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RESEARCH ARTICLE

Quality analysis, Anti-bacterial activity and Chemical Characterization of Ethnobotanical (Hawan) Medicinal Fumes.

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Abstract. Background: In Vedic literature, the traditional ritual Hawan is said to purify the environment and cure diseases in an eco-friendly way. Previously studies have shown potential of herbal fume for anti-microbial activity as well as for therapeutic purposes along with reduction in the air pollutants post Hawan. However, there was no study which had tested and compared herbal fumes of hawan in control and natural environment for air-quality, anti-bacterial activity and for presence of bio-compounds present in time-dependent manner simultaneously. This work aims to evaluate air quality of hawan and simultaneously evaluate bio-compounds present in it to find its potential for integrative medicine. This work aims to investigate its efficacy in air purification and potential application in modern integrative medicine. Methods: Chambers for performing Hawan under natural and controlled experimental conditions were constructed. Hawan was performed for 30min in a chamber. Chamber air and Hawan herbal fumes were sampled before, during (30 min) and at end (24-72 hours) to find viable bacterial load of air, chemical bio-actives (phyto-constituents), and air quality markers (gases and pollutants) by standard techniques and Gas Chromatography Mass Spectroscopy (GC-MS) analysis along with analysis of pyrolysed Hawan ingredients. OneWay ANOVA with post hoc analysis by Tukey's test was performed for comparison of air quality parameters during different experiments. Results: Transient sharp increase in CO, CO2, NOx levels (albeit within permissible limits) observed during Hawan was followed by decrease below baseline after 24hrs of Hawan in open-door natural conditions. Statistically significant persistent reduction (88-90%) ($p<0.0001$) in the viable bacterial count of air compared to control was observed upto 72 hours after Hawan, when all pathogenic bacteria present in air were eliminated, leaving few *Bacillus spp.* known to be beneficial for human health. GCMS analysis of Hawan medicinal fumes and pyrolysed Hawan ingredients revealed presence of an array of bioactive compounds known to have antimicrobial, anti-oxidant, air cleansing and nutritional activity and health benefits. Conclusion: Thus, through temporal and simultaneous analysis of Hawan air quality, its anti-microbial activity and phytochemical characterization, the study provided evidence-based-support to multifaceted potential of Hawan as described in ancient literature which can be put to immense use for therapeutic utility in modern time.

Keywords. Air quality, Anti-bacterial activity, Chemical Characterization, Hawan, Medicinal fumes

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Introduction

With the emergence of serious problems like multi-drug and pan-drug resistant pathogens due to irrational use of antibiotics and chemicals, hospital acquired infections [1, 2], escalating health care costs, the attendant morbidity and mortality, environmental pollution and toxic accumulations[3], it is evident that alternative approaches towards holistic health extend their scope in modern health care system. Hence, there has been a rekindling of interest in revival of traditional medical knowledge that are effective, and at the same time environment friendly, with long lasting holistic beneficial effects. It is in this perspective, that the ancient technology of Hawan assumes paramount significance. The physical process of Hawan involves oblation and sublimatization of various medicinal-odoriferous-nutritious natural substances in the consecrated fire produced by burning specific medicinal wood in an inverted pyramid shaped structure (altar), along with chanting of hymns. This leads to controlled chemical processing, sublimation, chemical conversion and/ or transformation into vapour phase of the oblated matter, leading to emanation of medicinal phytochemical fumes [4, 5] herein after called as Hawan Medicinal Fumes.

Recent studies have shown therapeutic potential of Hawan Medicinal Fumes in various non-communicable diseases like cancer, diabetes [6, 7], as also in treatment of pulmonary tuberculosis [8, 9], along with encouraging results of reduction in the bacteria and air pollutants post Hawan [10–14]. However, there was no study which had tested hawan thorough controlled (to overcome dilution and dispersion effects) and natural conditions for comprehensive simultaneous time-dependent effect on air quality, antibacterial activity, public health safety hindering its possible applicability in modern medicine. Hence, to address this knowledge gap and to conduct evidence-based-research, this study was undertaken keeping in mind its possible use in modern integrative medicine.

Materials and Methods

Hawan experiments were performed using specially designed experimental chamber setup for three different purposes i.e. to determine its– (i) impact on air quality (gases, air pollutants, and toxic substances), ii) anti-bacterial activity on bacteria in air and iii) characteristic chemical constituents of Hawan Medicinal Fumes as well as raw materials used in Hawan.

Experimental chamber and its design

To perform the experiments under controlled, reproducible conditions, a Hawan chamber of space 38m3 (9.6'X10'X14', with a 6'x 3.5'door and sitting arrangement) for performance of Hawan, with a motorized chimney hood and duct connected to other chamber (Fumes Chamber), of 3.4 m3 (4.8'X5'X5') for collecting fumes was constructed. Both the chambers were of glass and aluminium make. The whole setup was designed within a brick and cement walled room (Experiment Room, ER) of 238m3 (30'X20'X14', with a door and a window) (Figure 1). The chamber was monitored through digital control panel for temperature (T), carbon-monoxide (CO) and relative humidity (RH). Provision of adequate ventilation was given in the Hawan chamber through HEPA filtered sterile air and used when needed. The experiment design is shown in Table-I.

Performance and standardization of Hawan experiments

Hawan ingredients

A mixture of different medicinal and odoriferous plant parts (herbal mixture k/a Hawan samagri, S), as recommended in literature for use in various fumigation procedures) [8, 15–17] was mixed with nutritionally rich substances, viz-honey (Dabur Ltd., India) and clarified cow's butter (ghee) (Amul Pvt. Ltd., India), traditionally used as oblations in Hawan [8, 14, 15]. The composition of this plant mixture was as used in earlier work and reported [9]. Briefly it included - *Acacia Arabica* (Babool) bark; *Achyranthes aspera* L. (Chirchita) seed; *Acorus calamus* L. (Vacha) root; *Albizia lebbeck* (L.) Benth (Sirish) bark; *Allium sativum* L. (Lehsun,

Garlic) tuber; *Areca catechu* .(Supari, Arecanut) fruit; *Argemone Mexicana* (Satyanaashi) all parts; *Azadirachta indica A. Juss.* (Neem) leaves; *Erberis aristata* (Daruhaldi) wood; *Brassiaca campestris L.* (Sarsoan, Yellow mustard) seed; *Butea monosperma Lam.* Taub. (Dhaak) seed; Calotropis procera (Aak) all parts; *Cassia tora L.* (Chakvad) seed; *Centratherum anthelminticum* (L.) *Kuntze* (Kalizeeri, Black cumin) seed; *Cinnamomum camphora* (Kapur, Camphor); *Commiphora mukul* (Guggal) latex; *Crocus sativus* L.(Kesar, Baby saffron) Stigma; *Curcuma longa* L. (Haldi,Turmeric) tube; *Cyperus scariosus* (Nagarmotha) root; *Datura metel* (Dhatur) leaves; *Elletaria cardamomum Maton.* (Elaichi, Green cardamom) seed; *Embelia ribes Burm. f.* (Vayviding) fruit; *Moringa oleifera Lam.* (Sahijan, Drumstick) bark; *Myristica fragrans Houtt.* (Jaifal, Nutmeg) fruit; *Ocimum basilicum* L.(Van Tulsi) leaves and aerial parts; *Ocimum sanctum* L. (Tulsi, Basil) leaves; *Pinus roxburghii Sarg.* (Cheed) wood; *Piper longum* L.(Peepal) fruit; *Plumbago zeylanica* L. (Cheeta) root; *Pongamia pinnata* (L.) *Pierre* (Dithori) seed; *Sisymbrium irio* (Khoobkala) seed; *Solanum surattense* (Kanthkari) all parts; *Styrasx benzoin* Dry (Lobaan) latex; *Surya robus* (Raal) latex; *Swertia chirayta* (Chirayata) all parts; *Tinospora cordifolia Miers* (Giloy) creepers; *Trachyspermum ammi* (Ajwain) seed; *Vitex negundo* L. (Sambalu) leaves and *Zingiber officinale Roscoe* (Soonth, zinger) rhizome. This was indigenously prepared.

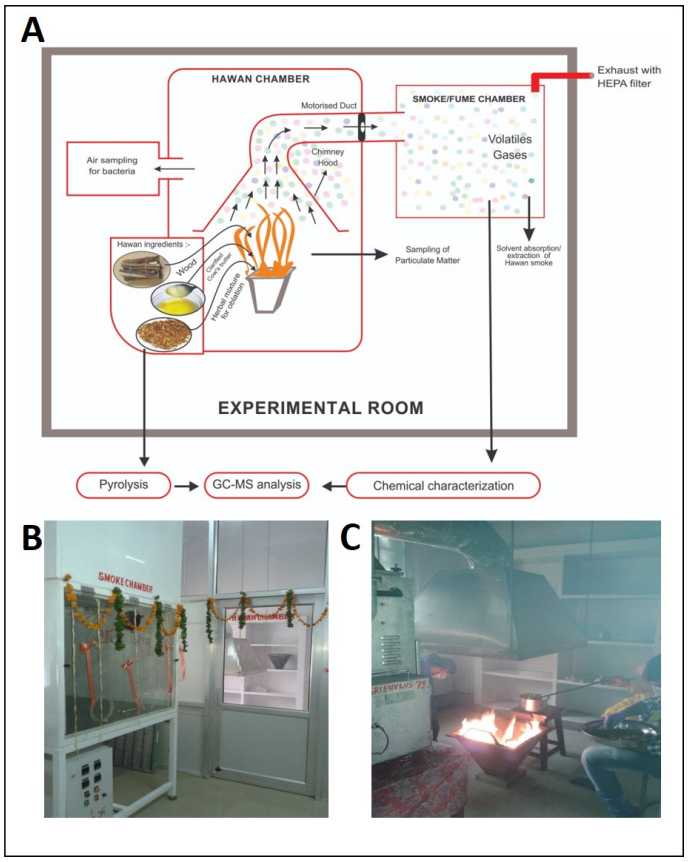

Figure 1: Design and Plan of chambers for experiments A) Schematic diagram of the experimental set up.: Hawan was performed in the Hawan chamber where air quality was monitored, while medicinal fumes collected in Smoke/Fumes chamber was used for chemical characterization; Phographic image of the experimental room set-up, C) Hawan Chamber with chimney hood and duct connected to Fumes Chamber within the Experimental Room; D) Performance of Hawan experiment in the Hawan Chamber.

Experimental Set-up Conditions	Natural Hawan [All experiments]	Control Hawan (Control condition) [Air Quality Study]	Control Hawan (Control condition) [Chemical Characterization]
Motorized Chimney and duct	OFF	OFF	ON
Exhaust	OFF	OFF	ON
Hawan Chamber door	OPEN	OPEN	CLOSED
Experiment room door	OPEN	CLOSED	CLOSED

Table 1: Chamber conditions for different experiments.

Performance of Hawan

Mixture of honey, Hawan Samagri (S) and ghee (G) was used as oblation onto the fire of *Ficus religiosa* (Traditional Indian name - Peepal) wood (W) in all the experiments.

For each Hawan experiment, total of 540 gm of herbal mixture and 540 gm of ghee was taken and around 430-540 gm of dry Wood was used. The ratio of wood:ghee:herbs-mixture in all experiments was 0.8-1:1:1. Herbal mixture (6gm) and ghee (6gm) was oblated every 15-20 seconds with chanting of Gayatri mantra, onto the fire produced by burning wood in the altar (inverted pyramid shaped vessel made of copper). Wood sticks (6-10 inch x 1-2 inch) were fed to fire periodically to keep the altar aflame at high temperatures, to ensure slow and complete combustion. Duration of each experiment was 30 minutes.

The altar was taken out on completion of each experiment and experiment room was subsequently kept closed till final sampling. Burning of wood alone and wood along with ghee were used as control experiments. After standardization of conditions, experiments were repeated in triplicates or more.

Control and Natural Hawan Conditions

In the study, Hawan performed under controlled conditions (in closed Hawan chamber to rule out dispersion and dilution effects) was termed as 'Control Hawan' and hawan performed simulating natural conditions (in partially open chamber, as traditionally done) was termed as 'Natural Hawan'. Their methodological variations with respect to experimental room and Hawan chamber is shown in Table 1.

For all experiments under natural conditions (NH) and for study of air quality and viale bacterial load, motorized chimney was kept switched off (i.e. fumes were not taken through the chimney to fumes chamber). Whereas for experiments to characterize Hawan medicinal fumes, fumes were carried through the motorized chimney and duct to the fumes chamber, where sampling was done.

i) Analysis methods of temporal effect of Hawan on air quality

Hawan fume is a collection of airborne solid and liquid particulates, semi-volatile compounds, gases, water vapour etc., along with some quantity of entrained air. Atmospheric air or hawan fume was sampled before (baseline), during, and at 24, 48 and 72 hrs after hawan to determine impact of Hawan with respect to time. Air quality markers, such as Particulate Matter (PM), gases [Sulfur dioxide (SO2), Nitrogen oxides(NOx), Carbon dioxide(CO2), Carbon monoxide(CO), Formaldehyde], and toxic substances [Pesticides, Polyaromatic Hydrocarbons(PAHs), Polychlorobiphenyls (PCBs), Oxidizing radicals/ions/molecules] were measured in the study. All equipment and methodologies used were as per guidelines of Indian National and international standards [18–21]. Table II showed the sample collection method and analytical techniques used for these parameters.

Particulate Matter analysis

Inside the Hawan chamber, PM was collected using modern Stack Monitoring equipment equipped with Respirable Dust Sampler (RDS) (Envirotech Instruments Pvt Ltd, New Delhi), with flow rate of 1-4m3/minute. It was kept two feet away (maximum proximity to source) from the altar inside the Hawan chamber. Particulate matter $\leq$ 10µ in size (RPM/ PM10) was collected by passing the

air/fumes through EPM2000 glass micro-fibre filters -IS:5182(P-23):2006 [18] and analysed by mass concentration using gravimetric method-IS:11255-part-I [19].

Gaseous analysis

For collection of various volatiles and gases modern Stack Monitoring equipment was used in fumes chamber. The initial flow rate of Low Volume Sampler was set at 40L/min to absorb essential oils which are easily absorbed in methanol. Later flow rate was set at 0.5L/min to enable less easily absorbable volatiles in other solvents/ solutions. Gas samples from fumes chamber was collected in tedlars (that makes any further reaction among gaseous mixture impossible) and bladders.

Parameters	Sample collection	Sample Analysis
Air Quality Index (AQI) for criteria pollutants		
Particulate Matter (PM)	Collection on Glass Micro fibre (GMF) filter.	Mass concentration by Gravimetric method
Sulfur dioxide (SO2)	Absorption of gaseous portion of fumes in 0.1M Trichloromercurate (TCM)	Colour development and measurement by UV-Visible spectrophotometer
Nitrogen oxides(NOx)	Absorption of gaseous portion of fumes in 0.1N NaOH	
Carbon dioxide(CO2)	Absorption of gaseous portion of fumes in 1N NaOH and collection of sample in bladders and teddler bags	Measurement by Titration and Orsat apparatus.
Carbon monoxide(CO)	Collection of sample in bladders and teddler bags.	Measurement by CO Analyser.
Pesticides, PAHs, and PCBs	Absorption of particulate portions of fumes with Dichloromethane as solvent	Analysis of final extract using GC-MS
Formaldehyde	Absorption of gaseous portion of fumes in ice cold water	Analysis of extract by HPLC
Oxidizing radicals/ ions/molecules	Absorption of gaseous portion of fumes in 1% Potassium iodide with starch indicator.	Visual comparison with reference standard
Chemical characterization of Hawan fumes and hawan ingredients		
Semivolatiles and other chemical bioactives	Absorption of gaseous portion of fumes in Hexane and Methanol.	Injection of extracted solution into the GC-MS and by normalization method
Pesticides, PAHs, PCBs, and Heavy metals	Extraction of herbal mixture (hawan samagri) with Dichloromethane as solvent using Soxhlet extractor.	Analysis of final extract using GC-MS
Minerals	Collection of particulate matter on GMF filter and acid digestion of particulate matter.	Analysis of final digested solution by AAS and ICP-OES.

Table 2: Sample collection method and analytical techniques used for various parameter PAHs- Polyaromatic Hydrocarbons, PCBs- Polychlorobiphenyls, AAS- Atomic Absorption Spectroscopy, HPLC- High Pressure Liquid Chromatography, ICP-OES- Inductively Coupled Plasma- Optical Emission Spectroscopy

ii) Chemical characterization methods for Hawan medicinal fumes

For extraction of chemical constituents, air/fumes after passing through Glass Micro fibre (GMF) was purged into methanol and other solvents (Table 2) and stored under refrigerated conditions. Qualitative analysis of solvent (methanol and n-hexane) extracted solutions of Hawan medicinal fumes was done by injecting in Gas Chromatography Mass Spectroscopy (GC-MS) and the library search report was generated using National Institute of Standards and Technology (NIST) library. Compounds that match 80% and above to the library were considered as present in the sample and selected. The percentage of each compound was reported by area normalization. Quantitative analysis of PM from Hawan medicinal fumes was done for determination of pesticides, Polychlorinated Biphenyls (PCBs) and Polynuclear Aromatic Hydrocarbons (PAHs). For this, collected PM was extracted with Di-chloro methane (DCM) and fil-

tered. After evaporating DCM, final volumes were made in hexane and analysed in GC-MS.

GC-MS analysis of pyrolysed Hawan ingredients

Quality evaluation of Hawan ingredients was done for compositional profile, residual pesticides and heavy metals by standard methods as given in Table 2. Pyrolyser analysis of Hawan ingredients (wood, ghee, Hawan samagri) was done individually as well as in combination to know the chemical composition of the fumes generated under strictly controlled conditions, simulating the temperature achieved in the altar and to delineate the contribution of each ingredient. For this, 0.2 mg of each sample was weighed in a pyrofoil and heated upto 590°C. The volatiles generated were injected into the GC-MS and the library search report was generated using NIST library. Compounds that match 80% and above to the library were considered and selected. The percentage of each compound was reported by area normalization.

iii) Air sampling and bacterial identification for determination of viable bacterial load

1m3 of Hawan chamber air was sampled onto nutrient agar (NA) plates using sieve type air sampler (model-LA881 HiMedia, India) before (baseline), during, and at 24, 48 and 72 hrs after experiments for natural hawan (NH), controlled hawan (CH), only wood (W), and wood plus ghee (W+G) experiments to know and compare the temporal effect of various fumes thus generated. Plates were then incubated at 35±2 C for 24hrs, and bacterial colonies grown were counted using digital colony counter. Isolated pure colonies were identified by Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) [22], (Bruker Daltonics GmbH, Bremen, Germany) using Flex control version 3.0. Control panel readings of T, CO and RH were recorded during experiments. Mean average values of all variables for each set of experiment were calculated.

Statistical analysis

Analysis of variances in levels of physical parameters (Temperature, Relative Humidity), gases, particulate matter, percent bacterial count reduction during different set of experiments were done by OneWay ANOVA with post hoc analysis by Tukey's test [23] to compare the means of all treatments to the mean of every other treatment. Differences in means, confidence interval (CI) levels and the adjusted p-values for all possible pairs were determined using R software version 3.6.2.

Results

I. Effect of Hawan on air quality

Level of air quality marker at different time interval in natural hawan conditions

Natural Hawan experiments best simulate the conditions of traditional Hawan. Hence, the results of air quality analysis during and at various intervals after natural Hawan, are summarized in Table 3. During natural Hawan, Total Suspended Particulate Matter (TSPM), Respirable Particulate Matter (RPM - PM10), Carbon monoxide (CO), NOx and CO2 levels were seen to rise sharply (Table 3), albeit well within Occupational safety and Health Association (OSHA) permissible exposure limits (PELs) for indoor air pollution and industrial emissions [24]. CO, and CO2 levels dropped below baseline and NOx became undetectable by 24 hours.

National Ambient Air Quality Standards (NAAQS) (applicable only for ambient air of outdoor spaces) [25], given in Table 3, are only for reference, and not for Hawan performed indoors as in the present study.

Difference in air quality markers among natural and controlled Hawan conditions

Differences in air quality markers during experiments for four groups i.e. natural Hawan (NH), controlled Hawan (CH), only wood (W), and wood plus ghee (W+G) (control conditions) are shown in Table 4. The results indicate statistically significant difference in the levels of Particulates and Gases (except CO2) between the different experiments (Table 4).

Parameter	Permissible Limits		Before, during and after natural hawan				
(Unit)	OSHA	NAAQS	Baseline	During	24h	48h	72h
Temperature range - T (C)	-	-	28-32	31-37	-	-	-
Average temperature Tav (C)	-	-	30	35.53 ± 0.25	30± 0.50	29± 0.25	28± 0.25
Average relative humidity - RHav	-	-	28	25.87 ± 0.23	29 ± 0.25	28 ± 0.28	28 ± 0.28
Particulates							
Respirable Particulate Matter RPM (µg/m3) (10µ)	5000	100	61	3810.33 ± 101.51	307± 5.52	95± 2.56	73± 2.52
Total Suspended Particulate Matter TSPM (µg/m3)	15000	-	83	4086.66 ± 101.04	312± 6.50	98± 3.50	76± 3.52
Gases							
NO× (µg/m3)	1870	80	7.8	71.67 ± 2.52	ND	ND	ND
CO (ppm)	5000	NS	428	2433.00 ± 260.04	341± 1.50	318± 1.00	310± 0.00
CO (ppm)	50	3.5	1.6	3.17 ± 0.06	1.4± 0.00	1.0± 0.00	0.9± 0.00
O (%)	NS	NS	20.9	20.8 ± 0.00	20.9± 0.00	20.9± 0.00	20.9± 0.00
SO (µg/m3)	5	80	ND	ND	ND	ND	ND
Viable bacterial load of air							
Bacterial reduction (%)	NS	NS	-	88.6± 4.52	89± 2.50	93± 2.00	90± 1.50
Chemical bioactives in air							
Volatile aromatic organic compounds	NS	NS	ND	Camphor , azulene	*Camphor, *azulene		
Quinones	NS	NS	ND	2,6-ditert-butyl benzoquinone	Nil		
Phenols	NS	NS	ND	2,6-ditert-butyl, 4-methyl and 2-methoxy (guaiacol)	*2,6-ditert-butyl-phenol		
Polyhydroxyalkanoates (PHAs)	NS	NS	ND	tetra-, penta-, hexa-, hepta-, octa-	Nil		
Methyl esters of fatty acids	NS	NS	ND	oleic, stearic, palmitic	*palmitic and *stearic		

Table 3: Temporal effect of natural Hawan on air quality markers, viable bacterial load of air and release of chemical bioactives. Results of air quality analysis before, during and after natural Hawan experiments showing air quality changes with respect to time. OSHA - Occupational Safety and Health Association; h – hour; NS - Not Specified; ND - Not Detected; These (camphor and azulene) VOCs have therapeutic properties and are used in medicine; *Detected at 24, 48 and 72 hours

During natural Hawan all air quality markers were within permissible limits at all times (Table 3 and 4). However, during wood and ghee (W+G) and control Hawan (CH), PM10 was above OSHA limits, it was observed that CO levels were seen to be comparatively much higher in experiments of only wood (W) and highest in wood plus ghee (W+G) experiments (Table 4). Similarly, NOx levels were highest in W experiments compared to other groups in closed-door conditions. Although CO2 levels were lowest during natural Hawan, levels observed during other experiments were not significantly different (Table 4). Interestingly all air quality markers of open-chamber natural Hawan remained significantly lower and within permissible limits than in closed-chamber control Hawan (Table 4). Also, both CO and NOx became undetectable after 24 hours in open-door natural Hawan conditions (Table 3) indicating public health safety and depolluting activity of Hawan in open-door settings.

To further evaluate differences between groups of different Hawan conditions, OneWay ANOVA with post hoc analysis by Tukey's test to compare means difference of air quality markers of all groups was done and the results are shown in Supplementary Table 1. It shows the significant difference in air quality between Hawan under controlled (CH) and natural (NH) conditions (emboldened), where all types of air quality markers i.e. PM (TSPM, SPM, RPM),

NOx, CO, and room temperature were significantly higher in CH except for O2. Similar results were obtained when natural Hawan was compared with fumes of only wood and wood plus ghee, all markers being much higher in non-NH experiments. This indicated public health safety of natural Hawan (open-door) conditions in contrast to closed-door settings.

Parameter (Unit)	Permissible Limits		Values during different fumes experiments (Mean ± SD)				P-value
	OSHA	NAAQS	Natural hawan (NH)	Wood (W)#	Wood+ Ghee (W+G)#	Control hawan (CH) #	
Average temperature Tav (C)	-	-	35.53 ± 0.25	36.80 ± 0.40	38.67 ± 0.32	40.60 ± 1.06	<0.0001
Average relative humidity - RHav	-	-	25.87 ± 0.23	26.67 ± 0.42	21.80 ± 1.97	27.53 ± 1.17	0.0015
Particulates							
Suspended Particulate Matter (SPM) (µg/m3)	NS	NS	276.33± 12.50	333.33 ± 14.05	366 ± 31.19	453.67 ± 29.26	0.0001
Respirable Particulate Matter RPM (µg/m3) (10µ)	5000	100	3810.33 ± 101.51	4489 ± 79.07	5956 ± 60.89	10657 ± 671.06	<0.0001
Total Suspended Particulate Matter TSPM (µg/ m3)	15000	-	4086.66 ± 101.04	4822.33 ± 92.87	6322 ± 50.86	11110.67 ± 643.90	<0.0001
Gases							
NOx(µg/m3)	1870	80	71.67 ± 2.52	191 ± 3.60	65.67 ± 2.08	103 ± 16	<0.0001
CO (ppm)	5000	NS	3.17 ± 0.06	4.03 ± 0.11	6.17 ± 0.06	4.73 ± 0.40	<0.0001
CO2 (ppm)	50	3.5	2433.00 ± 260.04	2329.67 ± 90.12	2343 ± 43.21	2194.33 ± 352.33	0.6464#
O2 (%)	NS	NS	20.8 ± 0.00	20.7 ± 0.00	20.7 ± 0.00	20.67 ± 0.057	0.0025
SO (µg/m3)	5	80	ND	ND	ND	ND	-
Viable bacterial load of air							
Bacterial Reduction (%) after 72 hrs	-	-	88.63 ± 3.69	34.67 ± 1.23	63.67 ± 2.08	93.33 ± 2.08	<0.0001

Table 4: Levels of air quality markers during four different types of experiments and comparison by OneWay ANOVA - Particulates, Gases and percent reduction in viable count of bacteria in air (BR) during four different groups of experiments i.e. natural Hawan (open-door conditions), wood, wood plus ghee, and control Hawan. #control conditions i.e. closed-chamber experiment; #- Statistically not significant

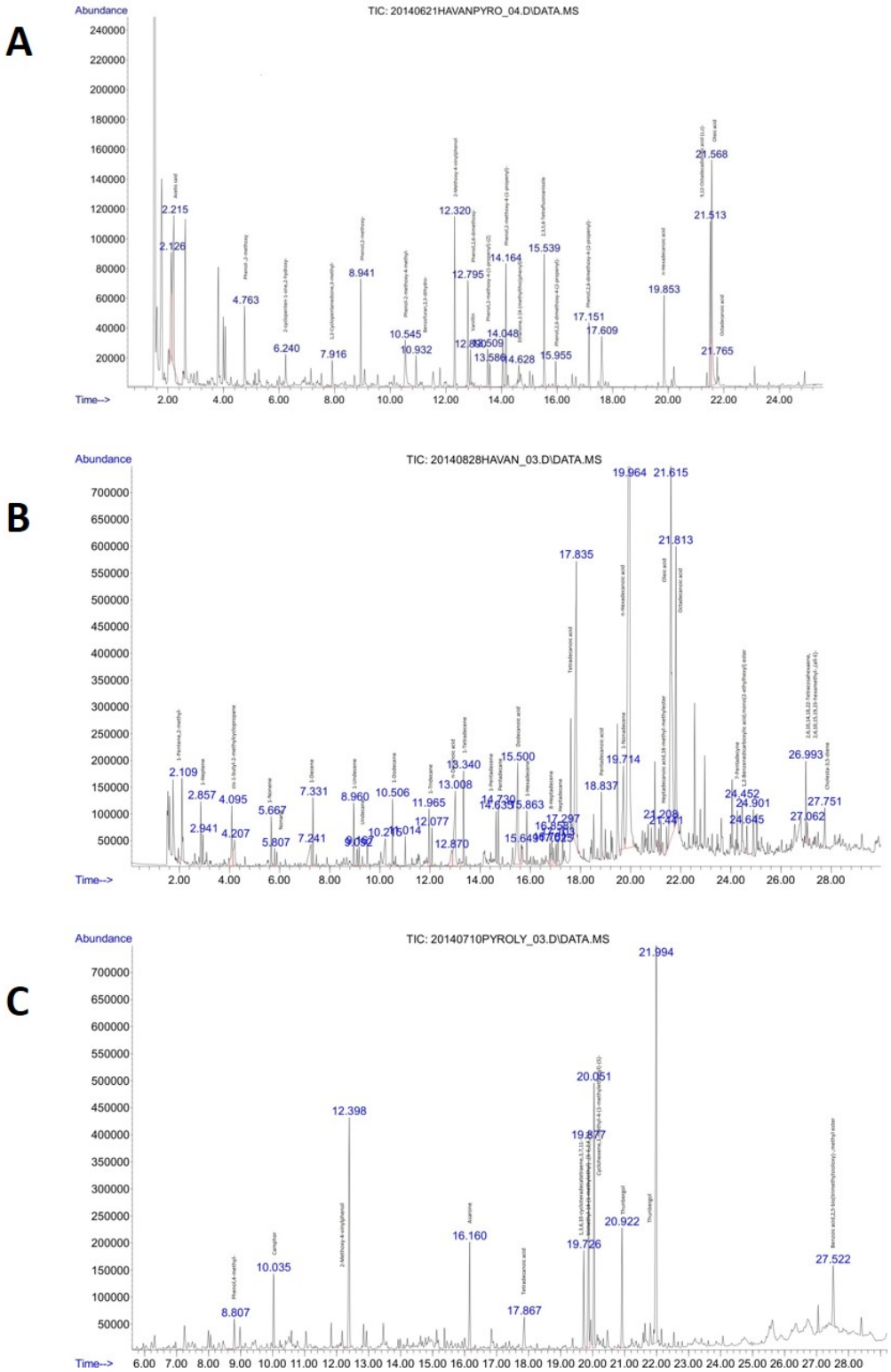

Figure 2A-C: Figure 2A-C showed GC-MS peaks of A) pyrolysed Wood (W), B) pyrolysed Ghee(G), and C) pyrolysed Samagri (S); while Figure2D showed GC-MS peaks of pyrolysed W+G+S.

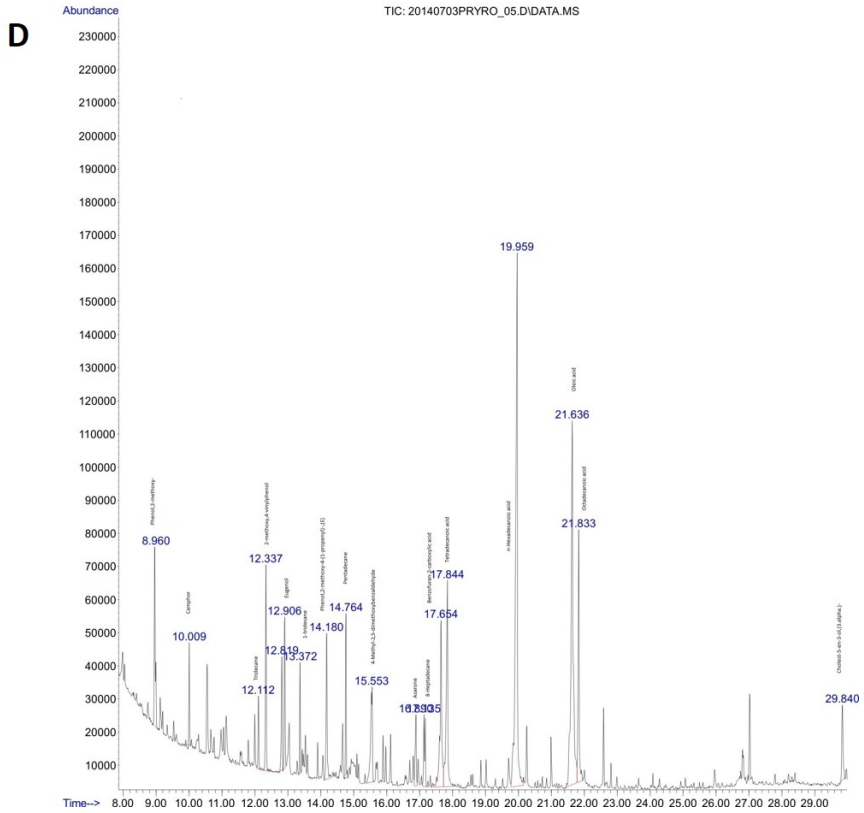

Figure 2D: Figure 2A-C showed GC-MS peaks of A) pyrolysed Wood (W), B) pyrolysed Ghee(G), and C) pyrolysed Samagri (S); while Figure2D showed GC-MS peaks of pyrolysed W+G+S.

II. Chemical Characterization of Hawan Fumes and Ingredients

Identification of chemical bioactives in pyrolysed Hawan ingredients

Besides, the presence of an array of bioactive compounds, quality evaluation of Hawan ingredients revealed absence of residual pesticides and heavy metals. GCMS chromatograms showing compound peaks identified in pyrolysis fumes of W, G, S and W+G+S are shown in Figure 2A-D respectively.

Table 5 shows chemical compounds identified by GC-MS in the pyrolysis fumes of different Hawan ingredients when analysed alone and in combination. Interestingly, fumes generated from pyrolysis of all Hawan ingredients (Wood + Ghee + Herbal mixture) yielded an array of medicinal compounds (n=20) (Figure 2D), with predominant (>60%) presence of (in decreasing order of concentration) n-hexadecanoic acid, oleic acid, tetradecanoic acid and octadecanoic acid (as also identified in methanolic extract of Hawan fumes). As shown in Figure 2C, herbal mixture (S) yielded unique compounds (n=9) namely, (in decreasing order of concentration) thunbergol, cembrene, D-limonene vinylguaiacol, benzoic acid,2,5-bis (trimethylsiloxy)-,methyl ester (also k/a gentisic acid(tms), borneol (naturally occurring form of camphor), asarone, tetradecanoic acid, eugenol, etc., which are known to have high therapeutic and medicinal value. Among these, thunbergol, cembrene, D-limonene and benzoic acid,2,5-bis (trimethylsiloxy)-,methyl ester were uniquely found only in pyrolysis fumes of herbal mixture(S) and absent from others. However, pyrolysis of Peepal wood yielded variety of phenols and other compounds of medicinal value (Figure 2A) along with an unique compound 2,3,5,6-Tetrafluoroanisole, known to have meropenem (potent broad spectrum antibiotic) and methimazole (used to treat hyperthyroidism) like ac-

Area % w.r.t (a/d)	Name of major compounds (source)	Known biological/ pharmacological activity (reference no.)
37.33	Thunbergol(d)	Anti-(oxidant, tubercular, rheumatic, scabies, inflammatory), analgesic [9, 30, 31, 33, 40, 41]
20% each	**n- Hexadecanoic (Palmitic) acid (a,b,c,e)**	Anti-oxidant [40, 41, 44], biopesticide [31, 33, 36], nematicide [33, 48], hypocholesterolemic [31, 33], lubricant [31]
	Oleic acid(a,b,c,e)	Anti- (inflammatory, androgenic, cancer), hypocholesterolemic [31, 42, 44–46]
14.28	Cembrene(d)	Anti-(bacterial, TB, Viral, fungal, cancer, inflammatory, oxidant) [30, 33, 41, 44, 46]
12.71	D-Limonene(d)	Has novel therapeutic actions in some CNS neoplasms especially gliomas, anti-angiogenic, biopesticide [41, 45, 46, 49]
9.8	Benzoic acid, 2,5-bis (trimethyl siloxy)-, methyl esterd,	Potent antifungal, used by African Xhose traditional healers for T/t of HIV, anti- (oxidant, microbial) [31, 33, 40]
6-8% each	Tetradecanoic (Myristic) acid (a,b,d)	Anti-(bacterial, tubercular, cancer) [9, 33, 42, 44]
	Benzofuran-2-carboxylic (coumarilic) acid (a,c)	Anti-cancer [33,41], Anti-(microbial, oxidant, inflammatory) [33, 44, 51]
	Octadecanoic (Stearic) acid(a,b,c,e)	Potent anti-fungal [9, 31, 33, 40**?** , 41]
	Octadecadienoic acid and esters (c*,e), PHAs	Biodegradable polymers, biomaterials for biomedical applications [50]
4-6% each	**Phenol, 2- methoxy(c,e)**, **Phenol,4- Methyl (d,e)**	**Anti-(microbial, oxidant)** [31, 40]
	4- methyl-2,5- dimethoxy-benzaldehyde(a,c,e)	Anti-tubercular [33, 40]
	2- Methoxy-4-vinylphenol (a,c,d,e)	Anti- (mutagenic, fungal, listerial) [33, 38, 41]
	Phenol, 2- methoxy-4-(1- Propenyl)-(E)- (trans isoeugenol)(a,c,e), **Borneol (a,d,e)**	Anti-inflammatory [41, 45], analgesic [31, 40, 41], structural analogues of natural anti-tumour agents and are 5-HT antagonists [40, 41, 46]
	Azulene(e)	Anti- (microbial, pruritic), anaesthetic, mucolytic [31, 40, 41]
	2,6-ditert butyl benzoquinone(c,e)	Anti-(inflammatory, microbial, ulcer, allergy, neoplastic) [41, 43], Anti-(microbial, inflammatory, oxidant), polymerization catalyst [53]
2-3% each	Phenol, 2,6 dimethoxy (a,c)	Anti-oxidant [31, 40]
	Eugenol(a,c,d)	Anti-(oxidant, microbial, tumour), biopesticide [31, 40, 41]
	Camphor(a,d,e)	Anti-(microbial, oxidant), expectorant [30, 31]
	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one(d)	Strong anti-oxidant and plant growth promoter [17, 28]
	Pentadecane(a,b,e)	Acaricide [40, 48]
	8- Heptadecene(a,b,e)	Anti-(microbial, oxidant), biopolymers [33, 40, 51]
	Heptadecane (a,b,e)	
	Decanoic (capric) (b,e)	Potent antimicrobial, reduces total HDL cholesterol ratio [40, 42]
	Dodecanoic acid (Lauric acid) (b,e)	Anti-(microbial, oxidant), promote healthy gut microbiome [31, 42]
	Cholest-5-en-3-ol, $(3\alpha/\beta)(a,b)$	
1 to $\leq$ 2%	Tridecane, 1- Tridecene (a,b), Vanillin (a,c)	Anti-(oxidant, microbial) [33, 41]
	Asarone (a,d)	Anti-(oxidant, helminthic), memory enhancer (β)[44, 46, 49]
	*2,3,5,6-Tetrafluoroanisole (c)	Anti-(oxidant, bacterial) (related to meropenem) [50]
10%	Unknown compounds (a,d,e)	

Table 5: GC-MS results of pyrolysed hawan ingredients and known pharmacobiological properties of identified compounds . a, compound identified on pyrolysis of W+G+S; b, compound identified on pyrolysis of ghee (G); c, compound identified on pyrolysis of wood (W); d, compound identified on pyrolysis of herbal mixture (S); e, compound identified on GC-MS of hawan smoke extract (emboldened to highlight significance); *Compound identified uniquely on pyrolysis of wood

tivity. Pyrolysis of Ghee (Figure 2B) yielded maximum compound peaks (n=46) consisting of predominantly (>63%) n-hexadecanoic acid, oleic acid, tetradecanoic acid and octadecanoic acid along with polyhydroxyalkanes (biodegradable biomaterials known to have varied biomedical use) and esters known to have medicinal value. 10.6% of compounds in pyrolysis fumes of herbal mixture and combination fumes could not be identified based on NIST library search results.

Identification of chemical bioactives in Hawan fumes

It is interesting to note that GC-MS analysis of Hawan fumes extract collected during Hawan showed presence of camphor, azulene, 2,6-ditert-butyl benzoquinone, phenols (2,6-ditert-butyl, 4-methyl and 2-methoxy); Polyhydroxyalkanes

(PHAs) and methyl esters (stearic, palmitic, oleic acids) (Table 3, 5), whereas, air collected after 24-72 hours of Hawan showed absence of PHAs, with presence of only camphor, azulene, 2,6-ditert-butyl-phenol, palmitic acid and stearic acid esters at 24, 48, and 72 hours. This indicates harmless combustion during Hawan and dynamics of photo-chemical oxidation-reduction reactions after Hawan (Table 3).

III. Effect of Hawan on viable bacteria in air

To examine effect of Hawan on viable bacteria in air, air sampling and bacterial identification was performed for four groups i.e. natural Hawan (NH), controlled Hawan (CH), only wood (W), and wood plus ghee (W+G) (control conditions). Percent reduction in viable bacterial count of air following exposure to natural Hawan at various time intervals is shown in Table 3, and that after 72 hours of exposure to the four different types of experiment is shown in Table 4. Graphical representation of reduction in viable bacterial count of air with respect to time following exposure to experiments of all four groups is shown in Figure 3.

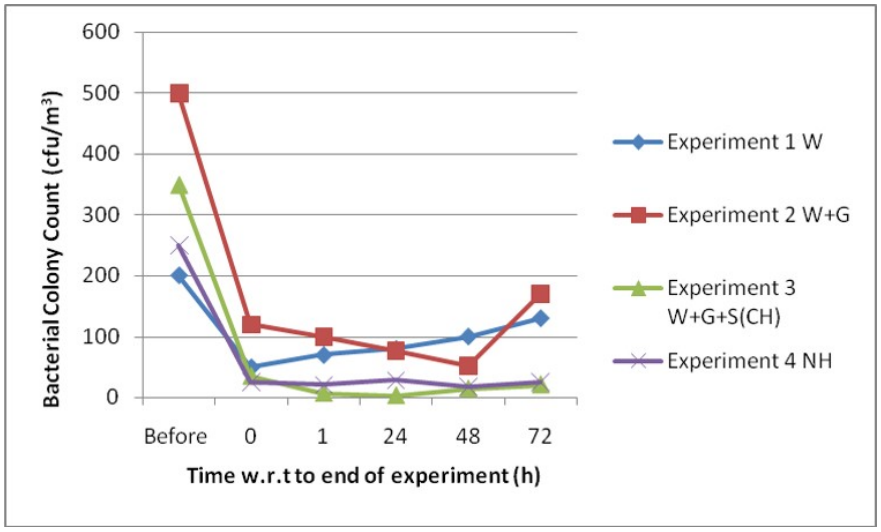

Figure 3: Graph showing temporal reduction in viable bacterial count at various time intervals after exposure to four different experiment groups. Wood (W), wood plus ghee (W+G), controlled Hawan (CH), and natural Hawan (NH) fume; cfu - colony forming unit. Before = One hour before end of experiment, i.e just before start of experiment.

At the end of natural Hawan, 88.6% reduction in viable bacterial count was observed and remained reduced by 89%, 93%, 90% at 24, 48, 72 hours respectively compared to the count before start of the experiment (Table 3 and Figure 3). Highly significant reduction in the mean value of viable bacterial count was observed during the different experiments, with the highest level of 89-93% reduction during NH and CH experiments (Table 4). Mean percentage reduction (Mean ± SD) in the bacterial growth following exposure to both Natural (88.63 ± 3.69) and Controlled Hawan (93.33 ± 2.08) were significantly higher ($p<0.0001$) than reduction due to fumes generated through wood only (W) (34.67 ± 1.23) or wood plus ghee (W+G) (63.67 ± 2.08) (Supplemental Table 1).

Identification of bacteria isolated from air before and after exposure to natural Hawan

MALDI-TOF MS identification of bacteria isolated from air of Hawan chamber before and after 72 hour of natural Hawan along with their counts are shown in Table 6. It is interesting to note that all types of bacterial species present before Hawan were absent at 72 hours post Hawan

except *Bacillus pumilus*, *Bacillus subtilis*, and *Bacillus mojavensis*.

Discussion

The fact that the quality of air we breathe should be of highest standard, free from all sorts of toxins, including microbes, was known to the ancient sages of India. They designed and propagated Hawan (also known as Havan, Yajya, Yagya, Yajna, Agnihotra, Homam) for prevention and cure of diseases by both purification of the environment and through bioactive compounds [4, 5]. Experimental studies show that the incidence of physical ailments, sickness and disease are reduced in the houses, where the Hawan is regularly performed because it creates a pure, nutritional and medicinal atmosphere [4, 5]. "Heal the atmosphere and the healed atmosphere will heal you"- was the Modus Operandi [26].

Bacterial Species	Before Hawan	#CFUs (%)	After Hawan (72h)	CFUs (%)
Gram Positive Cocci (25-30%)				
Staphylococcus aureus	+	5-6	-	-
Staphylococcus auricularis	+	03	-	-
Staphylococcus haemolyticus	+	03	-	-
Staphylococcus lentus	+	01	-	-
Staphylococcus epidermidis	+	5-6	-	-
Staphylococcus hominis	+	3-5	-	-
Staphylococcus xylosus	+	01	-	-
Stomatococcus mucilaginosis	+	01	-	-
Micrococcus luteus	+	5-6	-	-
Gram Positive Bacilli (50-55%)				
Corynebacterium urealyticum	+	3-5	-	-
Bacillus cereus	+	8-10	-	-
Bacillus megaterium	+	5-8	-	-
Lysinibacillus sphaereus	+	02	-	-
Bacillus licheniformis	+	5	-	-
Bacillus pumilus	+	5	+	32-36%
Bacillus subtilis	+	8-10	+	45-50%
Bacillus mojavensis	+	2-3	+	12-14%
Agromyces salentinus	+	2	-	-
Bacillus atrophaeus	+	3-5	-	-
Gram Negative Bacilli (15-20%)				
Aeromonas salmonicida	+	5-6	-	-
Pseudomonas stutzeri	+	2-4	-	-
Sphingomonas paucimobilis	+	3	-	-
Acinetobacter haemolyticus	+	3	-	-
Enterobacter aerogenes	+	2	-	-
Brevundimonas diminuta	+	2-3	-	-
Gram Negative Cocci		3-5%		-
Neisseria animolaris	+	2	-	-
Sthmon paucimobilis	+	1-2	-	-
Moraxella grp.	+	1-2	-	-

Table 6: MALDI-TOF MS identification of bacteria isolated from Hawan chamber air before and after 72 hours of natural Hawan; #CFU- Colony Forming Unit, as percent fraction of total colony forming units grown

Inhalation and absorption through skin of bioactives released in Hawan are the primary modes of entry into the human body [27]. In the current scenario, there is paucity of development of newer potent antimicrobial agents and treatment methodologies to combat the escalating burden of drug resistant microbial infections. Also, emerging and re-emerging diseases, malfunctioning of body organs due to increasing severity of pollution, and increasing burden of

psychiatric ailments highlight the need of novel approaches. Pulmonary drug administration is being projected by researchers as the prime mode of drug delivery in the future [28]. Hence, it seems prudent to rediscover this ancient vedic science where pulmonary inhalation of phytochemical fumes is traditionally used for health purposes. To re-evaluate the ancient method of Hawan, in terms of physico-chemical and biological parameters, the current study was done using internationally accepted standard methods. Scientific data was obtained on chemical composition of Hawan medicinal fumes and its air purifying, antibacterial effects, to explore its possible use in modern integrated medicine.

In this study, a chamber for undertaking antibacterial and chemical studies on Hawan under controlled conditions was designed (Figure 1) for scientific investigation of the traditional use of Hawan for indoor air purification. Apart from significant reduction in viable bacterial count of air persisting upto 72 hours (Figure 3), beneficial changes in air quality (in terms of post Hawan reduction in levels of NOx, CO, CO2, and persistence of beneficial volatiles in air upto 72 hours) (Table 3-5), and characterization of phyotoconstituents (bioactive compounds) in the Hawan medicinal fumes along with raw materials used in Hawan (Table 5), using modern scientific methodology is reported comprehensively for the first time in literature to the best of our knowledge.

Levels of TSPM, PM10, NOx, CO and CO2 were found to increase sharply during natural Hawan (Table 3), albeit well within OSHA PELs [24] for indoor and occupational exposure (Table 3). Also, analysis of the PM of Hawan medicinal fumes showed absence of toxic chemicals and presence of an array of beneficial bioactives (Table 5), clearly supporting the safety of natural Hawan for therapeutic use.

Exogenously administered nitrous oxide (NO) has been reported to be useful in the treatment of Adult Respiratory Distress Syndrome (ARDS) and especially in persistent pulmonary hypertension of the newborn [29]. NO is also known to be involved in host defense mechanisms, inhibit platelet aggregation and function as a neurotransmitter [29]. Although, what proportion of the NOx gases released was NO could not be determined in this study, the personnel comfort throughout the NH experiments suggests its major presence.

Slight increase in levels of CO2 is reported to increase cerebral blood flow and enhance inhalation of aroma chemicals aiding in treatment of mental disorders, apart from strengthening CO2 cycle [27, 29]. Thus short exposure to amounts of CO2 formed during Hawan may enhance inhalation and absorption of volatile bioactives like camphor (known to have soothing, calming, mucolytic, anti-inflammatory effects,etc [29].) identified in Hawan fumes (Table 3,5), which is used in medicine for its calming influence in hysteria, nervousness, neuralgia and also in infectious fevers like pneumonia [30, 31].

Small amounts of CO is also known to have endogenous anti-inflammatory, anti-apoptotic and anti-proliferative effects, reported to have therapeutic role in treating asthma, endotoxemic shock, etc [32]. Although identification of the exact role of gases released during Hawan was beyond the scope of this study, it can be inferred from the above discussion that contradictory to general belief, the amount of NOx, CO, CO2 released (all within OSHA PELs) in combination with volatile bioactives released during Hawan appear to be beneficial for public health and promises to have therapeutic value for human. The calming and soothing effects experienced post Hawan could be attributed to this.

Sharp reduction below baseline levels of CO, CO2, NOx and viable bacteria in the room air following Hawan, persisting for 72hours post Hawan (Table 3, Figure 3), also indicates its depolluting potential. Other researchers have also reported similar persistent decrease in PM and CO2 levels following indoor Hawan [10, 11]. This probably involves complex interactions between the volatile organic compounds (VOCs), gases and bacteria present in the air during and post Hawan. Sharma PK et al. also reported significant and persistent reduction in criteria pollutants following Hawan, after generating artificial pollution indoors [12]. Depolluting effects of Hawan when performed indoors has also been

reported by Saxena M et al [13]. The molecular basis for these observations has been clearly identified in this current work.

On performing Hawan in closed room (CH), TSPM, PM10, NOx, CO, CO2 levels and T were seen to be much higher (statistically significant), with PM levels beyond OSHA PELs, apparently due to concentration of fumes. This also explained the greater (though statistically non-significant) bactericidal effect in CH compared to NH (Supplementary Table 1). Also, O2 level was found to be significantly lower during CH, compared to NH. Hence, it is advisable that Hawan should always be performed in a well ventilated space when involving human subjects. On the contrary, exposure to Hawan medicinal fumes in a closed setting for specific purpose can be used to kill bacteria/ decontaminate spaces and inanimate objects, as also reported by other researchers [12, 14, 33, 34]. It will be appropriate to state that more experiments with panel of airborne pathogens will be desirable to know the spectrum of activity of Hawan which otherwise appears to be quite broad in this study.

Increase in ambient temperature due to release of heat during Hawan might seem to enhance the bactericidal effect, predictably by increasing penetration, solubilization and dispersion of released chemical actives. However, since significant reduction in bacterial count was observed even during natural Hawan, where the ambient temperature of the room did not exceed 37 C (Table 3) and post Hawan upto 72 hrs (room temperature 30ºC), the bactericidal effect can be primarily attributed to the chemical bioactives identified in the Hawan medicinal fumes (Table 3,5). This can be explained by persistence of antibacterial compounds like camphor, azulene, 2,4-ditert-butyl-phenol, palmitic and stearic acid esters in chamber air for atleast 72hrs following Hawan, probably as suspended colloids. Further supporting this inference, is the fact that natural Hawan showed statistically significant higher bactericidal effect (NH- 89%-93%) with persistence upto 72 hrs, than only wood (W) (35%) or wood plus ghee (W+G) (64%), where temperatures were significantly higher. W and W+G experiments also resulted in much higher levels of gases especially NOx (maximum in W), CO (maximum in W+G) over and above OSHA permissible limits, highlighting the prudence and benefit of using wood, ghee and herbal mixtures in combination in Hawan, .

Significant and persistent reduction in bacterial load of air upto 30days after Hawan fumigation in a closed room was reported by Nautiyal et al [14], although corroboration of the effect with bioactives in Hawan medicinal fumes was not done. In contrast to our findings, they did not observe any antibacterial effect due to burning medicinal wood (however, they used mango wood) alone. Sharma PK et al reported increase in bacterial load (>100%) following exposure to fumes of plain wood, coal, tyres and polybags, etc (non- Hawan experiment) [12]. Noxious levels of CO, CO2 , NOx as well as SO2 were released in these non- Hawan experiments which persisted in the room for days after the experiment. After thus creating artificial pollution, they demonstrated 79% reduction in pathogenic bacteria in air by treatment with Hawan, with effects lasting upto 48hrs. Hence it is clear that all types of combustion do not bring about persistent reduction in pathogenic bacteria as does Hawan. The fact that medicinal nature of the ingredients used and the controlled chemical processing in fire in Hawan leads to release of medicinal phytochemicals that bring about depollution of air was thus proved in the current study.

Interestingly all potentially pathogenic bacteria present in chamber air including *Corynebacterium* sp. (organisms with more complex cellular structure resembling mycobacteria) [35] were killed following Hawan, leaving behind only few *Bacillus* spp. known to be generally non-pathogenic to human and beneficial especially to soil and plants [36–38]. This effect can be attributed to the release of bioactives of high medicinal value like camphor, thunbergol, cembrene tetradecanoic acid, vanillin derivatives, etc. (Table 5) with known broad spectrum antibacterial and potent antitubercular action [31]. This is of significance, as it elucidates the molecular basis of efficacy of Hawan medicinal fumes in the inhalational therapy of pulmonary tuberculosis as reported [8, 33] in literature. Sig-

nificant in-vitro tuberculocidal activity of this herbal mixture extract when tested against clinical *Mycobacterium tuberculosis* (Mtb) strains has also been reported [9] corroborating with the observations. This finding is thus of importance in the preparation of novel antitubercular compounds from Hawan ingredients and development of inhalational therapy. Also, since 10% of compounds present in hawan medicinal fumes, fumes of herbal mixture alone and that combined with wood and ghee, could not be identified based on NIST library search, discovery of new chemical entities (NCEs) with broad spectrum bactericidal effect seems a real possibility that needs to be further investigated in well designed experiments.

B.subtilis and *B.pumilus* (known to possess high resistance to environmental stresses) were found to persist in air 72hrs post Hawan (Table 6). These are plant growth-promoting rhizobacteria (PGPR) known to have fungicidal, bactericidal and plant growth promoting properties, being employed in rhizoremediation [36]. *B.pumilus* is reported to have potential for use as an alternative antibacterial agent for treatment of infection with Methicillin Resistant Staphylococcus aureus (MRSA) and Vancomycin Resistant Enterococci (VRE) [37]. *B.subtilis* and *B.mojavensis* are endophytes known to promote healthy immune system and have strong antifungal activity [38] as well. Processing of specific plant ingredients through fire turning them to nano-particles and acting to alter biome was recently reported by Chun S et al [39]. The same mechanism occurring in Hawan may explain the beneficial biome alterations observed. These findings highlight and support the multifaceted beneficial role of Hawan in contrast to any currently available disinfection/ or fumigation methods, opening up further avenues for research, discovery and development of novel disinfectants/fumigants/ therapies.

GC-MS analysis of Hawan fumes and pyrolysis fumes of Hawan ingredients revealed presence of an array of bioactive compounds mainly n-Hexadecanoic acid, oleic acid, tetradecanoic acid, octadecanoic acid, 4-Methyl-2,5-dimethoxybenzaldehyde, 2-Methoxy-4-vinylphenol, coumarilic acid, eugenol, camphor, vanillin, asarone, myristic acid, thunbergol, cembrene, D-limonene, siloxanes, vinylguaicol, asarone, etc (Table 5). These are known to have potent antimicrobial [9, 39–43, 45], anti-oxidant [41, 43, 44], anti-inflammatory [41, 43–46], analgesic [41, 43, 44], hypocholesterolemic [41, 45], anti-hypertensive [47], anti-cancer [39, 43–45], biopesticide [48], nematicidal [48] effects of various intensities and are generally known to be safe for human use [41]. The pharmacobiological effects and therapeutic uses of these bioactives as summarised in Table 5 highlight the multifaceted beneficial role of Hawan. The pleasant odour enriched environment experienced following Hawan may promote neurogenesis as observed in rats [47], as well as exert beneficial CNS and ANS effects [49]. Pyrolysis of *Ficus religiosa* (Peepal) wood yielded variety of phenols (known to have antioxidant, antiseptic and disinfectant properties), polyunsaturated fatty acids (known to be beneficial for health and act as drug carriers). 2,3,5,6-Tetrafluoroanisole, an antioxidant- antibacterial, related to meropenem (potent broad spectrum antibiotic) and methimazole (used to treat hyperthyroidism) like activity [50], was uniquely identified in wood pyrolysis fumes, suggesting the medicinal value of burning Ficus religiosa wood.

Oleic, stearic, palmitic acids and their methyl esters identified in Hawan medicinal fumes are medium and small chain fatty acids known have antimicrobial, anti-oxidant, anti-inflammatory activity and promote healthy gut microbiome and metabolism bestowing multiple health benefits. They aid in penetration of drugs through skin and mucous membrane and are indispensable ingredients of many pharmaceutical products [42]. Camphor, the principal bioactive identified in Hawan medicinal fumes and also from pyrolysis fumes of W+G+S is known for its potent antimicrobial, antiseptic, antipruritic, analgesic (topical), anti-inflammatory, expectorant, cough suppressant and anticancer effects [22, 23]. Azulene, identified in Hawan medicinal fumes has been widely used for hundreds of years in antiallergic, antibacterial, and anti-inflammatory therapies. Also, due to physicochemical prop-

erties, azulene and its derivatives have found many potential applications in technology, especially in optoelectronic devices and have potential for use in various areas of medicine, including anti-inflammatory with peptic ulcers, antineoplastic with leukemia, antidiabetes, antiretroviral with HIV-1, antimicrobial, including antimicrobial photodynamic therapy, and antifungal [43].Polyhydroxyalkanoates (PHAs) identified in Hawan medicinal fumes during Hawan are biodegradable polymers considered potential biomaterials for future biomedical applications including drug delivery systems [51]. It is interesting that these are known to be synthesized naturally by bacteria under unfavourable growth conditions like physical or nutritional stress. Such stressful conditions were indeed evidenced during Hawan, explaining the synthesis of these PHAs by the stressed bacteria in air. This also corroborates with the significant reduction in viable bacterial load of air following Hawan, indicating their conversion to PHAs. 2,4-bis (tert-butyl)-phenol identified in the Hawan fumes and Hawan ingredients was also reported by Nair RR (2016) [52]in the alcoholic extract of Hawan samagri. This compound is known to have antioxidant and anti-inflammatory effects and has effective action against an agriculturally important fungus, *Fusarium oxysporum*[53]. However in excess concentration it can cause skin and mucosal irritation. 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one identified in W+G+S pyrolysis fumes is a potent antioxidant and plant growth promoter [17, 28, 34].

Since a plethora of bioactive compounds known to have multi-dimensional health benefits were identified, the utility of Hawan may well extend beyond mere air purifying effect, as investigated in this study. The origin of these compounds was traced to all the Hawan ingredients, with herbal mixtures contributing to bioactives of very high therapeutic value like thunbergol, cembrene, and limonene; clarified cow's butter (Ghee) contributing to most of the beneficial fatty acids and esters and *Ficus religiosa* (Peepal) wood contributing compounds of high medicinal value, especially compounds with antimicrobial and disinfecting properties (eg-guaiacol, 4-methoxy phenol,etc.), albeit in smaller proportions. Contrary to general belief, all VOCs identified in Hawan fumes like camphor [29–31] and azulene [43] are of high therapeutic value known to be beneficial to health; whereas benzene, toluene, ethylbenzene and xylenes (o-, mand p-) (BTEX), considered indicators of toxic VOC exposure [54] were all absent in air during and after Hawan proving its public health safety. Also contrary to general belief, pollutants of major public health concern including particulate matter, carbon monoxide, nitrogen dioxide were all found to be well within PELs during natural Hawan and were significantly reduced at 72hrs post Hawan, proving again public health safety of Hawan for its therapeutic uses. However, a word of caution is needed regarding ensuring thorough ventilation and choice of pure herbal ingredients during Hawan to ensure air quality index and public health safety. Further toxicity studies are warranted to know therapeutic dosage limits for therapeutic purposes.

In India, Hawan inhalational therapy is reported to be used since ancient times to cure various diseases especially tuberculosis, cardiac ailments, skin, psychiatric and neurological diseases [8, 15, 27, 33, 34, 40]. Our study has elucidated much of the chemical basis for all these observations. Hawan can be further explored and adapted for prevention as well as therapy of various ailments and hospital acquired infections. Research on the sonic signals encoded in the hymns though not investigated in this study, may further reveal effects unknown till date. This suggests that, several compounds and varied processes occurring in Hawan, may be working together to bring about the desired effect, which is a subject of further research.

The present study supports Hawan as a technique to reduce airborne viable bacterial load, reduce pollutants post Hawan and increase the nutrient-medicinal value of air in an eco-friendly way, with release of a unique combination of bioactive compounds known to have multi-dimensional health benefits.

Compliance with ethical standards: Not required.
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