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Anti-microbial activity of Herbal *Dhoopa* on clinical isolates of common nosocomial pathogens – An invitro study

Research Article

Anusha Naikwadi[1], Archana Panchaxarimath[2*]

1. Student, 2. Professor & HOD Department of Swasthavritta &Yoga,
SBSS Krishna Ayurvedic Medical College and Hospital Sankeshwar Belagavi, Karnataka India.

Abstract

Traditional practices such as *Dhoopana* (fumigation) are utilised to disinfect and reduce microbial levels within the environment. This study aims to create herbal *Dhoopa* sticks and test the antimicrobial activity against bacteria, which often cause nosocomial infections. Methods: *Dhoopa* sticks were made by blending *Guggulu*, *Aguru*, *Sarjarasa*, *Vacha*, *Shweta Sarshap*, *Saindhav*, *Nimba*, and Ghee with cow dung and milk. These sticks were then air-dried for five days at 45 degrees Celsius and stored. Antimicrobial activity for *Dhoopa* was tested on 10 clinical isolates namely *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Proteus mirabilis, Staphylococcus aureus*, *Salmonella typhi*, *Streptococcus pneumoniae*, E Coli, *Streptococcus pyogenes*, *Acinetobacter* spp and *Klebsiella pneumoniae*. The Agar Plates prepared were exposed to *Dhoopana* for 2 hours, then incubated at 37 degrees Celsius for 24 hours. Efficacy of *Dhoopana* was assessed based on viability of organisms. Results: Visible growth was absent in 6 sub cultured plates and present in 4 plates after exposure to *Dhoopana* Conclusion: Study suggest that *Dhoopana* is effective as antibacterial against *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Proteus mirabilis*, *Staphylococcus aureus*, *Salmonella typhi* and *Streptococcus pneumoniae* and using *Dhoopa* sticks for fumigation can effectively prevent and control nosocomial infections caused by these pathogens.

Keywords: Disinfection, Antimicrobial, Nosocomial Infection, *Dhoopana, Ayurveda,* Fumigation.

Introduction

Dhoopana (fumigation) a broadly practiced strategy in ayurveda for disinfection, involves utilizing medicinal substances to restrain the development of pathogenic organisms effectively. Exploring traditional practices like *Dhoopana* is fascinating and enriching, providing valuable insights into ancient methods of disinfection and health care. *Dhoopana* is utilized for the treatment of mending wound, *Yonivyapada* (Gynaecological clutters), *Karnarogas* (Ear disarranges). *Nasarogas* (infections of Nose) *Gudarogas* (infections of Rectum and Anus), to purify *Bheshajagara* (Drug store), *Sutikagara* (Accouchment ward) etc. It is additionally utilized to disinfect medical instruments and delivery rooms. Since Vedic period, the practice of *Homa-Havana* and *Yagnya* has been predominant, including the sterilization of air through *Agnihotra* (daily ritual oblation put into the fire) the refinement of the domestic and its environment through *Dhoopana*. *Harita Samhita* devotes a particular chapter to expand on the points of interest of the Homa custom [1]. The specify of *Guggulu* in *Atharva Veda* and fumigation details like *Nandaka Dhoopa* for various effects, including enhancing reproductive health, highlights the diverse applications of these practices in ancient traditions. It is interesting how traditional writings like *Yogaratnakara* too detail specific formulations like *Nimba Kashta Dhoopa* for contraception (2). The historical insights into such practices contribute to our understanding of the wealthy cultural and medicinal heritage (3).

Even though various disinfection methods are generally followed as per modern science in most of the domestic and professional setups, they have their own disadvantages, like unfavorable impacts on human, animal, and plants on long term utilize. *Ayurveda* prescribes *Dhoopana* as a method of disinfection for various chambers like *Shastragara*, *Kumaragara* (child abode / nursery), *Sutikagara* and for instruments like *Yantra* and *Shastras*, the methods are *Payana* (tempering of instruments), *Dhoopana*, *Parisheka* (Streaming), *Agnitapana* etc. *Sushruta* has also explained the method of treating *Vrana* (wound), *Vranitagara* and *Shastrakarmaghruha* by *Dhoopana* (4). The fumigation through herbo-mineral drugs has huge potential to fix the issue of hospital acquired infections (nosocomial infections) (5). The traditional technique of *Dhoopana* have been used in the process of disinfection to play down microbial stack to a nonpathogenic level within the environment. Traditional practices emphasised the importance of disinfection of *Vrana* (wound) along with the room and beddings of the

*** Corresponding Author:**
Archana Panchaxarimath
Professor & HOD, Department of Swasthavritta &Yoga, SBSS Krishna Ayurvedic Medical College and Hospital, Sankeshwar Belagavi, Karnataka India.
Email Id: drarchanavp2@gmail.com

patient by *Rakshoghna Dhoopa* in the form of fumigation with drugs like *Guggulu* (*Commiphora mukul Engl*.), *Aguru* (*Aquilaria malaccensis*), *Sarjarasa* (exudate of *Shorea robusta*), *Vacha* (*Acorus calamus Linn*.), *Shweta Sarsap* (*Brassica campestris*), *Saindhav* (rocksalt), *Nimba* (*Azadirachta.indica*), and *Ghrita* (ghee) (6). The available references suggests that the drugs mentioned by *Acharya Sushruta* are effective as antimicrobial. Current study was undertaken to develop *Dhoopa* stick to facilitate easy application of fumigation in hospital setups and to evaluate its antimicrobial activity on clinical isolates of nosocomial pathogens.

Aims and Objectives

Evaluation of antimicrobial activity of *Dhoopa* sticks against common nosocomial pathogens.

Materials and Methods

Place of study: SBSS Krishna Ayurvedic Medical College and Hospital (KAMCH) Sankeshwar.

Type of study: Invitro Study

Duration of Study: 3 months

Collection of raw materials

The raw materials such as *Agaru*, *Sarjrasa*, *Guggulu*, *Nimbatwak*, *Shewta Sarshap*, *Saindhav,* Vacha, and cow dung powder were procured from SBSS KAMCH Pharmacy in Sankeshwar. The raw drugs *Agaru*, *Sarjrasa*, *Guggulu*, *Nimbatwak*, *Shewta Sarshap* and *Vacha* were authenticated by experts in department of dravyaguna vignan of SBSS KAMCH Sankeshwar. Milk and Ghee were purchased from the local market in Sankeshwar, District Belagavi, Karnataka.

Table 1: The details of ingredients of herbal *Dhoop*

SL. No	Ingredient	Scientific Name/ Common Name	Part used	Quantity
1	*Agaru*	*Aquillaria agallocha Roxb.*	Heart wood	2g
2	*Sarjrasa*	*Vateria indica Linn.*	Gum	2g
3	*Guggulu* Powder	*Commiphora mukul (Hook.ex Stocks) Engl*	Resin	2g
4	*Nimba twak*	*Azadirachta Indica A.*	Bark	2g
5	*Shweta Sarshap*	*Brassica campestris Linn.*	Seed	2g
6	*Saindhav Lavan*	*Rock Salt*	-	2g
7	*Vacha*	*Acorus calamus Linn.*	Root	2g
8	*Ghrita*	Ghee	-	10 ml
9	*Dugdha*	Milk	-	QS
10	*Gomaya churna*	Cow dung powder	-	8g

Method of Preparation of *Dhoopa* Sticks

All the ingredients are taken in a clean mortar, mixed with cow milk, & ghee followed by trituration with pestle until it forms a paste. Semi-dried cow dung powder was then added to this mixture and macerated again to achieve a fine paste consistency. *Dhoopa* sticks were meticulously crafted using a rectangular plastic cube, which was cut open from the apical side to facilitate shaping. These *Dhoopa* sticks underwent a drying process lasting five days in an oven set at 45 degrees Celsius, after which they were stored in an airtight container(7). The *Dhoopa* sticks prepared were of uniform size (5cm×2cm×2cm) weighing approximately 3 grams.

Figure 1: Method of preparation of *Dhoopa* Sticks (a) Cow Dung Powder (b) All Ingredients (c) Trituration (d) Prepared *Dhoopa* sticks (e) *Dhoopa* Sticks Dried in the Micro wave (f) Burning of *Dhoopa*

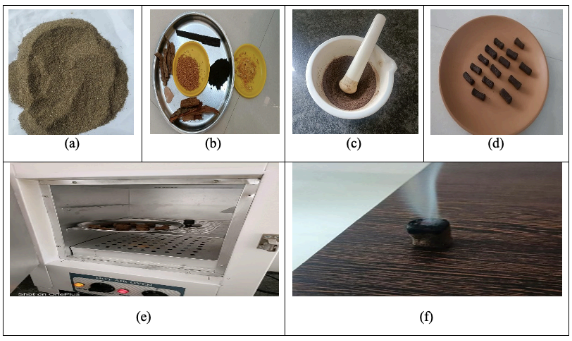

Figure 2: Exposure of agar plates to *Dhoopana*

Methodology of Antimicrobial Study

Total 10 clinical isolates (7 gram-negative and 3 gram-positive) were obtained from clinical microbiology section after its identification was done. Then isolated microorganisms which were identified by biochemical tests were subjected to the study.

Antimicrobial activity was determined by cup plate method. Prepared Mullar Hinton agar plates were inoculated with test organism and then with the help of a sterile borer cavities were made. Later *Dhoopana* stick was lit and was kept in a glass chamber measuring 12inch×18inch×12inch, along with the culture plate and left it untouched for 2 hours (8). Total 4 *Dhoopa* sticks were used to fumigate the chamber for 2 hours. After 2 hours the culture plates were taken out from glass chamber and then sub cultured onto freshly prepared culture plates. Plates which were sub cultured were incubated for 24 hours at 37°C to check for the viability of organisms. After incubation the plates were read for the visible growth of organisms. Effect of *Dhoopana* was assessed based on whether growth is observed in sub-cultured plates. The absence of growth after treatment indicates that the treatment was able inhibit the growth of microorganisms.

Results

Out of 10 clinical isolates of bacterial species 7 were gram-negative and 3 gram-positive. After exposure to *Dhoopa* in glass chamber growth was present in 4 sub cultured plates and absent in 6. The *Dhoopana karma* was effective for 4 gram-negative bacteria (*Pseudomonas aeruginosa, Proteus vulgaris, Proteus mirabilis, & Salmonella typhi*) and 2 gram-positive bacteria (Staphylococcus aureus & *Streptococcus pneumoniae*). The results of *Dhoopana* before and after exposure is as shown in table 2.

Table 2: Effect of *Dhoopa* on clinical isolates of bacterial species

S.No	Organism	Gram stain	Before	After
1	*Escherichia coli*	Gram-Negative	Growth Present	Growth Present
2	*Pseudomonas aeruginosa*	Gram-Negative	Growth Present	No Growth Seen
3	*Proteus vulgaris*	Gram-Negative	Growth Present	No Growth Seen
4	*Proteus mirabilis*	Gram-Negative	Growth Present	No Growth Seen
5	*Streptococcus pyogenes*	Gram-Positive	Growth Present	Growth Present
6	*Staphylococcus aureus*	Gram-Positive	Growth Present	No Growth Seen
7	*Salmonella typhi*	Gram-Negative	Growth Present	No Growth Seen
8	*Acinetobacter spp*	Gram-Negative	Growth Present	Growth Present
9	*Streptococcus pneumoniae*	Gram-Positive	Growth Present	No Growth Seen
10	*Klebsiella pneumoniae*	Gram-Negative	Growth Present	Growth Present

Discussion

Fumigation is a widely known method of disinfection adopted to reduce the nosocomial infections which are the major health care associated problems worldwide. Current study was aimed to evaluate antimicrobial activity of *Dhoopana* against commonly found gram-positive and gram-negative bacteria which are responsible for nosocomial infection. Clinical isolates (7 gram- negative and 3 gram-positive) were obtained as per availability due to time restriction in the study. When checked for the viability of organisms after getting exposed to *Dhoopana* fumes for 2 hours, growth was present in *E Coli*, *Streptococcus pyogenes*, *Acinetobacter spp* and *Klebsiella pneumoniae*, where as in remaining 6 sub cultures growth was absent (both gram positive and negative bacteria). The *Dhoopa* sticks were developed from *Rakshoghna Dravya* mentioned by *Acharya Sushruta* along with cow dung powder and milk. After trituration of ingredients proper consistency and shape for the stick was achieved. Drying them in oven facilitated complete burning by when ignited. The classical references on these drugs described properties like *Krimighna*, *Vishanut*, Detoxifier, Aromatic and antimicrobial, hot and penetrating in nature, helps in mitigation of *Vata-Kapha Dosha* (9,10).

Table 3: Established activity of study drugs against micro-organisms (11,12)

S.No	Drug	Established activity against micro-organisms
1	*Agaru*	Antimicrobial activity against *K. pneumonia* than *Juniperus oxycedrus*
2	*Sarjrasa*	High concentration of bioactive components such as alkaloids, glycosides, phenols, tannin, steroids and terpenoids which contribute to high antibacterial activities
3	*Guggulu*	Broad spectrum antimicrobial activity – *Bacillus megaterium, Micrococcus luteus, Enterococcus faecalis, Stasphylococcus aureus* and fungal strains of *Aspergillus niger A. flavus, Candida albicans* and *Microsporum Phylum*
4	*Nimba twak*	Antimicrobial property against *streptococcus pyogenes*, *S aureus*, *Sepidermidis* and *P. aeruginosa*
5	*Shweta Sarshap*	*Nematotes*, parasites and broad-spectrum antimicrobial and antifungal properties
6	*Vacha*	Exhibited potent antiviral activity against herpes virus

The research conducted on these drugs clearly demonstrated their antimicrobial properties. The drugs used in the formulations are volatile in nature, hence would be an advantage in lowering microbial contamination in air on difficult to reach surfaces. A research study on Efficacy of *Dhoopana* powder containing ingredients mentioned in table no.1 was conducted for Operation Theatre (O.T) fumigation. The swab reports of O.T. were like advanced procedure of formalin fumigation & gave Satisfactory results (13). The research findings on *Nimba* fumes against *Streptococcus pyogenes* after 10-Minute exposure showed 100% inhibition and 50% inhibition after 5-minute exposure, it also showed inhibition of *S. aureus*, *S. epidermidis*, and *P. Aeruginosa* under same study setting (14,15). Preliminary phyto-chemical screening of *Sarjarasa* (*Vateria indica)* exhibited the presence of very high concentration of bioactive components which contribute to high antibacterial activities (16). The species of *Sarshapa* (*Brassica*) contain contrasting profile of glucosinolates which have biocidal activity on different pathogens including bacteria and fungi (17). The properties of ingredients of *Dhoopa* sticks, like *Ruksha* (dryness), *Ushna* (heat), *Laghu* (lightness), *Visada* (clear), *Teekshna* (penetrating nature) removes the moistness and humidity of the environment (18). The fumes and carbon dioxide released during *Dhoopana* creates an anaerobic atmosphere which ultimately destroys the substratum for the survival of the microbes (19). In present study *Dhoopana* was effective against the bacteria viz, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Proteus mirabilis*, *Staphylococcus aureus*, *Salmonella typhi*, and *Streptococcus pneumoniae*. These are most found organisms in hospital setup and responsible for Nosocomial Infection. The study signifies the effectiveness of *Dhoopa* as antimicrobial and its importance in prevention of hospital acquired infection.

The development of *Dhoopa* sticks for fumigation facilitates easy application of fumigation and shelf life of these sticks will be more compared to the conventional method of using *Churna*. These *Dhoopa* sticks can be stored in airtight container or plastic ziplock bags for longer period and used for fumigation whenever required. Due to limitation in time, current study was carried out on clinical isolates of nosocomial pathogens. In future researches can be conducted with same formulation to evaluate the microbial load in hospital wards and isolation rooms. The study may be undertaken in future to standardize the quantity of *Dhoopa* sticks and time required to disinfect wards and rooms.

Conclusion

Fumigation with *Dhoopa* sticks is effective as antibacterial against *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Proteus mirabilis*, *Staphylococcus aureus*, *Salmonella typhi*, and *Streptococcus pneumoniae* which are commonly found in hospital setup and are major cause for nosocomial infection. These *Dhoopa* sticks can be used for fumigation of hospital wards, isolation rooms and to disinfect the fomites for prevention and control of nosocomial infection.

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