a significant antimicrobial effect of Dhoopana in comparison to control during the study and follow-up period up to six days against bacteria and fungus ⁽²⁾. In our study, we have observed good antimicrobial activity against airborne microorganisms. We observed a significant reduction in microbial load 66.12% in plates (environment), 55.38% (Main lab), and 60% (LAF room) after exposure of the plates to dhoop in laminar airflow (Fig- 4&5, Table-2).

Table No. 2: Antimicrobial effect of prepared dhoop.

Sr. No	Area	Microbial colonies on Nutrient agar plates without exposure to dhoop	Microbial colonies on Nutrient agar plates without after exposure to dhoop	Reduction of microbial load (%)
1	Environment	248	164	66.12%
2	Main Lab	65	36	55.38%
3	LAF room	15	9	60%

% Reduction of microbial load= 100/number of microbial colonies before exposure to dhoop X number of microbial colonies after exposure to dhoop.



Figure No. 4: (a) Colonies on agar plates (Environment) before exposure of plates to dhoop and after exposure of plates to dhoop (b) Colonies on agar plates (Main lab) before exposure of plates to dhoop and after exposure of plates to dhoop



Figure No. 5: Colonies on agar plates (LAF room) before exposure of plates to dhoop and after exposure of plates to dhoop

Ayurveda is the ancient science of life that has always focused on the nurture and maintenance of good health in the people. The Ayurveda system works by maintaining a balance between the body, mind, soul, and spirit of an individual. Fumigation process Dhoopana as described in Ayurveda is a method of drug delivery through inhalation. This process has some advantages such as ease of drug administration, more bioavailability of the drug, and higher drug penetration power in the blood-brain barrier⁽⁶⁾.

Fumigation using the fumes of formaldehyde along with potassium permanganate is the most commonly used and accepted method. Although this method has been associated with several health hazards. Raut and co-workers (2011), in their study, evaluated incense sticks made from woods, barks, gums, and volatile oils for antimicrobial activity. These workers demonstrated that formulated herbal-based incense stick has shown significant antimicrobial effect against airborne microorganisms. The incense sticks used by these workers were easy to prepare, user-friendly cost-effective, and safe in comparison to fumigation using formaldehyde. These workers suggested their use for fumigation of hospital wards, homes, and laboratories⁽⁹⁾. We have also observed significant antimicrobial effects of herbal dhoop against airborne microorganisms in our study. The dhoop was prepared from easily available and cheap raw materials.

Dhoopana Chikitsa (Medicinal Fumigation) is one of the most important practices in the Indian ayurvedic system. This is also used in various ailments and many Dhoopa Yogas practices as described in Ayurvedic Samhitas. The roots of Dhoopana can be found in the ancient Vedic period and was in routine use at that time. Many researchers in modern times have also authenticated its ability of air purification and sterilization of the environment. As per many studies, this technique was found very effective in preventing many health

problems. Also, Kashyap Samhita has described several Dhoopana formulations and their use for the treatment of many ailments. Mahesh and co-workers (2016), has conducted studies to assess the efficacy of this technique ⁽⁴⁾. In the present study, herbal dhoop prepared from herbs and cow products such as cow dung, milk, and ghee was used and evaluated for its efficacy in the removal of airborne microorganisms using Dhoopana practice as described in Ayurveda.

Lad and Palekar (2016), has also evaluated a herbal dhoop prepared from cow dung and herbs for its efficacy in removal of microorganism from the air. These workers demonstrated that herbal dhoop has great potential in the removal of microbes from the air and can be used in eco-friendly fumigation of hospitals, hotels, and labs ⁽⁷⁾. In our study we also used cow products and herbs for the preparation of dhoop and observed significant antimicrobial activity.

Palekar and co-workers (2018), worked on minimization of the use of chemicals or disinfectants by applying Dhoop formulation for removal of microbes from the environment and as a natural remedy to treat cough and cold. These workers prepared a herbal Dhoop using cow dung, cow ghee, camphor, Ajwain seeds (*Trachyspermum ammi*), and Dill (*Anethum graveolens L.*). These workers have observed a significant antimicrobial activity of the prepared Dhoop against respiratory pathogens such as *Staphylococcus aureus* and *Klebsiella pneumonia* ⁽⁶⁾. The dhoop prepared in the present study showed a significant effect in the removal of microorganisms from the air and thus may serve as an alternate to the chemical-based formulations and products for use as dhoop and fumigation agent.

CONCLUSION

In India, several traditional techniques such as Dhoopana and Havana are described in Ayurveda and Vedic system to minimize microbial load in the environment. In the present investigation, herbal dhoop was prepared from herbs and cow products such as cow dung, milk, and ghee and evaluated for its efficacy in the removal of airborne microorganisms. We have observed good antimicrobial activity against airborne microorganisms with a significant reduction in microbial load 66.12% in plates (environment), 55.38% (Main lab), and 60% (LAF room) after exposure of the plates to dhoop in laminar airflow. This herbal dhoop is prepared from easily available and cost-effective raw materials and has a pleasant odor and smell. This dhoop may serve as an alternative to the chemical-based incense sticks and other

products used as dhoop for religious practices and fumigation of offices, hospitals, and other areas.

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