



Effect of Plant-Products Fumigation on Air-Borne Microbes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background and Objective: Purity of the ambient air is essential for our health and well-being. One of the main factors influencing air quality is the presence of microbes. Fumigation of herbs has been recommended in Unani medicine to purify the air. Hence, the present study was aimed to evaluate the effect of selected Unani herbs fumigation on air-borne microbes.

Methods: In this study, the effect of fumigation with Unani Medicinal herbs powder on air-borne microbes was assessed using differences in total colony counts of microbes in pre and post fumigation samples. Microbial load in the air was quantified using the passive open-air petri plate method. Formalin and potassium permanganate served as positive control, while tamarind wood charcoal fumigation served as negative control.

Results: Fumigation with Unani medicinal herbs powder at a dose of 45 grams was found to be the most effective in reducing the microbial load of the air. Significant reduction in aerial microbial colonies was observed with fumigation at 30 and 45 gms in the fumigated areas ($P < 0.05$).

Conclusion: It can be inferred from the findings of the present study that the test drugs fumigation efficiently reduces the air-borne microbes, hence may be recommended for air disinfectant. However,

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various factors that were not considered in this study, such as the effect of temperature and humidity on disinfection efficiency of fumigants should be addressed in further studies.

Keywords: Unani medicine; fumigation; air disinfection; air-borne microbes.

1. INTRODUCTION

On average, a person inhales approximately 14,000 litres of air per day. Therefore, clean air is a basic requirement of life and is essential to human health and well-being [1]. Since people spend the majority of their time indoors, indoor air quality has a significant impact on our health and lives [2]. Recognizing the importance of clean air in health promotion, several editions of air quality guidelines were issued by World Health Organization (WHO). In 2006, global update on air quality guidelines issued by W.H.O. highlighted the significant impact of indoor air pollution on health [3].

One of the most common factors affecting indoor air quality is the presence of bioaerosols. Bioaerosols are particulate matter usually associated with compounds of biological origin such as bacterial cells and cellular fragments, fungal spores and fungal hyphae, viruses, and by-products of microbial metabolism [4]. These are considered to be responsible for approximately 5 to 34% of indoor air pollution and are produced by a combination of natural and anthropogenic activities. Some of the microbial aerosols produced during these activities may be infectious. Exposure to these microbial aerosols can result in a variety of adverse health effects such as infectious diseases, acute toxic effects, allergies, and cancer [5,6].

Therefore, reducing microbial aerosols in indoor environments is essential for the prevention of various airborne diseases. Various methods such as air filters, chemical fumigants are used to reduce microbial aerosols [7]. However, in developing countries, chemical fumigation such as hydrogen peroxide, formalin is frequently used for air disinfection, which is associated with various toxic side effects [8]. Hence, there is a necessity to find natural and safe alternative method of air disinfection.

Fumigation of plant-based products has been described by Unani scholars as a method to purify the indoor air of residential places [9-14]. The air purification method described in Unani literature corresponds to environmental disinfection procedures of modern science.

However, the efficacy of fumigation using these plant products in improving the microbiological quality of air has not been scientifically validated.

Therefore, the present study was carried out to validate the traditional fumigation method using some Unani medicinal herbs to improve the microbiological quality of air in relation to formalin, so that ancient knowledge of Unani scholars can be unfolded to practical application. Herbs used in this study were selected from a renowned Unani treatise, Kitābal-Manşūri, authored by Zakariya Rāzī in 9th century A.D. concerning their availability and potential.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

The *in-situ* experiment was designed to compare the efficacy of traditional fumigation of Unani medicinal herbs with formalin plus potassium permanganate fumigation in improving the microbiological quality of indoor air. The efficacy of fumigation on aerial microbes' population was assessed through differences in total colony counts (CFU) of microbes in pre and post samples. The experiment was conducted in three different locations of the National Institute of Unani Medicine campus in the months of September to December 2020. Prior to the start of the study, air samples were collected from various locations and the microbial load in the air was estimated; Places with the highest microbial load were chosen for the study. The dimensions of the three selected locations were 1200, 900, and 700 cu. ft. respectively.

2.2 Air Sampling

Passive open-air petri plate exposure method was used to quantify the microbial load in the air. Air sampling was done before fumigation and at periodic intervals, after fumigation (1hr, 4hrs and 12 hrs). For sampling pre-cultured, sterilized petri-plates were placed in the desired locations according to the 1/1/1 scheme (1 m above the floor, about 1m away from walls, for 1 hour) after closing the doors of the rooms [15]. To isolate the aerobic and anaerobic bacterial colonies, Luria Bertani (LB) medium and for fungi, potato

dextrose agar (PDA) was used. The petri-plates were then sent to the laboratory for the estimation of total colony count (CFU), where these were incubated at a certain temperature for a certain time. The LB plates were kept in the incubator at 37°C for 24 hours and PDA plates at 25°C for 72 hours [16]. Different bacterial and fungal colonies were noted after incubation. Each fumigant was tested in three different locations. The room was kept open for two days after each experiment, before the new experiment, to eliminate the effect of the previous fumigation.

2.3 Experimental Materials

2.3.1 Test formulation

Medicinal Plants for fumigation were selected from a classical Unani text Kitābal-Manṣūri authored by Zakariya Razi [11]. These include Qusṭ (*Saussurea lappa*) root; Kundur (*Boswellia serrata*) oleo-gum resin; Mī'asā'ila (*Liquidamber orientalis*) oleo-gum resin; 'Ūd (*Aquilaria agallocha*) root; Ṣandal (*Santalum album*) wood; Kāfūr (*Cinnamomum camphora*) extract; and Murr (*Commiphora myrrh*) oleo-gum resin. All of these herbal medicines were purchased from Bengaluru's local market. The regional research institute of the Central Council for Research in Ayurvedic Sciences (CCRAS), Ministry of AYUSH, Government of India had verified the samples' identity and purity (Authentication/ SMPU/RARIMD/BNG/2020-2021/727). The voucher specimen (89/TST/Res/2021) has been deposited at the Herbarium of the National Institute of Unani Medicine for future reference.

These crude herbal drugs were thoroughly cleaned and dried before being pulverized to a coarse powder. Sandal, 'Ūd, and Qusṭ were powdered using an electric grinder, whereas Kāfūr, Mī'asā'ila, Kundur, and Murr were ground in a mortar and pestle. The coarse powder was filtered through a 40-mesh screen. After that, an equal amount of each drug's powder was taken, mixed and tested for fumigation in varying doses (5, 10, 15, 30, and 45 grams).

2.3.2 Control

Fumigation with formalin and potassium permanganate (35ml/10gram per cubic meter space) was used as a positive control [17], while fumigation with tamarind wood charcoal (200 grams) was used as a negative control.

2.4 Method of Fumigation

Fumigation was carried out using the traditional method (charcoal burning). About 100 grams of tamarind wood charcoal was burnt with the help of methylated spirit and when it became red hot, the prepared dry powder of the test drug was sprinkled over the burning flame, just then, it started emitting smoke. This smoke was allowed to spread in the room for a sufficient period of time; after 15 minutes of fumigation, 1 hour of duration was given for germicidal activity [16]. The fumigation area was completely sealed during the procedure so that smoke could not escape. Fumigation of the test drug and plain charcoal was done in this manner. Whereas the positive control, formalin and potassium permanganate was fumigated according to the standardized method [17-19].

3. RESULTS

The efficacy of different fumigants on microbial colonies in different areas is presented in Table 1-3. The aerial microbial population has been presented in the form of colony-forming units (CFU). Reduction in microbial colonies due to fumigation has been presented in the form of a percentage reduction. For intragroup comparison, Friedman test for repeated-measures was used to determine the significant effect of each fumigant in reducing the microbial colonies. For intergroup comparison, the kruskal-wallis test was used. P-value less than 0.05 was considered significant.

Fumigation with Unani formulation at 30 gms ($P=0.058$) and 45 gms ($P=0.01$) dosage resulted in a significant reduction in microbial colonies in area 1 (1200 cu. ft. room). Intergroup comparison revealed a significant effect on microbial colonies at 4 and 12 hours after fumigation ($P=0.057$) (Table 1). In area 2 (900 cu. ft. room), the only fumigation of Unani formulation at 45 gms resulted in a significant reduction in microbial colonies ($P=0.01$); however, on the intergroup comparison, the effect was found to be insignificant ($P>0.05$) (Table 2). Fumigation with Unani formulation at 30 gms ($P=0.056$) and 45 gms ($P=0.01$) dosage resulted in a significant reduction in microbial colonies in area 3 (700 cu. ft. room). The effect was found to be significant at 1 hour ($P=0.051$) and 4 hours ($P=0.055$) after fumigation in an intergroup comparison (Table 3).

Table 1. Comparison of the effect of different fumigants on aerial microbial population (CFU) in Area 1 [1200 cubic feet]

Fumigants	Sampling Time							Significance*
	Before Fumigation	After Fumigation						
		1 hrs	4 hrs		12 hrs			
CFU	CFU	%	CFU	%	CFU	%		
Tamarind Wood Charcoal [#]	22	22	0	24	+9	23	+4.5	P=0.819
Unani Formulation (5gms)	53	50	5.66	50	5.66	52	1.88	P=0.135
Unani Formulation (10gms)	61	48	21.3	49	19.6	57	6.55	P=0.156
Unani Formulation (15gms)	38	24	36.8	29	23.6	33	13.16	P=0.135
Unani Formulation (30 gms)	35	11	68.57	14	60	18	48.57	P=0.058*
Unani Formulation (45gms)	58	11	81.03	15	74.14	18	68.97	P=0.001*
Formalin and potassium permanganate	29	22	24.13	24	17.24	9	68.97	P=0.156
Significance	P=0.21	P=0.064		P=0.057*		P=0.057*		

CFU=Colony Forming units. [#]Increase in microbial colony counts observed. % indicates reduction in aerial microbial colonies. *P< 0.05 considered significant

Table 2. Comparison of the effect of different fumigants on aerial microbial population (CFU) in Area 2 [900 cubic feet]

Fumigants	Sampling Time							Significance*
	Before Fumigation	After Fumigation						
		1 hrs	4 hrs		12 hrs			
CFU	CFU	%	CFU	%	CFU	%		
Tamarind Wood Charcoal [#]	21	21	0	23	9.5	23	0	P=0.768
Unani Formulation (5gms)	53	52	1.8	51	3.7	53	0	P=0.682
Unani Formulation (10gms)	57	50	12.2	49	14.03	54	5.2	P=0.120
Unani Formulation (15gms)	42	24	42.8	26	38.09	32	23.8	P=0.120
Unani Formulation (30gms)	28	7	75	11	60.71	14	50	P=0.061
Unani Formulation (45gms)	60	10	83.3	12	80	15	75	P=0.001*
Formalin and potassium permanganate	34	27	20.5	23	32.23	10	70.5	P=0.120
Significance*	P=0.168	P=0.065		P=0.063		P=0.060		

CFU=Colony Forming units. [#]Increase in microbial colony counts observed. % indicates reduction in aerial microbial colonies. *P< 0.05 considered significant

Table 3. Comparison of the effect of different fumigants on aerial microbial population (CFU) in Area 3 [700 cubic feet]

Fumigants	Sampling Time						Significance	
	Before Fumigation	After Fumigation		4 hrs		12 hrs		
	CFU	CFU	%	CFU	%	CFU		%
Tamarind Wood Charcoal [#]	29	28	3.4	28	3.4	30	+3.4	P=0.112
Unani Formulation (5gms)	52	49	5.7	51	1.9	52	0	P=0.145
Unani Formulation (10gms)	59	49	16.9	49	16.9	54	8.4	P=0.112
Unani Formulation (15gms)	38	22	42.1	28	26.3	33	13.1	P=0.120
Unani Formulation (30gms)	35	11	68.5	14	60	18	48.5	P=0.056*
Unani Formulation (45gms)	54	11	79.6	16	70.37	18	66.66	P=0.001*
Formalin and potassium permanganate	29	24	17.2	23	20.6	9	68.9	P=0.120
Significance	P=0.266	P=0.051*		P=0.055*		P=0.06		

CFU=Colony Forming units. [#]Increase in microbial colony counts observed. % indicates reduction in aerial microbial colonies. *P< 0.05 considered significant

4. DISCUSSION

The present study compared the effect of Unani Medicines fumigation on reducing the microbial load in ambient air in relation to positive (formalin plus potassium permanganate) and negative (tamarind wood charcoal) controls; in order to validate their air disinfection efficiency and reveal the potential of these plant-products fumigation as an alternative to conventional chemical fumigants.

The results of this study showed that fumigation of the negative control had no effect on the population of aerial bacteria and fungi, whereas the effect of test formulation fumigation was dose-dependent, with its effect increasing as the dose of the test formulation was increased. Test formulation at 45gms dose had the greatest effect on bacterial and fungal colonies. The effect of fumigation with formalin and potassium permanganate was found to be maximum after 12 hours of fumigation; whereas its effect was found to be very little at 1 and 4 hours of fumigation.

The results of other studies are not directly comparable with our study because, to the best of our knowledge, this is the first study that examines the effect of Unani Medicines fumigation in improving the microbiological quality of air. Although, some AYUSH researchers [15-16,20-22] have attempted to validate the use of fumigation with herbal products as an air disinfectant; however, the drugs they have used were different from our test formulation.

Bisht et al. [20] reported that among the various tested herbs, 15gms of *Cedrus deodara* resulted in around 96% reduction in airborne bacteria after 45 minutes of fumigation [20], Nautiyal et al. [15] and Samanth et al. [21] demonstrated that 500gms havansamagri (mixture of more than 50 odiferous and medicinal plants) reduced the airborne bacteria by 94% and 95% respectively within 1 hour of fumigation. Bhatwalkar et al. [22] demonstrated the use of 3gms powder of four herbal drugs reduced airborne bacteria by nearly 60-70% after 12 hrs of fumigation. Whereas in the present study, fumigation with the test formulation powder reduced airborne bacteria and fungi colonies by 75% and 81.5 percent respectively, in much larger settings (1200, 900, and 700 cu. ft. vs 594 cu. ft.) than used by Bisht et al., at a much lower dose (45 gms vs 500gms) than used by Nautiyal et al. and Samanth et al.,

and in a shorter period of time than Bhatwalkar et al. (1 hr after fumigation vs 12 hours).

From the Unani medicine point of view, the effectiveness of the test formulation in reducing aerial microbial population may be attributed to the cold and dry temperament, as well as the *dāfi'-i-ta'affun* (antiseptic) and *māni'-i-'ufūnat* (disinfectant) properties [23,24]. According to the Unani medicine, hot and moist air, particularly humid air, provides a favourable environment for microbial growth. As a result, measures such as increasing the dryness of the air should be taken to inhibit microbial growth. All the components of the test formulation are dry (*Yābis*) and some are cold (*bārid*) in temperament, both of which are contrary to microbial growth [25]. While, according to modern medicine, the presence of antibacterial phytochemicals in these plants may explain their efficacy in air disinfection. These plants are rich in a wide variety of bioactive compounds such as terpenoids, alkaloids, flavonoids, tannins and phenolic compounds, which have been found *in-vitro* to have antimicrobial properties [26].

Boswellic acids (BAs), which are pentacyclotriterpenoids, are the bioactive phytoconstituents of *Boswellia* that are considered to be responsible for its antimicrobial properties [27]. Mansumbinone, 3, 4-seco-mansumbinoic acid, β -elemene, and T-cadinol are four antimicrobial terpene compounds isolated from *Commiphora*; among these, 3, 4-seco-mansumbinoic acid has the most potent antimicrobial activity [28]. The presence of alcoholic compound elemol in *Costus* has been attributed to its antimicrobial activities [29]. The active antimicrobial compounds of *Liquidambar* are phenolics and terpenes in nature. The major terpenes identified in its essential oil are terpinen-4-ol, α -terpinol, sabinene and γ -terpinene [30]. Phytochemical studies revealed the presence of several phenylpropanoids and sesquiterpenoids including α - and β - santlalom in the sandal. The antibacterial activity of α - and β - santlalom has been demonstrated in various studies [31]. The presence of alkaloids and saponins may be attributed to the antimicrobial activity of *Aquilaria* [32].

Formalin as a comparative disinfectant was also used by Bisht et al. [20] in their study and reported that its fumigation completely destroys aerogenic microbes within 15 minutes of fumigation. However, the present study found that fumigation with formalin and potassium

permanganate had the greatest effect on aerial microbes after 12 hours of fumigation. Our findings are contrary to the findings of Bisht et al. [20]; variation in findings could be due to the differences in fumigants dose and fumigation method. While this finding is consistent with formalin fumigation standards, which state that the results of formalin fumigation can be better achieved after 12-24 hours of fumigation.

Three types of colonies, yellow, white, and orange were seen in the air samples of the study area. On sequencing, it was found that the bacterial species representing the yellow, white, and orange colonies are *Neomicrococcus lactis*, *Micrococcus lylae*, and *Kocuria rosea* respectively. This finding is in line of previous studies findings. Kookan et al. reported that two thirds of the environmental isolates in studies were micrococci [33]. According to Gorny et al. [34], the majority of bacteria found in indoor air are *Micrococcus*, *Staphylococcus*, and *Pseudomonas* [34]. However, only the *Micrococcus* species of bacteria were isolated in this study. The passive air sampling method and the study settings may be the major factors that can be attributed to this finding. Because only 5% of the total bacterial species present in the air can be cultured using the passive sampling method [35]. In the present study, air samples were collected from areas with very low human occupancy, and the literature indicates that human occupancy is the most important factor influencing the total number and community structure of bioaerosols in the indoor environment [36].

On analyzing the effect of fumigation on these bacterial colonies, it was found that fumigation with test formulation at 45 gms dose had the highest effect. The bacterial species isolated from the air sample belong to the micrococcus genus. These organisms are generally of low virulence and considered to be harmless commensals of skin and oropharynx but may cause opportunistic infections in immunocompromised individuals [37]. Therefore, it is assumed that researchers have rarely used these organisms in antibacterial studies. However, the effects of test formulation drugs on other gram-positive airborne pathogens have been extensively researched. Hence, on that basis, it can be said that the test formulation have acted on these bacteriae in the same way as it does on other gram-positive bacterial population [38,39].

The data of the current study clearly indicated that fumigation of Unani medicinal herbs is more effective than conventional chemical fumigants in improving the microbiological quality of air by reducing the total colony count of microbes (CFU). The findings of this study not only validate the claim of Unani scholars about the efficiency of the test formulation in air purification but also add an Unani formulation to the list of potential herbal air disinfectants that can be used for air disinfection after further researches.

5. CONCLUSION

It can be concluded from the findings of the present study that fumigation with Unani medicinal herbs (Kāfūr, Kundur, Mī'asā'ila, Murr, Quṣṭ, Ṣandal and 'Ūd) is effective in reducing the microbial load of air. The effect of fumigation with these medicinal herbs powder was found to be dose-dependent with the maximum effect occurring at a dose of 45 gms. However the effect of certain factors such as temperature and humidity on disinfection efficiency was not considered. Hence, the authors recommended that this ancient concept should be further evaluated in the light of modern medical science and can be utilized for air disinfection if found suitable.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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