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Preparation and Evaluation of Antimicrobial Herbal based Incense Sticks for Fumigation against infectious bacteria

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ABSTRACT

The most widely used method for fumigation is using the fumes of formaldehyde along with potassium permanganate, although this method is the most accepted, it is the cause of several health hazards. In this study a herbal based incense stick was formulated using authentic samples of herbal powders of various woods , barks , gums along with volatile oils using mould method, the herbal incense sticks were evaluated for its antimicrobial activity and appearance. The formulated herbal based incense stick not only showed good antimicrobial activity against airborne microbes but also these incense sticks were easy to prepare , user friendly, economical and did not lead to health hazards as observed with the fumes of formaldehyde. Thus it was concluded that herbal incense sticks can be comfortable used for fumigation of hospital wards, microbiological labs as well as homes.

Key words: Herbal incense stick, Fumigation, *Dhoops*, antimicrobial activity, Bacteria, airborne pathogen.

INTRODUCTION

The concerns about microbiological hazards to the health of laboratory workers in lab activities has persuaded the government and the official bodies to investigate the causes of these infections aswell as the prophylactic steps that can be taken against common laboratory infections[1], such as tuberculosis, diphtheria and streptococcal infections[2].

Generally the fumigation of microbial laboratory can be done with formaldehyde along with potassium permanganate, this destroys all microbes present in the lab effectively but it has several adverse reactions such as irritation to the eyes, sulphydryl poisoning, protein aggregation and the effects can also be responsible for inducing cancer [3].

Since ancient times human beings have used smoke of medicinal plants for curing disorders. The great saints used to perform rituals to purify the environment by burning wood and odoriferous medicinal herbs [4].

Throughout the medieval period, including the times of the bubonic plague caused by *Yersinia pestis*the main prophylactic measure against infectious diseases was fumigation by burning incense herbs and aromatic essences [5].

Recent studies have proven the efficacy of holy stick fumigation against infectious bacteria [3] as well as efficacy of medicinal smoke on airborne bacteria [4], in spite of the positive results of these studies formulation of antimicrobial incense sticks for fumigation has not been carried out.

In this study we have formulated herbal based incense sticks made from powders of wood, barks, gums and essential oils, we have evaluated the efficacy of this stick as a mode of fumigation in laboratories, the effect of adding different oils in the formulation on the antimicrobial effect has been reported to enable a comparative study.

EXPERIMENTAL SECTION

Formulation of Herbal Incense stick:

The experimental work started with first collecting the raw materials which were purchased from local market. The general procedure followed for the formulation of herbal based incense sticks having antimicrobial activity was as follows:

The base material (sandalwood powder, charcoal powder, jigit powder, white wood powder) was mixed, quantity taken according to two formulations, v.i.z, A & B (Table 1 & 2) with water. Pure ghee was added to one of the formulations to see if it has any effect on the activity. After getting a wet mass, volatile oils [neem oil, eucalyptus oil, lavender oil] were added after making three equal parts of the wet mass. Different oil was added to each part. Perfumes used in industries for good fragrance were added in one of the formulations. Thus with two formulations, six sets of incense sticks were obtained. After adding volatile oils, incense sticks were prepared by using Mould method. In this method, the wet mass was filled into the cone shaped mould, which is made of plastic. The mould was kept aside for some time and then the open end of the mould was tapped on a flat surface. The cone shaped incense stick, which is still wet, comes out. The stick was kept in sunlight for 2 days for drying.

The incense sticks thus formulated were evaluated for their appearance by taking into account the evenness of the surface and their shape.

Table : 1 Formulation (A) of Herbal Incense Stick

Formulation A (for 25 g)	
Ingredient	**Quantity Taken**
Charcoal powder	5 g
Jigit powder	5 g
White wood powder	10 g
Sandalwood powder	2 g
Benzoin (crushed, passed through #36)	1 g
Gum benzoin (crushed, passed through #36)	1 g
Sohra (crushed, passed through #36)	1 g

Table: 2 Formulation (B) of Herbal Incense Stick

Formulation B (for 25 g)	
Ingredient	**Quantity Taken**
Charcoal powder	5 g
Jigit powder	5 g
White wood powder	10 g
Sandalwood powder	3 g
Benzoin (crushed, passed through #36)	0.5 g
Gum benzoin (crushed, passed through #36)	0.5 g
Sohra (crushed, passed through #36)	1 g

NOTE- Formulation B without perfume, Formulation B without ghee.

Evaluation of antimicrobial activity:

The evaluation of the herbal incense sticks for their antimicrobial activity was carried out in a smoke chamber taking into consideration the size and capacity to produce fumes of these sticks, 90mm petri plates were used with nutrient agar (from Hi-MEDIA Laboratories Pvt. Ltd, Mumbai, India), an air sampler was prepared as described in the study conducted by Nautiyal et al on the effect of medicinal smoke on air borne bacteria[4], this was placed in the path of smoke produced by the incense sticks to filter the carbon content and nullify the antimicrobial effect produced by it. An agar plate was exposed to the smoke in the smoke chamber for 10 minutes . It was then removed and incubated for 48 hours at 38^{o}C (pre exposure plate) . After 10 minutes the windows were closed and the incense stick was burnt for 10 minutes, the fumes of the stick were allowed to fumigate the chamber for 10 minutes . After 10 minutes a new agar plate was exposed to the fumes of the incense stick in the chamber for 10 minutes, after 10 minutes the plate was taken out of the chamber and incubated for 48 hours at 38^{o}C (post exposure plates). The difference in the microbial counts of the plates indicated the antimicrobial activity of the incense sticks. Evaluation was carried out for 6 sets of incense sticks.

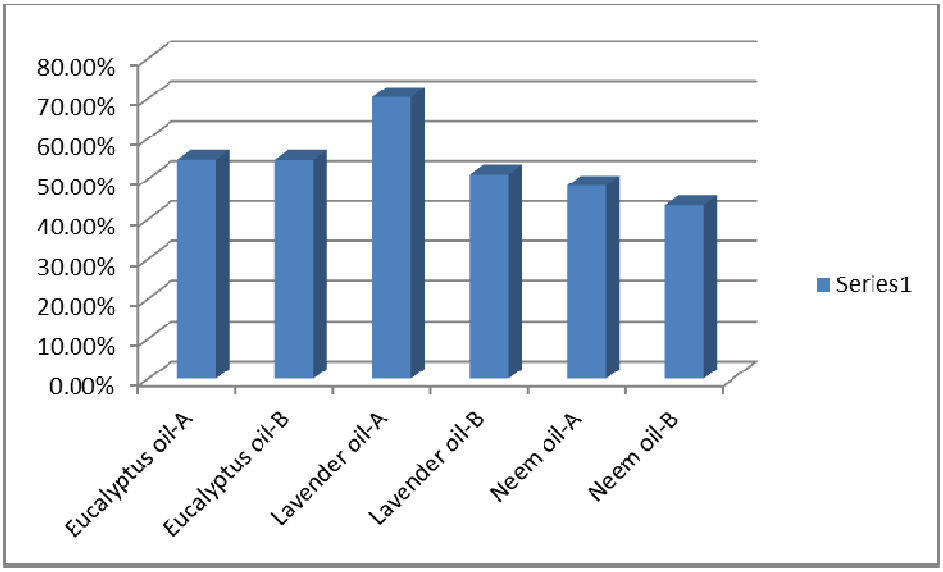
RESULTS AND DISCUSSION

From the results it was found that formulation A containing Lavender oil showed maximum inhibition (70.35%) while the least activity was shown by formulation B containing Neem oil (48.38%), the formulation A & B containing Eucalyptus oil showed intermediate activity which was almost the same for both formulations (54.69 % & 54.679 % respectively).

Thus from the observations it was concluded that fumigation with formulations containing Lavender oil had the highest percentage of inhibition followed by Eucalyptus oil and then Neem oil.

Formulation A containing ghee showed better activity compared to formulation B which was prepared without ghee , thus implying that presence of ghee plays a significant role in enhancing activity. The antimicrobial activities of the 6 sets of incense sticks can be shown in the form of graph (Fig 1), while the results are stated in Table 3.

Fig :1 Percent inhibition of Formulations A & B containing Lavender oil, Neem oil and Eucalyptus oil



X-axis represents volatile oil contained in the incense stick with Formulation type, while Y-axis represents % antimicrobial activity exhibited by the fumes of incense stick.

Table :3 Results of Microbiological evaluation

Sr. No	Formulation	Microbial Count (CFU)		% Antimicrobial activity
		Pre-exposure	Post-exposure	
	Formulation A			
1	Lavender oil	145	43	70.35 %
2	Eucalyptus oil	128	58	54.69 %
3	Neem oil	29	15	48.27 %
	Formulation B			
4	Lavender oil	47	23	51.07 %
5	Eucalyptus oil	150	68	54.675 %
6	Neem oil	189	107	43.38 %

The study of the ethnopharmacological aspects of smoke by Nautiyal et al showed that a room fumigated with medicinal smoke showed absence of pathological bacteria such as *Corynebacteriumurealyticum, Curtobacteriumflaccumfaciens, Enterobacteraerogenes (Klebsiellamobilis), Kocuriarosea, Pseudomonas syringaepvpersicae, Staphylococcus lentus, and Xanthomonascampestris , Tardicrescens*even after 30 days in an open room[4].

A study carried out showed that dhoop sticks of various combinations produce fumes of sweet aroma to inhibit levels of contamination and risk of infections , these were found to be effective against pathogens like *Pseudomonas aeruginosa, Streptococcus aureus , Escherichia coli* [3].

Another study has reported antifungal activity of plant extracts and smoke on fungal pathogens such as *Bipolarissorokiniana, Fusariumoxysporum*[6], this was found to have several advantages over the conventional method of disease control caused by fungal pathogens.

Inspite of these studies the formulation of herbal incense stick for the specific purpose of fumigation had not been carried out. Formaldehyde fumigation is a simple and easy procedure for fumigation but is inherently hazardous to health.

The incense sticks formulated showed satisfactory degree of inhibition along with the advantages of these being non-toxic, economical and easy to prepare thus these can effectively replace the more hazardous methods of fumigation.

The Future scope of this study will enable more accurate scope of the utility and use of herbal incense sticks, this includes isolation and identification of the bacteria inhibited by these herbal incense sticks along with their commercialization for use in sterilization of microbiological laboratories, in hospitals for maintaining well-being of patients in wards and even for maintaining general health and disease free atmosphere.

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