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Phytochemical Profiling and Antibacterial Evaluation of *Havan* Fume Extract Using GC-MS and *In Silico* Docking

Vikash Rana[1], Manish Dev Sharma[2], Indra Rautela[3], Naina Srivastava[4], Ritu Rani[4], Vijaylaxmi[5], Himani Badoni[6], Shweta Sahni[7] and Atal Bihari Bajpai[5]*

[1]Department of Botany and Microbiology, Gurukula Kangri (Deemed to be University), Haridwar - 249404, Uttarakhand, India

[2]Department of Biotechnology, School of Basic and Applied Sciences (SBAS), Shri Guru Ram Rai University, Patel Nagar, Dehradun - 248001, Uttarakhand, India

[3]Department of Biotechnology, School of Applied and Life Sciences (SALS), Uttaranchal University, Dehradun - 248001, Uttarakhand, India

[4]Department of Botany, D.A.V (P.G.) College, Dehradun - 248001, Uttarakhand, India

[5]Department of Botany, D.B.S. (P.G.) College, Dehradun - 248001, Uttarakhand, India; dratalbajpai@gmail.com

[6]Department of Botany, Mahayogi Gurugorakhnath Government Degree College, Bithyani, Yamkeshwar, Pauri Garhwal - 246001, Uttarakhand, India

[7]Department of Microbiology, School of Basic and Applied Sciences (SBAS), Shri Guru Ram Rai University, Patel Nagar, Dehradun - 248001, Uttarakhand, India

Abstract

Background: *Havan* has been a common thing to do in India for a long time. *Havan samagri* is made with mango *samidha* and other important medicinal herbs that are used in *havan*. It cleans the air by cutting down on the number of germs in the air. **Aim:** This study examined the utilisation of 31 botanical components, including *guggal*, *giloya*, *amla*, *bahera*, *til*, *bach*, *nagkesar*, and *harad*, in the formulation of *havan samagri* and its potential efficacy against airborne microorganisms. **Methods:** Four air-isolated bacteria, *Priestia endophytica*, *Bacillus subtilis*, *Deinococcus ficus*, and *Staphylococcus caprae,* were evaluated against the ethanolic and aqueous fume extracts of *havan samagri* and GC-MS analysis was done of ethanolic extract of *havan* fume and molecular docking was examined. **Results:** The ethanolic and aqueous fume extracts of *havan samagri* were the most effective against *Deinococcus ficus* and *Staphylococcus caprae*, respectively. After 5 h, the CFU count had dropped by 62.50% and 59.29%, respectively, on the plates that had been exposed to *havan* and those that had not, inside and outside of *Yagyashala*. The GC-MS test of the ethanolic extract showed 20 chemical constituent peaks. Acetic acid (39.63%) was the highest, followed by asarone (15.29%). The molecular docking of the asarone compound revealed its greatest efficacy against the bacterial isolates *Deinococcus* sp. and *Bacillus subtilis*. Asarone had a binding energy of 6.1 kcal/mol against *Bacillus subtilis* and 5.9 kcal/mol against *Deinococcus* sp. **Conclusion:** Everyone can use scientific inhalation treatment to treat respiratory illnesses with daily *havan* performances. Further research is needed to produce new products from existing conventional residential air purifiers in order to better benefit the general public and rural poor people.

Major Findings: The main chemical compounds found in *havan samagri* were asarone and acetic acid, according to GC-MS analysis of the ethanolic fume extract of *havan*. The ligand asarone molecule exhibited the highest action against bacterial isolates, specifically *Bacillus subtilis* and *Deinococcus* sp., according to molecular docking. According to a molecular docking investigation, the pathogenic proteins of *Deinococcus* sp. and *Bacillus subtilis* had a binding affinity of 5.9 kcal/mol and 6.1 kcal/mol, respectively, with the asarone molecule.

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*Author for correspondence

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1. Introduction

The *Rigveda*, which is the oldest book of knowledge still in existence, tells how the great *Rishis* (saints) of the Saraswati-Indus civilisation (7500 BC) used the *agnihotra-yagya* rite to clean the air. They did this by singing Vedic mantras from the Rigveda and burning *yagya samagri*, which is a mix of mango *samidha* and fragrant and healing herbs[1]. This ceremony is sometimes called *havan*, *homam*, or *agnihotra* when it is done on a small scale. There are a number of volatile oils in the *havan* parts that turn into gases when heated. The fumes that come out at high temperatures are a simple way to get a drug into the body through the nose that works quickly. These volatile fumes can treat SARS-CoV-2, CoV2, Ebola, tomato flu, Japanese encephalitis, and many other respiratory problems like asthma, TB, acute sinusitis, and chronic bronchitis[2]. Mango *samidha*, the main ingredient in *havan samagri*, burns to make formic aldehyde, a gas that kills harmful microorganisms and cleans the air. To keep the fruit fresh, mango *samidha* is burned to make formic acid. Formaldehyde is also used to kill germs on walls and ceilings[3]. People know that the medicinal herbs used in *samagri*, like *guggal*, *nagkesar*, *giloya*, *jaiphal*, *tagar*, *chandan*, *kapur*, *tulsi*, *brahmi*, and others, have antibacterial properties. Another study is support both medicinal and smelly plant fumes changed how well the pathogen worked as an antibacterial agent[4]. When cow ghee, grains, sugars, and herbs are burned as offerings to the fire in *havan kunda*, chemical reactions happen. It makes gases that can kill germs, like buta-propiolactone, formaldehyde, ethylene oxide, and propylene oxide. The decrease in microbial count is caused by both the fumigation effect and the control of microorganisms' metabolic activity[5]. The camphor tree, which is closely related to cinnamon and has long been thought to have healing properties, produces *kapur*, which is also known as *Cinnamomum camphora*. If you want to feel better, camphor is a powerful psychic healer that can lift your spirits, especially to a higher, more ethereal level. Camphor is often used in alternative medicine to treat colds and the flu because its strong, unpleasant smell helps clear the airways. Camphor can also help with joint and muscle pain[6]. People say that the *guggal* plant cleans the air. The *guggal* (*Commiphora mukul*) plant burns and makes a gas that absorbs smells in the air[7]. People often use the medicinal herb *brahmi* as a health tonic and to help them remember things. Brahmi is a great way to boost brain function and memory. It helps with short-term memory, long-term memory, and keeping power. *Brahmi*'s cooling properties help to keep the mind calm and free of stress. It also helps people sleep better[7,8]. *Havan* is a medical fume that contains volatile combustion products from pyrolysis, oxidation, dehydration, and hydrolysis[9]. *Havan* is a scientific method that keeps the levels of CO_2 and O_2 in the atmosphere stable[10]. Recent studies show that *havan* and medicinal herb fume may help clean the air by getting rid of harmful air pollutants and microbes that are in the air. The present study represents *in silico* analysis, phytochemical profiling, and antibacterial assessment of *havan* fume Extract.

2. Material and Methods

2.1 *Havan* Ingredients

Materials for formulation of *havan samagri* were purchased from Vijay Herbal Automation Centre, Hanuman Garhi, Kankhal, Haridwar**.** The wood which is used as a fuel for the fire at the time of performing *havan* is known as *samidha*. The *samidha* is collected from an insect-free plant twig of mango (*Mangifera indica*) from Aman Chowk, Gurukula Kangri (Deemed to be University), Haridwar. *Havan* ingredients were divided according to their properties, such as the flammable properties of white crystal *kapur* and *devdar* wood powder. The antimicrobial properties of medicinal herbs, *i.e.*, *guggal*, *giloya*, *jaiphal*, *brahmi*, *harad*, *chandan*, *amla*, *tulsi*, *bach*, *nagkesar*, *jatamansi*, *bael*, *bahera*, *etc.*, used in the *havan samagri* are well known; therefore, the antimicrobial effect of *havan samagri* fumes against aeromicroflora shall be studied (Table 1).

2.2 Formulation of *Havan Samagri*

The *havan* ingredients were collected and kept separately in different labelled polythene bags. Medicinal plants *giloya*, *jaiphal*, *brahmi*, *laung*, *elaichi*, *munakka*, *harad*,

Table 1. *Havan* ingredients, active metabolite and their pharmacological properties

S. No.	*Havan* ingredient	Active metabolite	Pharmacological properties	References
1	*Loban*	Benzoin	Antidepressant, anxiolytic	[11]
2	*Jatamansi*	Calarene, patchouol, α-gurjunene, aristolone, β-maaliene, spathulenol	Increase in seizure threshold, inhibit the electroshock convulsions	[12]
3	*Guggal*	Terpenes, sesquiterpenoids, cuminic aldehyde, eugenol and the ketone steroids Z and E	Antibiotic, anti-inflammatory	[13]
4	*Kapur*	Camphor, linalool, 1,8-cineole, nerolidol, safrole, orborneol	Anti-fungal, anti-cancer, anti-infective, anti-inflammatory	[14]
5	Cow dung cakes	Potassium, nitrogen, smaller quantities of sulphur, calcium, magnesium, manganese	Antibacterial, antifungal, antiviral	[15]
6	*Ashwagandha*	Alkaloids, flavonoids, phenolics, saponins and glycosides	Antibacterial, antioxidant, anticancer, cardioprotective	[16]
7	Sandalwood	Tricyclic α-santalol, β-santalol	Antibacterial, fungistatic, anti-inflammatory	[17]
8	Mango wood	PGG, polyphenolics, flavonoids	Increase GABA levels, anticonvulsant action	[18]
9	Cow ghee	Ketones	Antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory and immune-restorative	[19]
10	Black sesame seeds	1-(5-methyl-2-furanyl)-1-propanone	Antimicrobial, anticancer, antioxidant, anti-inflammatory	[20]
11	Saffron	Crocetin, picrocrocin, safranal	Increase in seizure threshold, antioxidant, antitussive, antidepressant	[21]
12	Nutmeg	Myristicin and macelignan	Inhibit seizures, reduce severity of seizures	[22]
13	*Nagarmotha*	Cyperone, selinene, cyperene, cyperotundone, patchulenone, sugeonol, cyperol, isocyperol and cyperone.	Anticonvulsant action, antimicrobial, antifungal	[23]
14	Clove	Eugenol, β-caryophyllene	Analgesic, antioxidant, anti-inflammatory, anxiolytic, anticonvulsant, increase GABA ergic and glycinergic activity	[24]
15	*Viabidang* fruit	Embelin, quercitol, barberine, embolic acid.	Antibacterial activity	[25]
16	*Nagkesar*	Sesquiterpene	Anti-pyretic, anti-inflammatory	[26]
17	*Phoolmakhane*	N- nornuciferine	Anticonvulsant action	[27]

amla, *jatamansi*, *nagarmotha*, *shatavar*, *tejpatta*, *bahera*, *bael*, *gokhru*, *nagkesar*, *bach*, *viabidang*, *nirmali* seed, *indrajaw*, *shankhpushpi*, *kalmegh*, *etc*., were taken in a *Sujata* mixer grinder and ground separately to obtain coarse pieces of *havan* ingredients. *Guggal* and *kapur* were both taken in a clean mortar and pestle and ground to obtain fine pieces of *havan* ingredients. All these ingredients were then mixed properly. After mixing all these *havan* ingredients in proper quantity ratio (Table 2) were packed in labelled polythene bags.

The neat, clean, dry and insect-free mango *samidha* were also cut separately into small sticks of varying size, about 12 to 16 cm in length and 3 cm in diameter[28].

2.3 Microbiological Test

2.3.1 Air Sampler Vacuum Cleaner

A large 10 l brand (*Agaro* 33398) air sampler vacuum cleaner with a powerful 1000 W motor and strong 16.5 kPa suction pressure was used for air sampling inside the Yagyashala of Gurukula Kangri (Deemed to be

Table 2. Plant ingredients for formulation of *havan samagri*

S. No.	Ingredient	Scientific Name	Quantity
1.	*Giloya*	*Tinospora cordifolia* (Thunb.) Miers	50 g
2.	*Guggal*	*Commiphora wightii* (Arn.) Bhandari	100 g
3.	*Brahmi*	*Bacopa monnieri* (L.) Pennell	50 g
4.	*Jaiphal*	*Myristica fragrans* Houtt.	10 g
5.	*Laung*	*Syzygium aromaticum* (L.) Merr. and Perry	10 g
6.	*Elaichi*	*Elettaria cardamomum* (L.) Maton	50 g
7.	*Munakka*	*Vitis vinifera* L.	20 g
8.	*Harad*	*Terminalia chebula* Retz.	20 g
9.	*Kapur (Camphor)*	*Cinnamomum camphora* (L.) J. Presl.	50 g
10.	*Amla*	*Phyllanthus emblica* Linn.	20 g
11.	*Jatamansi*	*Nardostachys jatamansi* (D. Don) DC.	20 g
12.	*Javitri*	*Myristica fragrans* Houtt.	20 g
13.	*Nagarmotha*	*Cyperus scariosus* R. Br.	20 g
14.	*Kapur kachri*	*Hedychium spicatum* Sm. in A. Rees	20 g
15.	*Til*	*Sesamum indicum* L.	10 g
16.	*Jaun*	*Hordeum vulgare* L.	60 g
17.	*Shatavar*	*Asparagus racemosus* Willd.	50 g
18.	*Tejpatta*	*Cinnamomum tamala* (Buch.-Ham.) Nees and Eberm.	50 g
19.	*Tulsi leaves powder*	*Ocimum tenuiflorum* L.	20 g
20.	*Bahera*	*Terminalia bellirica* (Gaertn.) Roxb.	30 g
21.	*Bael*	*Aegle marmelos* (L.) Correa	30 g
22.	Red sandalwood powder	*Pterocarpus santalinus* L. f.	30 g
23.	*Devdarwood* powder	*Cedrus deodara* (Roxb.) G. Don	100 g
24.	*Gokhru*	*Tribulus terrestris* L.	20 g
25.	*Nagkesar*	*Mesua ferrea* L.	30 g
26.	*Bach*	*Acorus calamus* L.	30 g
27.	*Viabidang*	*Embelia ribes* Burm. f.	10 g
28.	*Nirmali* seeds	*Strychnos potatorum* L. f.	20 g
29.	*Indrajaw*	*Holarrhena pubescens* Wall.	20 g
30.	*Shankhpushpi*	*Convolvulus prostrates* Forssk.	20 g
31.	*Kalmegh*	*Andrographis paniculata* (Burm. f.) Nees	10 g
Total Weight			**1 Kg**

University), Haridwar. 500 g *havan samagri* and 250 g mango *samidha* were used for performing *havan*. After completion of the *havan*, the fumes were collected through the outlet of the vacuum cleaner attached to a 250 ml flask filled with 100-100 ml ethanol and distilled water. The *havan* fumes through vacuum cleaner air samples were collected for 5 min, and this process was repeated for each solvent. The *havan* fumes after each 5 min time interval were shaken for mixing it completely to dissolve the *havan* fumes inside each solvents[29].

2.3.2 Antibacterial Activity of Havan Fumes Extract

2.3.2.1 Selected Pathogens

Air-isolated bacteria were selected for this study. Four bacterial isolates with their respective accession number, *i.e.*, *Staphylococcus caprae* (PP412045),

Deinococcus ficus (PP345434), *Priestia endophytica* (PP382224) and *Bacillus subtilis* (PP345587), were taken after sequencing was performed at Biocart India Pvt. Ltd., Bangalore.

2.3.3 Preparation of Inoculum

A single colony was transferred to Muller-Hinton broth medium and incubated in a controlled shaker at 120 rpm at 37°C overnight with the help of an inoculum loop from a stored slant culture. These bacterial suspensions were transferred in sterile Muller-Hinton Broth (MHB) medium and retained the inoculum concentration of 10^8 CFU mL^{-1} by obtaining 0.132 absorbance at 600 nm wavelength according to the McFarland 0.5 standards.

2.3.4 Antibacterial Assay (Well Diffusion Method)

A well diffusion method has been used for the antibacterial activity of ethanol and aqueous *havan* fumes extracts. The antibacterial assay was carried out against four bacterial air isolates. Four plates (Ethanol and Aqueous *havan* fumes extracts, positive control, *i.e.,* an antibiotic disc and negative control, *i.e.*, DMSO) for each bacterium were prepared with 100 µL of respective inoculum on Muller-Hinton agar medium. Three wells (except the positive control antibiotic disc) were placed on each plate with their combinations. The three wells (size 6 mm) were made on each plate by using a cork borer. One antibiotic disc, Ciprofloxacin (CIP), was taken as a positive control with 5 µg/disc concentration. Dimethyl sulphoxide, *i.e.,* DMSO, was taken as a negative control. Every plate was analysed in triplicate, and the mean value of the inhibition zone was observed in millimetres (mm) with standard error after a 24 h incubation period at 37°C.

2.4 Quantitative Estimation of Antibacterial Activity of *Havan* Fume by Exposed Settle Plate Method

2.4.1 Description of the Study Site

The experiment was conducted at the Laldevi Yagyashala in Gurukula Kangri (Deemed to be University). The *Yagya kund* was positioned in the centre of the *yagyashala*, and mats were spread around it along with four sitting *asana*, each in a different direction. The plate was set on a 30 cm stool that was situated at a distance of four corners from the *Havan Kund*, and the measurement was 2 m.

2.4.2 Description of Experimental Design

The *havan* experiment was conducted in three different time periods: pre-havan (9:30 AM), post-*havan* (9:50 AM) and 5 h after post-*havan* (2:50 PM). The bacterial CFU count and their density/100 cm^3 were studied on nutrient agar Petri plates. The plates were exposed for 10 min inside and outside the *yagyashala* in three different time periods before (9:30 AM), after (9:50 AM) and 5 h after post-*havan* (2:50 PM). The Petri plates placed inside and outside the *yagya* were collected after air sampling, properly labelled and incubated in a BOD incubator at 37°C for 24 h for bacterial growth[5].

2.4.3 Record of Microflora

A complete record of data on the number of colonies of each plate was kept. The percentage density of bacterial propagules/100 cm^3 and the percentage reduction of bacterial CFU count difference between before (9:30 AM) and after *havan* (9:50 AM, 2:50 PM) was calculated.

2.5 GC-MS Analysis of *Havan* Fume Extract

Havan fumes extract was prepared through the outlet of a vacuum cleaner attached to a 250 ml flask filled with ethanol. The duration for collection of *havan* fumes through vacuum cleaner air sampler was 5 min. The ethanolic solvent was used to shake the flask for 5 min in order to extract the *havan* fumes, which eventually dissolved as an ethanolic fumes extract. Before *havan,* no bioactive compound was found at the *yagyashala* site after GC-MS through vacuum cleaner solvent trap, work as positive control[29].

2.5.1 Sample Preparation

After sample preparation of ethanolic solvent fumes extract, it was analysed by using the GC-MS with AOC-20i Splitless Auto injector at Patanjali Food and Herbal Park Pvt. Ltd., Laksar Road, Padartha, Haridwar. The analysis was completed using the experimental parameters of SH-Rxi-5Sil MS (5% biphenyl, 95% dimethyl polysiloxane) for the column with 30 m length, 0.25 mm diameter and 0.25 µm film thickness. After that, a 100 mg to 200 mg sample was dissolved

in Ethanol/Methanol/Ethyl acetate/Hexane (as per solubility) and, if required, further diluted by taking 1 ml to 10 ml solvent and injected into GC-MS.

2.6 Molecular Docking of Major Compound against Air-isolated Bacteria

One such computational technique that forecasts the preferred orientation of a molecule to another when they are joined to create a stable complex is called molecular docking. Protein-protein docking and small molecule-protein docking, also referred to as ligand-protein docking, are the two main types of docking, or molecular docking[30].

2.6.1 Ligand and Protein Preparation

2.6.1.1 Ligand

First, download the test ligands' native SDF format 3-D conformation structures from PubChem. The ligands were transformed into PDB format using the PyMol program. Following that, AutoDock Vina was used to store the ligand structures in PDBQT format for further investigation.

2.6.1.2 Protein

Bacterial protein 1xp8 and Alp7A were searched and downloaded in PDB format using Protein Data Bank (PDB) software. Protein amino acid sequences were saved for further docking analysis.

Alp7A - Bacterial actin-like protein in *Bacillus subtilis* that is involved in plasmid pLS20 segregation and in creating a spindle-like structure.

1xp8 - X-ray crystal structure of the RecA protein in *Deinococcus* sp. RecA is a key protein in repairing double-stranded DNA breaks through homologous recombination.

3. Results and Discussion

3.1 Antibacterial Activity of *Havan* Fume Extract

Four air isolates were used to test the ethanol (EtOH) and aqueous (Aq) *havan* fume extracts' antibacterial activity (Table 3). The zone of inhibition for *Staphylococcus caprae* ranged from 14.33±0.33 mm to 16.00±0.57 mm, with ethanolic fume extract showing the highest effective zone of inhibition at 16.00±0.57 mm and aqueous fume extract at 14.33±0.33 mm. The zone of inhibition for *Deinococcus ficus* ranged from 15.33±0.33 mm to 16.00±0.57 mm. Aqueous fume extract was the most effective, exhibiting a zone of inhibition of 16.00±0.57 mm, followed by ethanolic fume extract at 15.33±0.33 mm. The zone of inhibition for *Priestia endophytica* ranged from 13.66±0.33 mm to 15.00±0.57 mm, with ethanolic fume extract exhibiting the most effective zone of inhibition at 15.00±0.57 mm, followed by aqueous fume extract at 13.66±0.33 mm. The zone of inhibition for *Bacillus subtilis* ranged from 15.33±0.33 mm to 15.66±0.33 mm, with ethanolic fume extract showing the most effective zone of inhibition at 15.66±0.33 mm and aqueous fume extract at 15.33±0.33 mm (Table 3). The many phyto-constituents found in *havan* fume may be the cause of these extracts' antibacterial properties (Figure 1).

3.2 Antibacterial Activity of Antibiotics

The antibacterial activity of the antibiotic Ciprofloxacin (CIP) and the negative control (DMSO) was evaluated against four air isolates. Ciprofloxacin is effective against all four air isolates and showed 34.00±0.57 mm, 30.66±0.33 mm, 32.00±0.57 mm and 42.00±0.57 mm zones of inhibition against *Staphylococcus*

Table 3. Antimicrobial activity of *havan* fume extract against isolated bacterial strains

Bacterial isolates	Zone of Inhibition (mm)			
	Ethanolic fumes extract	Aqueous fumes extract	Ciprofloxacin	DMSO
Staphylococcus caprae	16.00 ± 0.57	14.33±0.33	34.00±0.57	00.00±0.00
Deinococcus ficus	15.33±0.33	16.00±0.57	30.66±0.33	00.00±0.00
Priestia endophytica	15.00 ±0.57	13.66±0.33	32.00±0.57	00.00±0.00
Bacillus subtilis	15.66±0.33	15.33±0.33	42.00±0.57	00.00±0.00

The values are the mean ± error (n = 3); diameter of disc: 6 mm. The antibacterial activity of ciprofloxacin is effective against all four isolates between 30.66±0.33 mm to 42.00±0.57 mm, respectively.

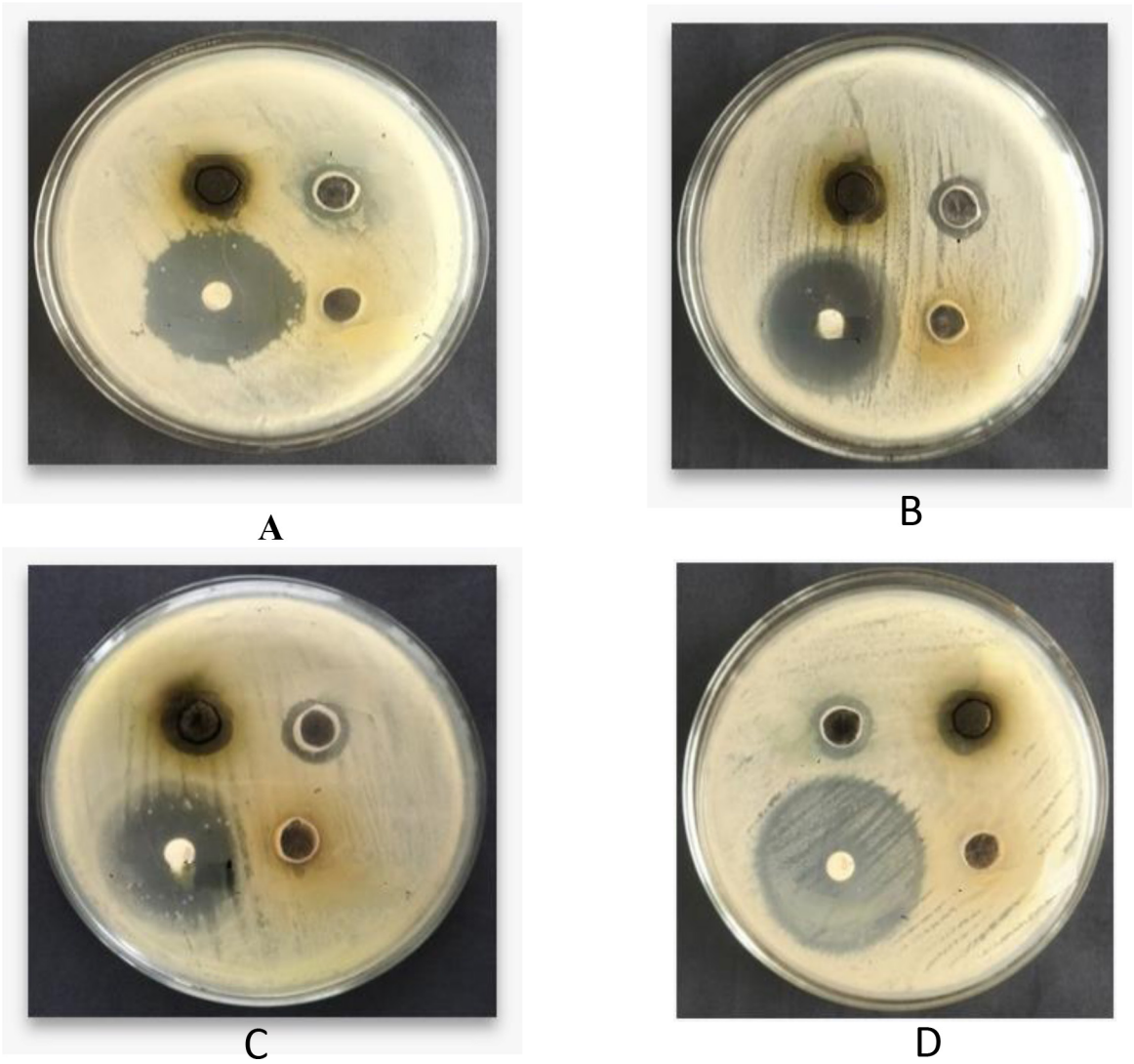


Figure 1. Antibacterial activity of hawan fumes extract against; **(A).** *Staphylococcus caprae* Abr-2, **(B).** *Deinococcus ficus* Abr-3, **(C).** *Priestia endophytica* Abr-4, **(D).** *Bacillus subtilis* Abr-6; Etoh: Ethanol, Aq: Aqueous, Positive control disc: Ciprofloxacin, Negative control: DMSO.

caprae (PP412045), *Deinococcus ficus* (PP345434), *Priestia endophytica* (PP382224) and *Bacillus subtilis* (PP345587), respectively.

3.3 Quantitative Estimation of Antibacterial Effect of *Havan* Fume at *Yagyashala* Site

Tables 4 and 5 show the results of NAM plates CFU mean of inside and outside *yagyashala* after 10 min of exposure at different time intervals during pre-*havan* (9:30 AM), post-*havan* (9:50 AM) and 5 h after post-*havan* (2:50 PM). After an incubation period of 24 h at 37℃, results were analysed by calculating the percentage reduction of CFU count between pre- and post-*havan* with respective temperature and per cent humidity conditions (Table 6). Unexposed plates with 0 CFU count work as positive control plates. Inside the *yagyashala* site, 45.84% and 42.23% reduction of CFU count were calculated between pre- and post-*havan* exposed plates on 13/07/2024 and 16/08/2024, respectively. 62.50% and 37.78% reduction of CFU count were calculated between pre-*havan* and after 5 h of post-*havan* exposed plates inside the *yagyashala*. Outside the *yagyashala* site, 34.12% and 27.58% reduction of CFU count were calculated between pre- and post-havan NAM plates during two different air sampling dates. 59.29% and

36.22% reduction of CFU count were calculated between pre-*havan* and after 5 h of post-*havan* exposed plates (Table 4 and 5). Further CFU count results were calculated into variation in density of bacteria/100cm^3 by using the density formula. Inside *yagyashala* pre-*havan* exposed plates, the bacterial density/100 cm^3 was calculated to be 60 and 56.25. On the post-*havan* fumigated plates, decreasement of the bacterial density/100 cm^3 was calculated to be 32.50, and further reduction of 22.50 and 35.00 bacterial density was also calculated on 5 h after post-*havan* plates during two different air sampling dates, respectively. Similarly, outside *yagyashala* pre-*havan* exposed plates, the bacterial density/100 cm^3 was calculated to be 126.60 and 96.67. On the post-*havan* fumigated plates, decreasement of the bacterial density/100 cm^3 was calculated to be 83.30 and 70.00 and further reduction of 51.60 and 61.67 bacterial density was also calculated 5 h after post-*havan* exposed plates during two different air sampling dates, respectively (Table 7).

Only slight variations in temperature and relative humidity were found in the climatic parameters recorded in the *Yagyashala* during the *havan* on

Table 4. Bacterial CFU mean and percentage reduction of CFU count between pre and post *havan*

Timing of havan Date-13/7/2024 ↓ / NAM plates position →	Inside *Yagyashala*		Outside *Yagyashala*	
	CFU mean	Percentage reduction of CFU count	CFU mean	Percentage reduction of CFU count
Pre-*havan* (9:30 AM)	12	45.84%	25.30	34.12%
Post-*havan* (9:50 AM)	6.50		16.67	
5 h after post-*havan* (2:50 PM)	4.50	62.50%	10.30	59.29%

*Mean of three Petri plates

Table 5. Bacterial CFU mean and percentage reduction of CFU count between pre and post *havan*

Timing of *havan* Date-16/8/2024 ↓ / NAM Plates position →	Inside *Yagyashala*		Outside *Yagyashala*	
	CFU mean	Percentage reduction of CFU count	CFU mean	Percentage reduction of CFU count
Pre-*havan* (9:30 AM)	11.25	42.23%	19.33	27.58%
Post-*havan* (9:50 AM)	6.50		14	
5 h after post-*havan* (2:50 PM)	7	37.78%	12.33	36.22%

*Mean of three Petri plates

Table 6. Metrological parameters of *yagyashala* at different time periods

Timing of *havan* period ↓	Date-13/07/2024		Date-16/08/2024	
	Temperature (°C)	Humidity (%)	Temperature (°C)	Humidity (%)
Pre-*havan* (9:30 AM)	28	18	29	23
Post-*havan* (9:50 AM)	28	18	29	23
5 h after post-*havan* (2:50 PM)	30	26	30	24

Table 7. Variation in density of bacteria/100 cm^3 inside and outside *yagyashala* NAM plates

Timing of *havan* period ↓	Date- 13/07/2024		Date-16/08/2024	
	Inside *yagyashala*	Outside *yagyashala*	Inside *yagyashala*	Outside *yagyashala*
Pre-*havan* (9:30 AM)	60	126.60	56.25	96.67
Post-*havan* (9:50 AM)	32.50	83.30	32.50	70.00
5 h after post-*havan* (2:50 PM)	22.50	51.60	35.00	61.67

July 13, 2024, and August 16, 2024, during the three observation periods: pre-*havan*, immediately following the ritual, and 5 h later.

3.4 Variations in Temperature

Throughout the *havan* procedure, the reported temperature stayed mostly constant. The temperature was 28°C on July 13, 2024, both before and after *havan*. After 5 h, it marginally rose to 30°C. In a similar vein, on August 16, 2024, the temperature rose to 30°C after 5 h from 29°C before and following *havan*. This pattern suggests that in the enclosed or semi-enclosed *yagyashala* habitat, *havan* had little effect on the ambient temperature. Since ambient temperature usually rises from morning to afternoon due to increased solar radiation, the minor increase seen after 5 h is probably due to normal diurnal temperature variation[31]. Furthermore, even though *havan* entails combustion, the size and duration of fire utilised in ceremonial contexts are insufficient to change the macro environmental temperature. This is consistent with research that found only small changes in temperature during yajna rites[32].

3.5 Changes in Humidity

Relative humidity likewise varied little before and after *havan*, but it did somewhat increase after 5 h, especially on July 13, 2024 (from 18% to 26%) (Table 6). This delayed increase in humidity could be due to midday evaporation processes; as the temperature rises, evaporation processes take place. Water vapour and combustion byproducts from *havan* (wood, ghee, and herbal combinations) are released, which could raise regional moisture levels. Changes in the ambient atmosphere as opposed to the *havan*'s direct, immediate effects. According to earlier research, *havan* smoke may contain aerosols and volatile organic compounds that interact with atmospheric moisture over time to affect microclimatic humidity. The lack of an instantaneous shift, however, implies that these impacts are progressive rather than instantaneous[33].

The data unequivocally show that Temperature and humidity are two meteorological characteristics that are

Table 8. Chemical compounds identified from ethanol extracts of *havan* fumes

S. No.	Compound	RI	MF	MW	%FID
1	2-(1-methylethoxy)-ethanol	708	$C_5H_{12}O_2$	104	5.93
2	Acetic acid	-	CH_3COOH	60	39.63
3	Ethyl(dimethyl)ethoxysilane	720	$C_6H_{16}OSi$	132	2.51
4	2,2-diethoxy-propane	761	$C_7H_{16}O_2$	132	5.63
5	Propylene Glycol	764	$C_3H_8O_2$	76	3.79
6	Triethylmethoxy-silane	858	$C_7H_{18}OSi$	146	2.67
7	3-Hydroxybutanoic acid	976	$C_4H_8O_3$	104	12.59
8	Octamethyl-cyclotetrasiloxane	994	$C_8H_{24}O_4Si_4$	296	0.48
9	1,4:3,6-Dianhydro-α-d-glucopyranose	1148	$C_6H_8O_4$	144	4.87
10	2,3-Anhydro-d-mannosan	1152	$C_6H_8O_4$	144	0.47
11	Silane, methyldiethoxyisopropoxy-	1155	$C_8H_{20}O_3Si$	192	0.99
12	7′-Oxaspiro[cyclopropane-1,4′-tricyclo[3.3.1.0(6,8)] nonan-2′-one]	1162	$C_{10}H_{12}O_2$	164	0.51
13	Tricyclo[4.2.1.1(2,5)]deca-3,7-diene-9,10-diol	1364	$C_{10}H_{12}O_2$	164	0.52
14	1,2,3-trimethoxy-5-(2-propenyl)- Benzene	1556	$C_{12}H_{16}O_3$	208	1.44
15	Diethyl Phthalate	1580	$C_{12}H_{14}O_4$	222	0.67
16	Asarone	1682	$C_{12}H_{16}O_3$	208	15.29
17	3-dodecylpyrrolidine-2,5-dione	1730	$C_{16}H_{29}NO_2$	267	1.20
	Total Identified				**99.19**

RI-Experimentally determined retention indices on SH-Rxi-5Sil MS (5% biphenyl 95% dimethyl polysiloxane) for column with 30 m length, 0.25 mm diameter and 0.25 µm film thickness; RI* Identified by retention index according to literature.

neither immediately nor significantly altered by *havan*. The environment doesn't change during the ceremony, suggesting that any biological or antimicrobial effects are probably caused by the chemical components of *havan* smoke rather than actual environmental changes. Changes that occur after 5 h are more likely to be caused by cumulative emission effects and natural atmospheric variation than by the ritual's direct thermal or hygroscopic impacts. Bioactive phytochemicals generated during burning, such as phenolics, terpenoids, and essential oils, mediate the biological efficacy of *havan* (*e.g.*, antibacterial activity, antioxidant potential), rather than temperature or humidity changes[34,35]. The trustworthiness of experimental results is strengthened by stable weather conditions, which guarantee that observed experimental effects can be attributed to chemical exposure rather than ambient complicating factors.

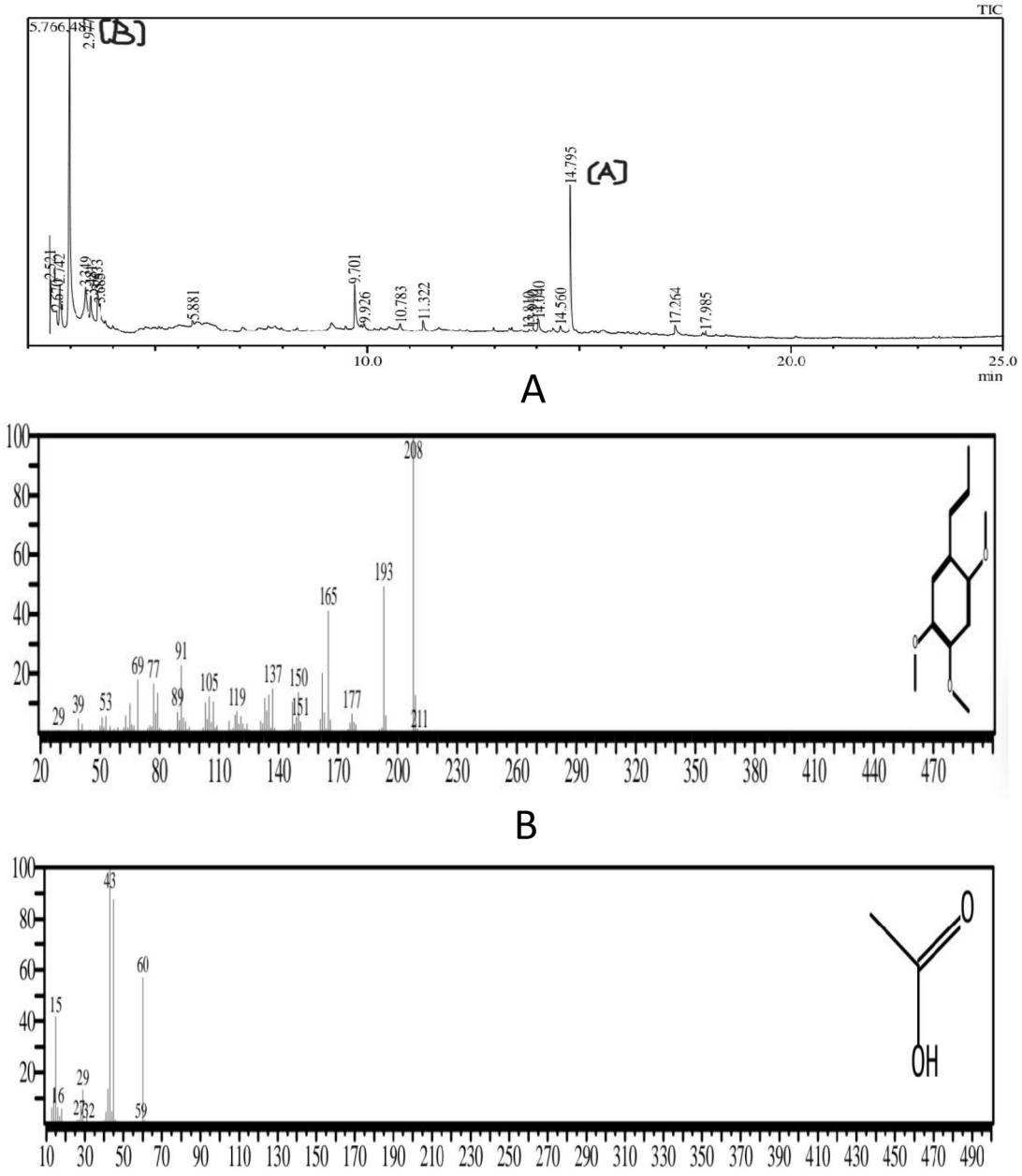


Figure 2. A:GC-MS chromatogram of ethanolic *havan* fume extracted compound with retention time peak. B: GC-MS chromatogram of Asarone with retention time (14.795) and base peak size 208.10. C: GC-MS chromatogram of Acetic acid with retention time (2.977) and base peak size 43.05.

3.6 GC-MS Compounds of *Havan* Fume Extract

The GC-MS analysis shows that the extract of *havan* fumes in an ethanol solvent trap has a total of 20 volatile chemicals. The GC-MS results were analysed using the National Institute of Standards and Technology (NIST) database in the United States. Table 8 shows the name, formula, area percentage, height percentage, and retention time of the volatile chemicals. The GC-MS analysis of the ethanolic *havan* fumes extract showed 20 peaks. The most important one was acetic acid (39.63%), followed by asarone (15.29%) (Figure 2), butanoic acid (12.59%), 1,4:3,6-dianhydro-alpha-d-glucopyranose (4.87%), propylene glycol (3.79%), triethylmethoxysilane (2.67%), diethyl phthalate (0.67%), and octamethylcyclotetrasiloxane (0.48%) (Table 8). Asarone, the main ingredient in the *havan* fume extract, had a retention time of 14.795. Asarone is a phenylpropanoid found in certain plants, utilised in various applications including fragrance oil, a prospective remedy for neurological disorders such as Alzheimer's disease, and a volatile fragrance oil employed to eliminate bacteria and pests. Octamethyl cyclotetrasiloxane and triethylmethoxysilane were the two main organosilicon compounds in ethanolic *Havan*a fume extract.

3.7 Physiochemical Properties of Active Volatile Compounds

Butanoic acid, which has the molecular formula $CH_3CH_2CH_2COOH$, is a straight-chain alkyl saturated fatty acid. It smells bad and is a colourless, greasy liquid. Although the acid is not common in nature, its esters are. It is a common industrial chemical that has a significant role in the digestive systems of mammals.

Asarone is a phenylpropanoid found in certain plants that has many uses, including as a fragrance oil, a potential treatment for neurodegenerative disease, *i.e.*, Alzheimer's disease prevention, and it is volatile fragrance oil used to kill pests and bacteria. By preventing *Aspergillus niger* from producing ergosterol, asarone demonstrates antifungal efficacy.

i. Acetic acid - Ethanoic acid, an organic acidic colourless liquid. It has been used as a component of vinegar. Commercially, it is used in the manufacturing of esters and many polymeric materials.
ii. 1,4:3,6-Dianhydro-alpha-d-glucopyranose- It is a natural carbohydrate anomer found in the plant cell wall cellulose component.
iii. Diethyl phthalate is a colourless, synthetic phthalate ester that tastes unpleasant and has a faint fragrance. It is a component of pesticide sprays and mosquito repellents, as well as a fragrance ingredient in cosmetics.
iv. Propylene glycol is a synthetic colourless, viscous liquid with a slightly sweet taste and almost no odour. Propylene glycol is a diol, having two alcohol groups.
v. Octamethyl cyclotetrasiloxane is an organosilicon compound. It is used as an emollient and solvent in cosmetics.
vi. Triethylmethoxysilane is a colourless, mildly scented liquid that is used as a silylating agent to create hydrophobic surface coatings.

3.8 CB-dock Online Tool-2

Asarone, also known as trans-2,4,5-Trimethoxypropenyl benzene, was docked with the proteins Bacillus subtilis (Alp7A) and *Deinococcus* sp. (1xp8) using the CB-dock online tool-2, which helps predict the ligand's optimal binding site with the protein. The CB-dock online tool-2 results offer an analysis of the binding energy involved in the creation of the protein-ligand structure complex as well as the apparent molecular interactions responsible for its activity. The docked protein of *Deinococcus* sp. (1xp8) had a binding affinity of -5.9

Table 9. The binding affinity of bioactive compound asarone with protein 1xp8 (*Deinococcus* sp.)

Ligand+Protein	Cur pocket id	Vina score (binding affinity)	Cavity score	Center (x, y, z)	Docking size (x, y, z)
Asarone+1xp8	C1	-4.8	897	29, 34, 9	19, 19, 32
Asarone+1xp8	C2	-5.9	449	22, 17, 3	19, 19, 19
Asarone+1xp8	C3	-4.8	312	20, 29, 31	19, 19, 19
Asarone+1xp8	C4	-4.4	261	20, 42, 21	19, 19, 19
Asarone+1xp8	C5	-4.5	194	15, 40, 28	19, 19, 19

Table 10. The binding affinity of bioactive compound asarone with protein Alp7A (*Bacillus subtilis*)

Ligand+Protein	Cur pocket id	Vina score (binding affinity)	Cavity score	Center (x, y, z)	Docking size (x, y, z)
Asarone+Alp7A	C1	-6.1	2306	-19, 2, -14	27, 26, 25
Asarone+Alp7A	C2	-5.3	352	-8, 10, -45	19, 19, 19
Asarone+Alp7A	C3	-4.8	185	6, 4, -7	19, 19, 19
Asarone+Alp7A	C4	-4.5	168	-10, 14, -14	19, 19, 19
Asarone+Alp7A	C5	-4.5	137	-18, -3, 2	19, 19, 19

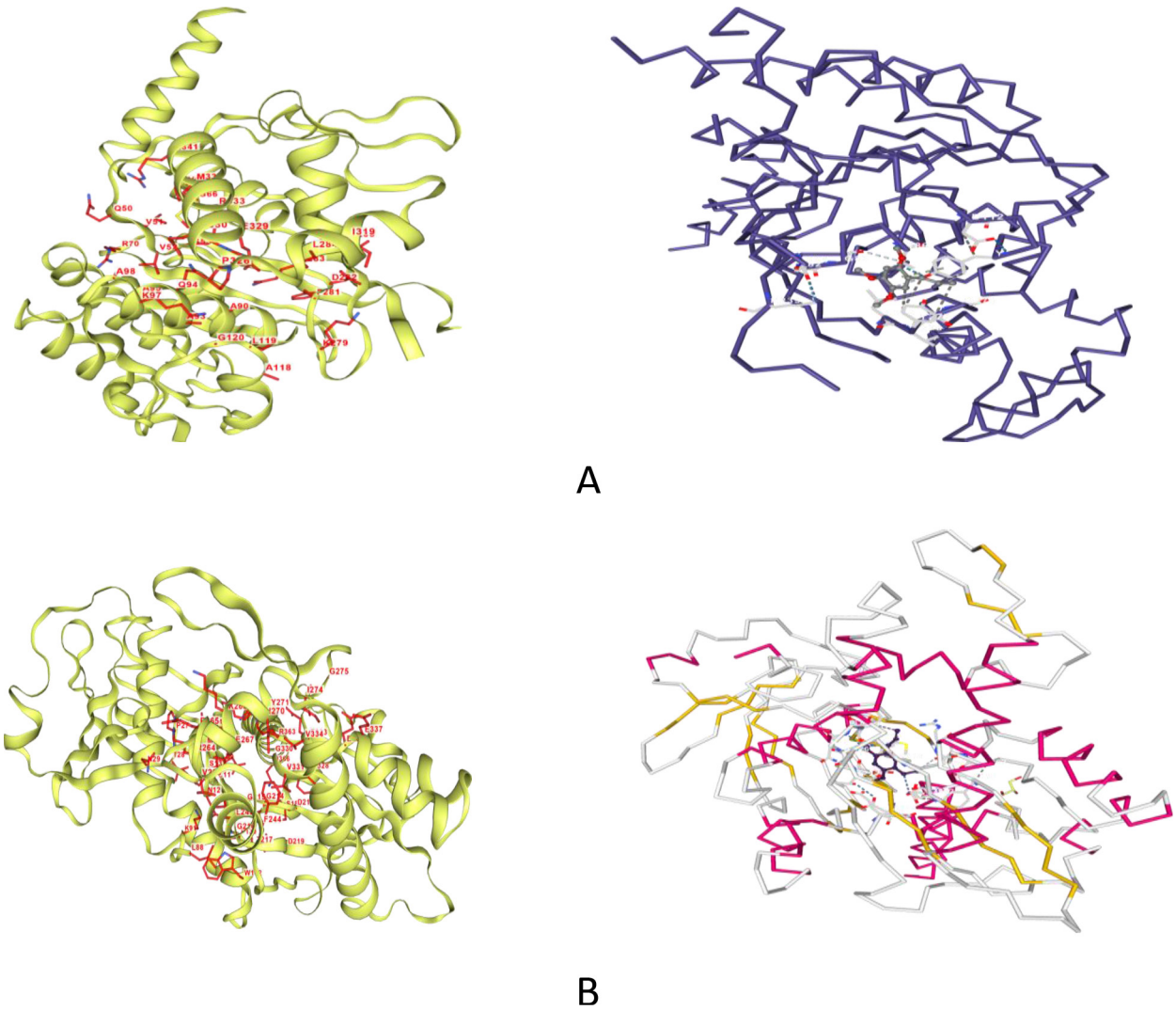


Figure 3. **(A).** Molecular docking of asarone + 1xp8 (*Deinococcus* sp.). **(B).** Molecular docking of asarone + Alp7A (*Bacillus subtilis*).

kcal/mol for asarone (Table 9 and Figure 3A). The protein *B. subtilis* (Alp7A) was docked with asarone, and the binding affinity was discovered to be -6.1 kcal/mol (Tables 9 and 10 and Figure 3B). Therefore, the bactericidal qualities of the ethanolic vapour extract of *havan samagri* are highly supported by molecular docking of the ligand asarone molecule with the protein of *Deinococcus* sp. and *B. subtilis*. Thus, it was

shown that using the medicinal fume of *havan samagri* can reduce the quantity of airborne germs in the air we breathe.

4. Conclusion

The current investigation supports the antibacterial effects of medicinal *havan* fume and its scientific validity. Initially, *havan* fume was collected using an air sampler vacuum cleaner to create an ethanolic and aqueous fume extract. In this research, the influence of fumes on air-isolated bacteria was investigated at Laldevi *Yagyashala* using the settling plate exposure method and well diffusion method. *Havan Samagri*'s ethanolic and aqueous fume extracts had the highest levels of effectiveness against *Deinococcus ficus* and *Staphylococcus caprae*, respectively. The bacterial CFU count was found to have decreased by a maximum of 62.50% and 59.29%, respectively, during the pre-*havan* and 5-h post-*havan* exposed plates inside and outside of *Yagyashala*. The main chemical compounds found in *havan samagri* were asarone and acetic acid, according to GC-MS analysis of the ethanolic fume extract of *havan*. The ligand asarone molecule exhibited the highest action against bacterial isolates, specifically *Bacillus subtilis* and *Deinococcus* sp., according to molecular docking. According to a molecular docking investigation, the pathogenic proteins of *Deinococcus* sp. and *Bacillus subtilis* had a binding affinity of 5.9 kcal/mol and 6.1 kcal/mol, respectively, with the asarone molecule. According to a recent study, the market for herbal ingredients and goods made from them, such as *havan samagri*, an air purifier for public and social welfare, is growing. Everyone can treat respiratory illnesses with scientific inhalation treatment by participating in daily *havan* performances. Further research is needed to create new goods from existing conventional residential air purifiers in order to better benefit the general public and rural poor people.

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Author Contribution

Vikash Rana: Experimental work, Drafting of research; Manish Dev Sharma: Manuscript revision and proofreading; Indra Rautela: Drafting of research findings, Naina Srivastava: Drafting of research findings; Ritu Rani and Vijayalaxmil: Draft table and Figure; Himani Badoni: Draft table and Figure; Shweta Sahni: Proofread manuscript; Atal Bihari Bajpai: Supervision of work. All authors have read and agreed to the published version of the manuscript.