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Inhibitory Effect of Herbal Formulation Fumigation Against *Staphylococcus Aureus*

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Abstract: ***Ayurveda, the ancient science of life, offers many time-tested methods to treat and prevent illnesses. One such method is herbal fumigation, known as Dhoopana karma. This practice serves two purposes: it can cure wounds and also act as a preventive measure through sterilization. In a recent study, researchers explored the preventive aspect by testing herbal fumigation against*** *Staphylococcus aureus****, a bacterium responsible for about 70% of post-surgical infections like sepsis and cellulitis. Drawing inspiration from the classical Ayurvedic text,*** **Sushrutha Samhitha,** ***which recommends fumigating post-surgical wards to prevent infections, the study aimed to evaluate the effectiveness of this traditional practice. The researchers prepared six petri dishes with diluted*** *S. aureus****. Three dishes were exposed to the herbal fumigation, while the other three served as controls with no fumigation. After 15 minutes of fumigation, all plates were incubated overnight. The results were striking the fumigated plates showed*** *no bacterial colonies****, while the control plates had a high count of*** *4,800 colony-forming units per milliliter****. This clear difference highlights the potential of classical herbal fumigation as a powerful preventive tool against surgical infections caused by*** *S. aureus****. This study not only validates an ancient Ayurvedic practice but also suggests a natural, effective way to reduce post-surgical infections, blending traditional wisdom with modern scientific inquiry.***

Keywords: Herbal fumigation, Dhoopana, antimicrobial herbal compound, mustard, neem leaves, cow's ghee, Rock salt

1. Introduction

Millions of microorganisms live all around and inside us, but only a small number cause diseases. Ayurveda, the ancient healing system, offers unique ways to treat and prevent these illnesses. One such method is fumigation, an age-old practice like Dhoopana, Homa, and Havana. These traditional fumigation techniques help reduce harmful microbes and prevent infections, a fact that modern microbiology supports. In Ayurvedic texts, fumigation is recommended for various uses such as sterilizing operation theaters, disinfecting post-surgical wards, and treating wounds. The great Ayurvedic surgeon Acharya Sushrutha described how fumigating post-operative rooms with natural antimicrobial substances like mustard seed, neem leaves, ghee, and rock salt can aid healing and infection control. (1)

Inspired by this, a recent study explored the antibacterial effects of these traditional ingredients against *Staphylococcus aureus*, a common culprit in post-surgical infections. This focus is important because infections caused by this bacterium, especially the Methicillin-Resistant *Staphylococcus aureus* (MRSA), have doubled in US hospitals between 2002 and 2008. (2) The Centers for Disease Control and Prevention (CDC) reports over 80,000 invasive MRSA infections and more than 11,000 related deaths annually. (3)

Currently, formaldehyde fumigation is widely used to sterilize surgical areas, but it is a known carcinogen and presents health risks with prolonged exposure. This has fueled the search for safer, effective alternatives. Herbal fumigation, rooted in Ayurvedic tradition, presents a promising and natural option for controlling infections without harmful side effects. (4)

In summary, the ancient wisdom of Ayurvedic fumigation, supported by modern research, offers a hopeful path toward safer infection control, especially in surgical care. Balancing tradition with science could lead to healthier and safer medical environments for all.

2. Materials and Methods

Culture- *Staphylococcus aureus*

The study began with a culture sourced from agillus diagnostic lab. This culture was carefully maintained for short-term use on Mannitol Salt Agar (MSA), where *Staphylococcus aureus* showed its distinctive golden yellow colonies. The bacteria were incubated overnight for about 15 hours at a steady temperature of 37°C. Before starting the experiments, the culture underwent thorough characterization using differential staining and biochemical tests to ensure accuracy.

All procedures from culturing to maintenance and experimentation were conducted under strict aseptic conditions to prevent contamination. Several media types played a key role in the study, including Mueller Hinton Agar (MHA), nutrient broth, and MSA. The controlled fumigation environment involved clay pots, a McIntosh and Filde's anaerobic jar, and a suction pump (Tarsons Rocky vac 400), creating ideal conditions for the study's needs.

Through careful preparation and precise conditions, the study ensured reliable and reproducible results, laying a solid foundation for further research on this bacterial culture.

3. Methodology

In a laboratory setting, a bacterial culture was carefully examined to identify its characteristics. The first step involved Gram staining, which revealed the bacteria to be Gram-positive. Under the microscope, the cells appeared as

round cocci clustered together in bunches, hinting at a specific type of microorganism. To further understand the organism, a catalase test was performed by exposing the culture to hydrogen peroxide. The appearance of effervescence confirmed the presence of the enzyme catalase, indicating the bacteria's ability to break down hydrogen peroxide.

Additionally, the culture was grown on Mannitol Salt Agar (MSA), where its growth suggested it could ferment mannitol, a sugar alcohol. Combining all these observations the Gram-positive cocci in clusters, catalase positivity, and mannitol fermentation led to a clear identification the bacterium was *Staphylococcus aureus*, a well-known pathogen with distinct laboratory features.

Culture methods

In a controlled laboratory setting, an overnight culture of *Staphylococcus aureus* was prepared and diluted in physiological saline to create different concentrations. Dilutions of 10^{-2}, 10^{-4}, and 10^{-6} were carefully plated on Mueller-Hinton Agar (MHA) plates, with 0.1 mL spread on each plate. Two sets of three plates, representing these dilutions, were prepared for both a control and a fumigation test, all under strict aseptic conditions.

The fumigation involved a traditional mixture of neem leaf powder, salt, mustard, and ghee, each weighed at 10 grams and blended. This mixture was heated in a specially designed mud pot, generating fumes that were directed through a sealed McIntosh and Filde's jar using a suction pump to maintain negative pressure and ensure the fumes filled the jar completely.

Before fumigation began, three inoculated MHA plates were placed inside the jar. The fumes were drawn through the jar for 15 minutes to saturate the environment, after which the pump was turned off, and the jar was sealed and left undisturbed for three hours to allow the fumigant to act fully on the bacteria.

After this exposure period, both the test and control plates were incubated overnight at 37°C. The following day, colonies were counted and expressed as Colony Forming Units per milliliter **(**CFU/mL**),** calculated by multiplying the number of colonies by the reciprocal of the dilution factor. This method provided a clear measure of the fumigant's effectiveness in inhibiting bacterial growth compared to the control.

4. Observation and Result

In a recent experiment, test plates were exposed to a fumigant to evaluate its effectiveness against microbes. After an overnight incubation, the test plates showed no colonies at all, a stark contrast to the control plates, which had 4800 colony-forming units per milliliter (cfu/mL). This demonstrated a significant reduction in microbial presence on the treated plates.

Over the course of three hours, the fumigant achieved a remarkable three-log reduction in microbial counts, confirming its strong antimicrobial action. These results were not only impressive but also consistent and reproducible, highlighting the fumigant's reliability in reducing microbial contamination.

Overall, the study clearly showed that exposure to the fumigant effectively eliminated microbes, offering promising potential for applications requiring stringent microbial control.

5. Discussion

Many modern medicines can cause unwanted side effects, sometimes serious ones. To reduce these risks, plant-based remedies offer a promising alternative. In this study, we explored three herbal drugs alongside one mineral drug to harness their natural healing powers.

Black mustard is known for its antimicrobial strength, mainly due to a compound called allyl-isothiocyanate. This substance fights germs by reacting with components inside microbial cells. Research shows that its effectiveness against certain molds improves when combined with other ingredients.

Neem leaves, from the Azadirachta indica tree, have long been valued for their antibacterial properties. They contain various natural chemicals like alkaloids, glycosides, flavonoids, and saponins, all of which help defend against harmful pathogens. These compounds contribute to neem's powerful medicinal qualities.

Salt, used as a mineral drug in this combination, also plays a crucial role. It disrupts microbes by disturbing their water balance, altering their ionic environment, and interfering with essential enzymes. Salt can even weaken the molecular structure of microbial DNA, making it harder for these organisms to survive (5).

Adding Go-Ghrita, a traditional preparation, enhances the overall potency of the medicine. It helps the medicine release fumes easily, which can boost its antimicrobial effect. By combining these herbal and mineral components, the medicine offers a natural, effective way to combat microbes while minimizing the harmful side effects often seen with synthetic drugs.

6. Scope for Further Research

- Impact of Herbal Fumigation on nosocomial Infection.
- Proportional efficacy of formaldehyde and herbal fumigation.
- Permutation and Combination of herbal fumigation and ratio of efficacy.
- Dynamic ingredient in each drugs and its class of compounds.

7. Conclusion

Recent research has highlighted the powerful antimicrobial effects of herbal fumigation. A simple mixture of mustard seed, neem leaf, ghee, and rock salt was tested and found effective against *Staphylococcus aureus*, a common cause of serious post-surgical infections like sepsis, toxic shock syndrome, and pneumonia.

This discovery suggests that such a natural fumigation method could serve as an alternative strategy to reduce infections in various settings, including hospital wards, homes, schools, and public spaces. By embracing this herbal combination, we may find a safer, accessible way to protect people from harmful bacteria and improve overall health.

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