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In-vitro evaluation of antimicrobial activity of Dhupana drugs on *Staphylococcus aureus*

Research Article

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Abstract

Multidrug resistance in the notorious bacterial strains is accounting for a tremendous increase in global mortality rate. The upcoming new generation antibiotics are granting a perceptive provision in this issue but the superbugs have enforced these antimicrobial agents to surrender by developing the innovative variation in their genes/ plasmids. So alternatives are desired to combat this problem. Herbal drugs may broaden this spectrum of antimicrobial agents by offering safer and promising approach. *Dhupana karma* is such a modality known for its antimicrobial activity due to its indication in infectious diseases as per classical texts. The present study included fumigation on *Staphyloccus aureus* with four *Dhupana* formulations to assess their antimicrobial activity. The assessment criteria taken was mean bacterial colony count after 20 min of fumigation compared with control (without fumigation). Statistical analysis was done using Anova test and Tukey Kramer comparison test. The % change in mean colony count of B1 *(S.aureus)* after 20 min of fumigation was found to be 76.61 % with drug D1 (*Nimbadi Dhupa*), 84.13 % with drug D2 (*Kumaragara Dhupa*), 80.51 % with drug D3 (*Dashanga Dhupa*) and 98.94 % with drug D4 (*Gana Dhupa*).

Keywords: Multidrug resistance, *Dhupana karma*, Antimicrobial agents.

Introduction

The effective prevention and treatment of an ever -increasing range of infections caused by bacteria, parasites, viruses and fungi is constantly being threatened by increasing antimicrobial resistance. It is a complex global public health challenge and is accelerated by the misuse of antimicrobial agents. The increasing waves of antibiotic resistance in *S. aureus set a* problematic example of this issue. Today, *S. aureus* is a growing issue both within hospitals and communities because of its internal virulence and ability to adjust to different environments. Despite the availability of effective antimicrobials, the mortality of *S. aureus* bacteraemia remains approximately 20–40% (1).

The upcoming antimicrobial drugs are becoming less effective or even ineffective resulting in global health security emergency that is rapidly beating the available treatment options. It is posing a great impact on particularly vulnerable and immunocompromised patients resulting in prolonged illness, life threatening infections acquired in hospitals and in the community and finally increased mortality. It causes the crucial necessity for development and implementation of effective strategies to curtail the emergence and spread of AMR.

Herbal therapies and drugs may contribute in this field. Ayurveda is rich in treatment modalities in the context of microbiology. Although under noticed, these can be used as add on therapy along with modern antibiotics. *Dhupana karma*, a traditional method of sterilisation, can be a boon and help in curtailing the problem of increasing trends of antimicrobial resistance. It is a technique of delivering the plant active constituents in form of fumes to the target sites.

In the present study, four Dhupana formulations were assessed in vitro for their antimicrobial activity on *S.aureus*.

Materials and methods

Dhupana drugs

The drug formulations were prepared at NIA Pharmacy under expert's supervision. The required drugs were taken in raw form and then air dried. The drugs were ground into coarse powder using pulveriser machine and mixed in equal proportions accordingly. The formulations were then stored in air tight containers at room temperature. Table 1. shows the ingredients of the respective 4 formulations.

Microorganism

The isolated strain of *Staphylococcus aureus* (ATCC 29213) purchased from Hi media Laboratories Pvt. Ltd. Mumbai was selected for the study. The bacteria was cultured on Mueller Hinton Agar at 37 °C for 24 h and then used for antimicrobial activity.

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Table 1: Showing the ingredients of four formulations

S.No.	Drug	Contents	Indication
D1.	*Nimbadi Dhupa* (2)	*Neem, Vacha, Kutha, Haritaki, Sarshapa, Guggulu, Ghrita*	*Jvara*
D2.	*Kumaragara Dhupa* (3)	*Yava, Sarshapa, Atasi, Hingu, Guggulu, Vacha, Choraka, Brahmi, Jatamansi, Ashoka, Rohini, Sarpnirmoka* (shedded snake skin), *Ghrita*	*Kumaragara Dhupana*
D3.	*Dashanga Dhupa* (4)	*Sarshapa, Kuth, Bhallataka, Vacha, Bastaloma, Tagara, Bhurjapatra, Guggulu, Ghrita*	*Sarvaroga*
D4.	*Gana Dhupa* (5)	*Akshat* (rice), *Jaatipushpa, Madhu, Sarshapa, Vacha*	*Sarvabhutaruja*

Fumigation chamber

A closed glass chamber (11cm × 12cm × 13 cm) was designed with a cover lid having a hole for entry of fumes into the chamber. The fumes were produced by burning powdered drugs over the hot plate with an inverted earthen funnel over it and were projected into the chamber through a rubber pipe connected with the funnel. Same set was designed for the control group (I), but no fumigation was done and thus it acted as negative control. A thick thermocol sheet was placed in between the chamber and the hot plate so as to nullify the direct effect of temperature by adjacent hot plate.

Antimicrobial activity procedure

In-vitro antibacterial activity was tested by fumigating the trial bacterial strain with the Dhupana drugs for a fixed duration. Each set of experiment was done in triplets as per the procedure. 3 swabs were dipped in bacterial suspension in test tube.2 were put in fumigation chamber and one was taken in control chamber. Fumigation was done with fixed concentration of fumes obtained by burning *Dhupana* drugs on hot plate (5gm powder/ 5 min) and projected to the swabs in the chamber for 10 min and 20 min. The first and second swabs were taken outside after 10 min and 20 min of fumigation respectively and all the three swabs were diluted in (3 ml) fresh peptone water separately. Inoculation was done on culture plates using loop method and the plates were incubated for 24 hrs. Bacterial colonies were counted and compared.

Observations and Results

Table 2. Showing the effect of D1 (*Nimbadi Dhupa*), D2 (*Kumaragara Dhupa*), D3 (*Dashanga Dhupa*) & D4 (*Gana Dhupa*) on mean colony count of B1 *(S.aureus)* after fumigation for 20 min.

C20 : bacterial colony count of control group (no fumigation) after 20 min.

F20 : bacterial colony count of trial group (fumigation done) after 20 min.

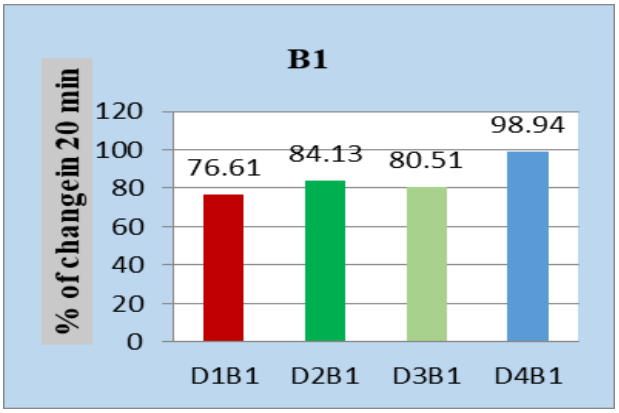
B1 (Bacterial strain 1) *(S.aureus)*	C20	F20	Difference in colony count after 20 min (C20-F20)	Mean	S.D	SEM
D1	209	34	175	213.00	41.940	24.214
	380	122	258			
	245	39	206			
D2	289	03	286	302.33	16.503	9.528
	415	96	319			
	374	72	302			
D3	209	09	200	239.67	34.385	19.852
	269	11	258			
	415	154	261			
D4	389	03	386	313.00	71.547	41.308
	249	06	243			
	311	01	310			

Table 2. Effect of D1 (nimbādi dhūpa), D2 (kumārāgāra dhūpa), D3 (daśāṁga dhūpa) & D4 (gaṇa dhūpa) on B1 *(S.aureus)* after fumigation for 20 min

One-way Analysis of Variance (ANOVA)

Comparison	Degree of Freedom	Sum of Squares	Mean of Squares	F	p
Between Groups	3	21083	7027.6	3.373	0.0751
Within Groups	8	16665	2083.2		
Variation among group means is not significantly greater than expected by chance					

Fig.1 Effect of fumigation with D1, D2, D3 and D4 in terms of % change in mean bacterial colony count of *S. aureus*.



The % change in mean colony count of B1 *(S.aureus)* after 20 min of fumigation was found to be 76.61 % with drug D1, 84.13 % with drug D2, 80.51 % with drug D3 and 98.94 % with drug D4. But intergroup comparison showed change in mean bacterial colony count was not significant statistically between various groups

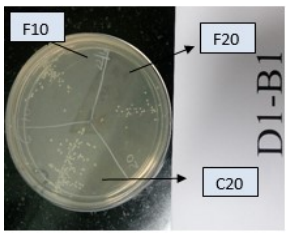


Fig.2 Fumigation by *Nimbadi Dhupa* on *S.aureus*

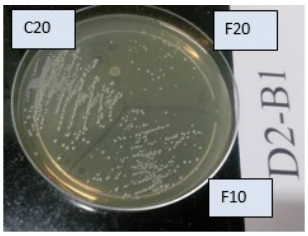


Fig.3 Fumigation by *Kumaragara Dhupa* on *S.aureus*

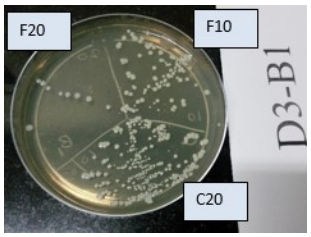


Fig.4 Fumigation by *Dashanga Dhupa* on *S.aureus*

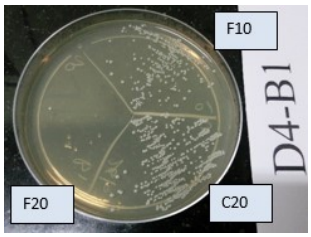


Fig.5 Fumigation by *Gana Dhupa* on *S.aureus*

Discussion

Nimbadi Dhupa

Fumigation for 20 min with this formulation resulted in remarkable difference of 76.61% in mean bacterial colony count of the experimental as compared with control group. The findings are supported by a previous study in which 93% sensitivity was seen with fumigation of neem leaves on *S.aureus* (6). Antimicrobial activity was found with extract form of various contents of *Nimbadi Dhupa* also. A study reported that the MIC of extract of *Vaca* rhizome was found to be 0.25 mg/mL for *S. aureus* with 1.62 cm zone of inhibition showing significant antibacterial activity (7). Nimbolide is also reported to have antibacterial activity against *S. aureus* and *S. coagulase* (8).

Results suggest that this drug formulation is having anti-microbial activity and is effective in reducing the bacterial load of *S.aureus* when administered through fumigation.

Kumaragara Dhupa

This formulation showed significant change in mean bacterial colony count after fumigation (84.13 % change in 20 min). The contents of *Kumaragara Dhupa* possess good antimicrobial efficacy against *S.aureus* with their proven efficacy in earlier studies. Mishra et al. reported the antimicrobial activity of aqueous and alcoholic extracts of asafoetida with MIC 6.25 (mg/ml) against *S.aureus* by agar well diffusion method.A. glauca oil exhibited variable degree of antimicrobial activity against *S. aureus* with the zone of inhibition of 22.8mm and the lowest MIC values 159.3 µg/Ml (9). Hari Venkatesh K R et al. reported that the zone of inhibition by methanolic extract of *Kaṣuki* (*Picrorhiza kurroa Royle ex Benth.)* rhizome was 12mm for *S aureus* by well diffusion method.

Results of this experiment are in accordance with previous studies. This trial suggests that *Kumaragara Dhupa* has statistically significant anti-microbial activity on *S.aureus* when given in form of fumigation.

Dashanga Dhupa

A remarkable difference of 80.51 % in mean bacterial colony count was observed after fumigation with this *Dhupa* for 20 min. The drugs present in *Dashanga Dhupa* have well established antimicrobial activity against *S.aureus* as supported by earlier studies. Mohanta *et al*. found that the petroleum ether (PEE) and aqueous extract fractions (AQE) of *Bhallataka* showed inhibitory activity against *S. aureus* (10 mm) at 100 mg/ml. The chloroform fraction of *Valeriana wallichi* exhibiting significant activity against *S.aureus* was reported in a study (10). Sidgi Syed Anwer Hasson

et al in a study found that 6000 µg/ML concentration of ethanol extract of was found to be bactericidal (11).

Gana Dhupa

Fumigation by *Gana Dhupa* on *S.aureus* showed a significant change in mean bacterial colony count after fumigation when compared with control group with % change of 98.94 % in 20 min fumigation. Many studies support the antimicrobial activity of contents of *Gana Dhupa*.

Laboratory studies have revealed that the honey is effective against methicillin resistant *S.aureus* MSA (12). Lusby PE et al conducted a research on manuka L. scoparium honey and found it to be effective against *S.aureus* (13). A Sabitha Rani et al reported that the MIC of extract of *Vaca* rhizome was found to be 0.25 mg/mL for S. *aureus* with 1.62 cm zone of inhibition showing significant antibacterial activity.

Results suggest that slightly prolonged exposure of Dhupa will show better results.

Conclusion

Fumigation of D1 (*Nimbadi Dhupa*), D2 (*Kumaragara Dhupa*), D3 (*Dashanga Dhupa*) & D4 (*Gana Dhupa*) on B1 *(S.aureus)* for 20 min when compared with control group shows that there was remarkable difference in mean bacterial colony. In *Gana Dhupa* change was 98.94 % followed by 84.13 % change with *Kumaragara Dhupa*, 80.51 % change with *Dashanga Dhupa* and 76.61 % with *Nimbadi Dhupa* (Fig.1). Intergroup comparison showed change in mean bacterial colony count was not significant statistically between various groups but on the basis of results it can be inferred effectiveness of various *Dhupa* on *S.aureus* is in order of *Gana Dhupa — Kumaragara Dhupa — Dashanga Dhupa — Nimbadi Dhupa.*

Thus *Dhupana Karma* can be used as add on therapy to oral antibiotics as it may reduce the dose along with enhancing the efficacy of the antibacterial therapy.

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