



Research Article

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KRIMIGHNA (ANTI-BACTERIAL) EFFECT OF DHUPANA (HERBAL FUMIGATION) IN LABORATORY SETUP

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ABSTRACT

Curcuma longa, *Acorus calamus* and *Embelia ribes* have anti-microbial activity against microorganisms when tested against their active ingredients. Present study examines the efficacy of these drugs when used as a fumigant. Herbal discs prepared out of these were burnt on red hot charcoal in Microbiology-Pathology laboratory set up and in OPD and were exposed to the herbal fumes for a period of fifteen minutes. Petri plates containing Nutrient agar were exposed to the air in respective areas for 2 minutes before fumigation and 15 minutes after fumigation. Later petri plates were incubated for 24 hours at 37 degree Celsius in an incubator. Observed bacterial colonies were identified by Gram's stain technique. This study was conducted for 3 times. Scanty to moderate growth of aerobic spore forming bacilli observed in the petri plates exposed to air before fumigation. No growth of organisms observed in the petri plates exposed to same air after fumigation. There is a significant decrease in the growth of airborne microorganisms after fumigation with *Curcuma longa*, *Acorus calamus* and *Embelia ribes* –Herbal discs. Hence these herbal drugs which are cost effective, non-irritant and aesthetic can possibly be used for regular fumigation to maintain sterile environment in Laboratory setup.

Keywords: Herbal drugs, fumigation, anti-microbial, petri plates, aerobic spore forming bacilli.

INTRODUCTION

Iatrogenic causes may affect patients as well as health care workers in a hospital set up. Hospitals provide a breeding ground for drug-resistant bacteria which can be transmitted due to poor infection control practices in the hospital. It is estimated that nosocomial infections cost \$ 4.5 billion and contribute to 88,000 deaths annually. Although infection control and hospital epidemiology activities have been the subjects of increasing scientific study over the past 30 years, efforts to lower infection risks have been continually challenged by the growing numbers of immune-compromised patients, antibiotic resistant bacteria, fungal and viral super-invasive functions. Most often, the mode of infection occurs through cross infections. Hence a sterile atmosphere like operation theatre or labour theatre in a health care setup is essential.¹

Fumigation is commonly practised in many developed nations in view of toxic nature of formalin. Too frequent use and inhalation of these is dangerous to health. Several new safe chemicals are emerging, but due to constraints of economy limit the use and closing of operation theatres for long hours can be managed with fumigation.²

In Ayurveda, we have some of the drugs which are known to have antibacterial effect. In this concern, an effective herbal fumigation containing drugs: Haridra (*Curcuma longa*), Vacha (*Acorus calamus*) and Vidanga (*Embelia ribes*) possessing germicidal and anti-microbial action is selected to see the effectiveness against airborne organisms.³

This study is aimed at making a routine practise of herbal fumigation in hospital laboratory set up, to maintain sterile atmosphere and to decrease the health hazards.

MATERIALS AND METHODS

Description of drugs

Haridra⁴

Botanical name: *Curcuma longa*

Family: Scitaminae / Zingiberaceae

Synonyms: Hattavilasini, nisha, krimighni, gouri, peeta

Varieties: Haridra, Daruharidra, vana haridra & amraharidra

Chemical constituents: curcumine, curcane

Useful part: Rhizome

Karma: Krimighna, vishaghni, mehaghni

Pharmacological actions: Hepato-protective, anti-allergic, antioxidant, anti-microbial, immune-stimulant.

Vacha⁵

Botanical name: *Acorus calamus*

Family: Araceae

Synonyms: Ugragandha, mangalya and karshani

Varieties: Vacha and Shweta vacha

Useful part: Rhizome

Chemical constituents: 1.5-3% volatile oil having asaraldehyde, asarone and eugenol.

Karma: Jantughna, jwaraghna, bhutaghna, mootravishodaka

Rogaghna: Krimi, moorcha, jwara

Pharmacological actions: Vermifuge, carminative, tranquilizer.

Vidanga⁶

Botanical name: *Embelia ribes*

Family: Myrsinaceae

Synonyms: Chitra tandula, shweta tandula

Varieties: *Embelia ribes*, *Embelia robusta*

Part used: Fruit

Chemical constituents: Embelin, Vilangine, quercitol

Karma: Vishaghna, Krimighna and jantu nashana; Indication: Krimi, udara, kushta
 Pharmacological actions: Antimicrobial, anti-helminthic, antifungal, anticonvulsant.

Requirements

Claypot, burning charcoal, Coconut shell, Ghee, camphor, Matchsticks, Chakrikas (cakes) made out of Haridra (*Curcuma longa*), Vacha (*Acorus calamus*) and Vidanga (*Embelia ribes*) Churna, Microscope, Gram stain reagents: Methylene blue, Grams iodine solution, Ethyl alcohol, Saffranin, Glass slides, Cover slips, Petri plates, Hot air oven, Incubator, Autoclave, Nutrient agar.⁷

Method of drug preparation

Above mentioned drugs are taken in powdered form separately. Under all aseptic precautions, these powders are mixed together and made into a fine paste with distilled water. Then it is made into chakrikas (cakes) of equal size weighing about 5 grams each. Then it is dried under sunlight for 2 days and later stored in air-tight container.

Microbiological Method

Preparation of Nutrient Agar (NA): First add 28 grams of Nutrient Agar powder in 1 litre of distilled water in a beaker. Heat this mixture while stirring to dissolve completely all the components. Later autoclave the dissolved mixture at 121

degree Celsius for about 15 minutes. After autoclaving, allow it to cool for some time but not solidify. Pour nutrient agar into each plate and keep these plates on the sterile surface until the agar has been solidified. Then close the lid of each Petri dish and store the plates in a refrigerator packed airtight to avoid contamination.⁸

Uses of Nutrient Agar: It is used frequently for isolation and purification of cultures. It is also helpful in testing antibiotic sensitivity.⁹

Method of drug fumigation and gram staining

Venue: Shree Dharmasthala Manjunatheshwara Ayurveda College and Hospital, Hassan.

Locations: Pathology- Microbiology Lab and Out Patient Department (OPD).

Nutrient agar petri plates were exposed to the air for 2min and incubated for 24 hours. Later dhupana has been done with five dried chakrikas nearly weighing 25 grams in each of the above said places for about 15 min, by using charcoal and coconut shells for burning with the help of camphor and ghee. After 15 minutes once again Nutrient agar petri plates are exposed in the same areas for about 2 min and subsequently incubated for 24 hours. After 24 hours- observed for the colony morphology and gram staining done separately. Observed in binocular compound microscope under oil immersion 100X and identified the morphological features of micro-organisms.¹⁰

OBSERVATIONS AND RESULTS

| Number of day | Day 1 | Day 2 | Day 3 |
|--------------------------------|---|--|---|
| Before Dhupana | Single Colony: Medium, creamy, round, opaque, smooth, elevated colony. | Colony 1: Large, irregular, whitish, creamy, opaque, raised colony. Colony 2: Large, round, translucent, raised, smooth colony. Colony 3: Small, round, whitish, opaque, raised, smooth colony. | Colony 1: Single, large, irregular, opaque, whitish, raised, smooth colony. Colony 2: Single, large, irregular, brownish, opaque, raised, smooth colony. Colony 3: Few, small, round, whitish, opaque, smooth colony. Colony 4: Pin point colony seen. Gram stain: Gram positive aerobic spore forming bacilli. |
| Result on gram staining | - | - | Gram stain: Gram positive aerobic spore forming bacilli. |
| After dhupana | No growths observed. | No growths observed. | Colony: Single, medium, round, translucent, raised, smooth colony. |
| Result on gram staining | Gram positive aerobic spore forming bacilli. | Gram positive aerobic spore forming bacilli | Gram positive aerobic spore forming bacilli. |



Picture 1: Haridra, Vacha and Vidanga churna



Picture 2: Chakrikas dried



Picture 3: Chakrikas in Airtight container



Picture 4: Dhupana Pot



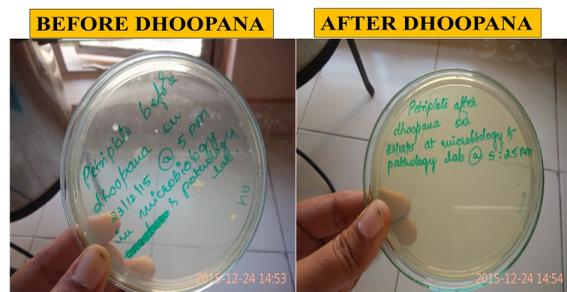
Picture 5: Burning of Herbal drugs

**PETRI PLATES BEFORE AND AFTER
DHUPANA IN OPD- 1st DAY**



Picture 6: Petri-plates before and after dhupana procedure

**PETRI PLATES BEFORE AND AFTER
DHUPANA IN MICROBIOLOGY LAB -2nd DAY**



Picture 7: Petri-plates before and after dhupana procedure

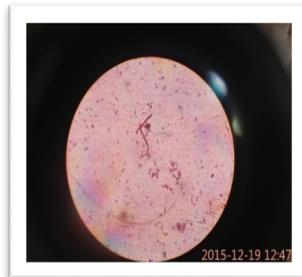
**PETRI PLATES BEFORE AND AFTER DHUPANA
IN MICROBIOLOGY LAB -3rd DAY**

BEFORE DHOOPANA

AFTER DHOOPANA



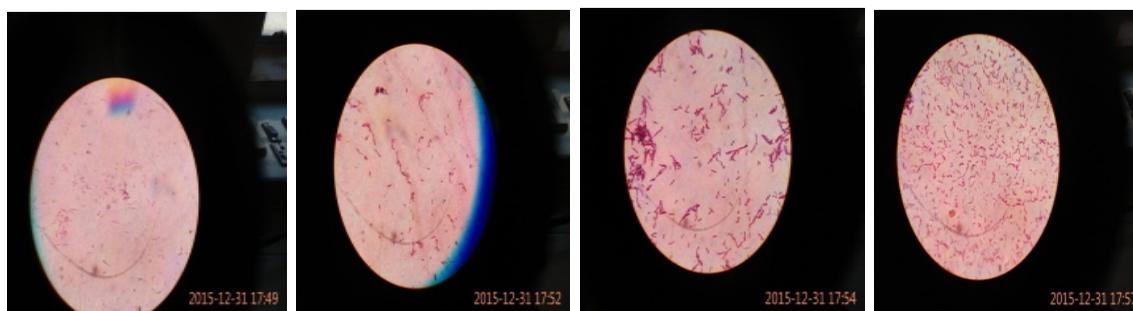
Picture 8: Petri-plates before and after dhupana procedure



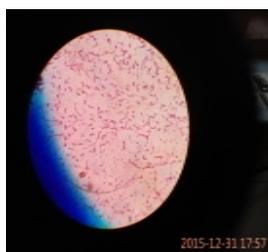
Picture 9: Day 1-Before dhupana (1 colony)



Picture 10: Day 2-Before dhupana (3 colonies)



Picture 11: Day 3-Before dhupana (4 colonies)



Picture 12: Day 3-After dhupana (1 colony)

DISCUSSION

There is a significant decrease in the growth of airborne microorganisms after dhupana with Haridra (*Curcuma longa*), vacha (*Acorus calamus*) and vidanga (*Embelia ribes*) because of their active constituents, tannins and essential oils like acolomone, aconone, calarene, camphene, euginol, quercitol, curcumene, camphor etc. Dhupana dravyas demonstrated antimicrobial property against gram positive aerobic spore forming bacilli which are common saprophytes present in air, water and soil. The smoke and fumes produced by these herbal drugs acts as germicidal and thereby do not produce toxic complications to the people around. Dhupana dravyas are cost effective, less irritant and are more aesthetic in comparison with chemical fumigation methods. Hence, they are very safe to use and are non-toxic in nature¹². One can use these drugs for regular fumigation in laboratory setup. In this study, fumigation was done first and then air samples were collected immediately after fumigation. Immediate response can be seen and prolonged effect is not yet being analysed. Future research scholars can take up measures on this.¹¹

CONCLUSION

Haridra (*Curcuma longa*), Vacha (*Acorus calamus*) and Vidanga (*Embelia ribes*) these three drugs proved their anti-bacterial effect on gram positive aerobic spore forming bacilli, which are commonly found in the environment. Hence, they can be used as an effective drug combination for the microbiota present in the environment, thus protecting us from the hospital acquired respiratory infections.

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