

# Anti –Microbial effect of three essential oils in treating Upper respiratory tract Infection via vapours generated by Pressurized steam chamber (PSC)

## Research Article

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### Abstract

Disinfection tunnel (DT) or sanitization tunnel used for disinfecting the persons by spraying with appropriately atomized virucide spray, Direct inhalation or spraying of disinfectants on people with chemical element and different toxicant chemicals may lead to eye and skin irritation and cause numerous allergic disorders. So this obstacle was overcome by Ideal flow control private limited by designing the Pressurized Steam Chamber (PSC) in which Natural oils was mixed in an emulsifier solution, and induced along with steam through multiple nozzles within the chamber, so that persons could get disinfected the entire body. Anti-microbial efficacy of our three essential oils were determined by standard method, ISO 4833-1:2013 by collecting sample through walk in/walk out chamber protocol. Our present study report of MSME (ministry of micro, small and medium enterprises) and SGS Chennai, reveals that in phytotherapeutic oils initial microbial load are found to be <1 CFU / mL, in emulsifier and in formulation (water + emulsifier + natural oils) the presence of bacterial colonies found to be 470000 CFU / mL and 20000000 CFU / mL whereas, the distillate collected from essential oils at 65° c shows the absence of microbial load. Further swab analysis report of 8 individuals states that samples (hand swab and surface swab) collected for the estimation of Total plate Count showed that there is a reduction in microbial load when exposed to the Steam generated by Pressurized Steam Chamber (PSC) at both the time intervals, 20 and 40 seconds. These findings confirm that three essential phytotherapeutic oils combined with steam have some potent activity against emerging disease.

**Key Words:** Disinfection chamber, Phytotherapeutic oils, Steam.

### Introduction

An outbreak of COVID-19, posing extreme threats to the human lives which were caused by Corona virus, a family of SARS, which is contagious. In 2003 other human corona virus was identified such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). In 2019, a new corona virus was evolved as corona virus 2 (SARS-CoV-2) causes a disease outbreak that originated in China. In the present scenario, due to a corona virus pandemic, WHO has declared the outbreak in Public Health Emergency of International Concern on 30 January 2020, and a pandemic on 11 March 2020, with the certain preventive measures like Social Distancing, Frequent Hand washing, wearing personal protective gadgets (face mask, face shield, gloves ) and through another effective method called disinfectant tunnels which is potentially effective in the context of a

prolonged pandemic of COVID-19. An ideal disinfectant for spraying in these tunnels should be nonvolatile, require less contact time, be harmless to mucous membranes and skin, and have virucidal and bactericidal activity. These disinfectants can destroy the outer envelope of the virus, only if allowed for a recommended concentration with a contact period of more than 60 sec. There have been a lot of confidence-building measures rapidly initiated in the current COVID-19 pandemic. Foremost among these measures, Steam chamber, a traditional technology which would be an effective alternative technology used as a purifier for an upcoming era. The power consumption, dosing tank, photo sensor, size of chamber, operating time, material of construction - Stainless steel fabrication, SS pipe, SS nozzles in portable structures are customized based upon the source of erection ( PSC- 6 : Humans , PSC-V-20: Two wheelers and cars, PSC-BL-25: Buses and trains, PSC-M-30: Metro trains, PSC-T-90: Trains).

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#### Review of literature

*Dr. Dilip Pawar* leading study investigator in Seven Hills Hospital, Mumbai conducted the study using steam by including two groups, *group 1*: 105 asymptomatic, mild, and moderate patients steam was given twice a day by the nasal route and for *group 2*: symptomatic patients and health coworkers steam was administered by nasal and oral route every 3 hours for 5

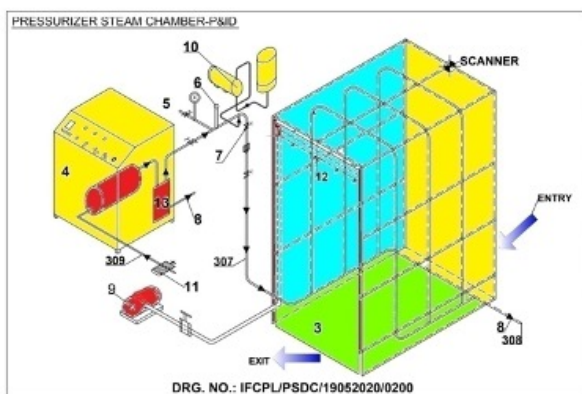
minutes. From an above-mentioned observational study result reveals that in group 1 patients didn't show any sign of progression to Covid-19 symptoms after 14 days to 2 months. In group 2 mild symptoms regressed in 3 days to normal and in moderate symptoms, it took 7 to 10 days to return to normal. Further, he concluded that steam temperature (70 to 80 degrees Celsius) would be unstable for the virus so that it will effectively kill SARS –Cov-2, and can be permitted as an adjunct to social distancing, sanitizers, masks, and PPE for effective treatment of covid-19 infection.

Due to its volatility, this essential oil could reach rapidly in an upper respiratory tract, lower respiratory tract by inhalation. *Gyorgyi Horwath et al* suggested that anti-viral activity of eucalyptus oil, tea grass oil, was tested by RT-PCR, Virus inhibition assay and the result reveals that, these two oils are effective virucidal agent by inhibiting the viral genome replication of HIV virus, Polio virus, dengue virus, Coxsackie B group virus.

*Ong G.H., et al* suggested that Antiviral effect of crude aqueous extracts of Neem leaves and Neem bark (*Azadirachta Indica*) were studied by in-vitro virus inhibition assay and the result reveals that Antiviral activity of Neem bark extract had antiviral activity at the concentration of 5.6 mg/ml.

In our present study other factors such as stability, exposure time, chemical composition, and concentration of essential oil in vapor phase incubation temperature, were tested through the reduction in bacterial count assay by Ministry of Micro, Small, and Medium Enterprises (MSME) and Société Générale de Surveillance (SGS) Chennai. In our further research work, an anti-viral efficacy of our compound was evaluated for baseline studies and the results are in progress for the validation study of the clinical trial with ethical committee approval.

**Fig 1: Flow chart of Pressurized steam Chamber**



**Materials required**

1. Electrical Boiler – 33KW.
2. Portable water.
3. Natural Extracts: Neem Oil, Eucalyptus Oil & Turmeric Oil along with emulsifier.
4. Dosing pump - 01 No
5. Water charging Pump – 01 No

**Dosing formulation**

- Turmeric oil - 4ml

- Eucalyptus oil - 8 ml
- Neem oil - 20ml

These oils are mixed in 4 L of water with an 8 ml emulsifier and dosed at 5 ml /min along with pressurized steam at ambient temperature at 65°C with humidity above 90%.

**Role of steam**

- Diaphoresis (sweating), Vasodilatory, analgesic, anti-spasmodic, sedative, anti-pyretic, expectorant effect.

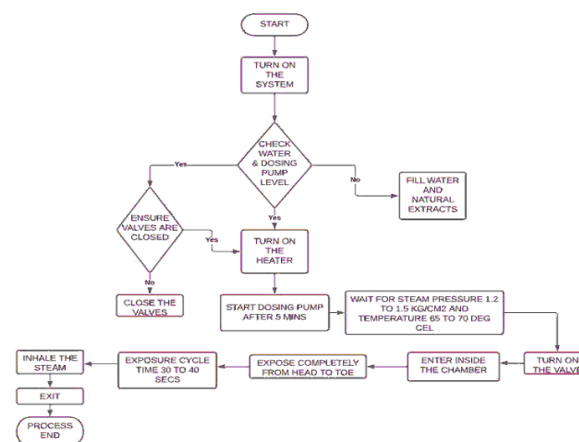
**Working Principle**

When a person walks near the chamber, the motion sensor detects before 2 to 3 feet and the sensor sends the signal to solenoid valves where the steam is produced at 140 to 150 °C Temperature and Pressure around 2.5 to 3 Kg/Cm<sup>2</sup> and stored at steam accumulator. Once, the above-mentioned criteria were fulfilled it starts opening the Steam, pass through PRV, and reduces the outlet pressure to 1.5 to 2 Kg/cm<sup>2</sup> and temperature around to 60 to 65 °C, dosing pump starts dosing the natural extract at the required PPM and sprayed through multiple nozzles within the chamber.

**Safety Features**

We use a safety valve in the main steam line which relieves during excess pressure as safety of the steam generator. Then a High Temperature & Pressure sensor, High & low water level sensor are used for Safety, 2 Pressure Gauges and 2 temperature Gauges are also used at Inlet and Outlet of the steam piping. The steam chamber is equipped with safety features like temperature sensor which has an automatic cut off/on when the steam temperature exceeds 70°-75°. Condensate water drops are being collected at the bottom of the drain pipe and by using Steam Trap valve the water drains out from system.

**Figure 2: Flow chart of the working principle**



**Methodology**

**Estimation of microbial load in the natural oil, emulsifier, formulation and distillate**

Initial microbial load in the natural oils, emulsifier, formulation, and distillate at 65° C was determined through total plate count by collecting the

swab samples in sterile containers before and after the disinfection at two intervals of 20 and 40 seconds for a period of three days to assess the efficacy of the steam chamber, PSC. A total of 55 swabs were taken along with control, each day to minimize the error. The swab sampling was done based on the standard method, ISO 18593:2014. The collected swab samples were placed inside a sampling box at 2-8 °C. After the completion of each day trial, the samples were brought to the SGS Chennai laboratory along with the travel controls and tested for the Total Plate Count to find out the microbial load by following the standard method, ISO4833-1:2013 (**horizontal method for enumeration of microorganisms that can grow and form colonies in a solid medium after aerobic incubation at 30 °C for 72 hrs**).

**Table1: Chemical composition of essential oil in emulsifier and water**

SI. No.	Natural oil	Water (L)	Emulsifier (L)	Quantity of natural oil added (L)
1	Neem oil	7	0.05	0.04
2	Eucalyptus oil			0.015
3	Turmeric oil			0.02

**Table 4: Day 1 (13.07.2020) Microbial load in the swab samples and the percentage reduction**

S . NO.	Type of Swab	Microbial load (Before and after disinfection, CFU/swab)				Microbial load Reduction %
		20 sec		40 sec		
		Before	After	Before	After	
1	Hand swab	230	-	-	-	34.78
2	Hand swab	-	150	-	-	
3	Hand swab	170	-	-	-	58.82
4	Hand swab	-	70	-	-	
5	Hand swab	-	-	1100	-	50.00
6	Hand swab	-	-	-	550	
7	Hand swab	-	-	130	-	30.77
8	Hand swab	-	-	-	90	
9	Hand swab	150	-	-	-	40.0
10	Hand swab	-	90	-	-	
11	Surface swab	80	-	-	-	100
12	Surface swab	-	<10	-	-	
13	Surface swab	150	-	-	-	46.67
14	Surface swab	-	80	-	-	
15	Surface swab	-	-	660	-	51.52
16	Surface swab	-	-	-	320	
17	Surface swab	-	-	110	-	100
18	Surface swab	-	-	-	<10	

**Table 5: Day 2 - Microbial load in the swab samples and the percentage reduction**

S . NO.	Type of Swab	Microbial load (Before and after disinfection, CFU/swab)				Microbial load Reduction %
		20 sec		40 sec		
		Before	After	Before	After	
1	Hand swab	610	-	-	-	78.69
2	Hand swab	-	130	-	-	51.22
3	Hand swab	410	-	-	-	
4	Hand swab	-	200	-	-	100.00
5	Hand swab	-	-	140	-	
6	Hand swab	-	-	-	<10	90.91
7	Hand swab	-	-	1100	-	
8	Hand swab	-	-	-	100	

## Results

**Table 2: Initial microbial load in the natural oil, emulsifier, formulation, and distillate**

S. No.	Sample name	Parameter	Method	Result
1	Neem oil	TPC	ISO4833-1:2013	<1
2	Turmeric oil	TPC	ISO4833-1:2013	<1
3	Eucalyptus oil	TPC	ISO4833-1:2013	<1
4	Emulsifier	TPC	ISO4833-1:2013	47000
5	Formulation	TPC	ISO4833-1:2013	20000000
6	Distillate at 65	TPC	ISO4833-1:2013	<1

### TPC: Total plate count

The initial microbial load in the natural oils, viz. Neem oil, Eucalyptus oil, and Turmeric oil are found to be <1 CFU / mL. Whereas, the emulsifier and the formulation (water + emulsifier + natural oils) used in the PSDC system showed the presence of bacterial colonies of 470000 CFU / mL and 20000000 CFU / mL, respectively. The distillate collected at 65 °C showed the absence of microbial load (<1 CFU / mL).

**Table 3: Control swab result**

SI. NO.	DAY	BATCH N O.	RESULT
1	DAY 1	13.7.2020- T1	<10 CFU / SWAB
2	DAY-2	14.7.2020 -T2	<10 CFU / SWAB
3	DAY-3	15.7.2020- T3	<10 CFU / SWAB

*Jegannathan Ganesan et.al., Anti – Microbial Efficacy of CO-AYUR Steam against Emerging diseases*

9	Hand swab	190	-	-	-	89.47
10	Hand swab	-	20	-	-	
11	Surface swab	50	-	-	-	100
12	Surface swab	-	<10	-	-	
13	Surface swab	260	-	-	-	76.92
14	Surface swab	-	60	-	-	
15	Surface swab	-	-	70	-	57.14
16	Surface swab	-	-	-	30	
17	Surface swab	-	-	80	-	62.50
18	Surface swab	-	-	-	30	

**Table 6: Day 3 - Microbial load in the swab samples and the percentage reduction**

S. NO.	Type of Swab	Microbial load (Before and after disinfection, CFU/swab)				Microbial load Reduction %
		20 sec		40 sec		
		Before	After	Before	After	
1	Hand swab	50	-	-	-	80.00
2	Hand swab	-	10	-	-	
3	Hand swab	70	-	-	-	71.43
4	Hand swab	-	20	-	-	
5	Hand swab	-	-	250	-	100.00
6	Hand swab	-	-	-	<10	
7	Hand swab	-	-	8400	-	78.57
8	Hand swab	-	-	-	1100	
9	Hand swab	190	-	-	-	89.47
10	Hand swab	-	20	-	-	
11	Surface swab	40	-	-	-	100
12	Surface swab	-	<10	-	-	
13	Surface swab	30	-	-	-	100
14	Surface swab	-	<10	-	-	
15	Surface swab	-	-	100	-	60
16	Surface swab	-	-	-	40	
17	Surface swab	-	-	20	-	100
18	Surface swab	-	-	-	<10	

The results from the analysis of swab samples (hand swab and surface swab) collected for the estimation of Total plate Count showed that there is a reduction in microbial load when exposed to the Steam generated by Pressurized Steam Chamber (PSC) at both the time intervals, 20 and 40 seconds.

**Table 7: The percent reduction of TPC at 20 and 40 seconds**

Samples	% Reduction @ 20s			% Reduction @ 40 s		
	Day 1	Day 2	Day 3	Day1	Day2	Day3
Hand swab	34.78	78.69	80.00	50.00	100.00	100.00
	58.82	51.22	71.43	30.77	90.91	78.75
	40.00	89.47	-	-	-	-
	Reduction range = 34.78% - 89.47%			Reduction range = 30.77% - 100.00%		
	Average percent reduction = 63.05			Average percent reduction = 75.04		
Surface swab	100.00	100.00	100.00	51.52	57.14	60.00
	46.67	76.92	100.00	100.00	62.50	100.00
	Reduction range = 46.67 % -100 %			Reduction range = 51.52% - 100%		
	Average percent reduction = 87. 26			Average percent reduction = 71. 86		

The results from the hand swabs collected before and after the exposure time of 20 sec., the percent reduction in the TPC ranged from 34.78% - 89.47% with an average reduction of 63.05% was observed. The results from the hand swabs collected before and after the exposure time of 40 sec., show the percent reduction in the TPC ranged from 30.77% - 100.00% with an average reduction of 75.04%.

**Protocol for Efficacy evaluation of Pressurized Steam Chamber (PSC) In MSME Fungus and bacteria**

1. Sanitizing against fungus and bacteria was checked in the interval of 20 and 30 seconds.
2. Developed Fungus and bacteria plates were placed in PSDC-6 with the above- mentioned intervals.
3. Sanitized plates after completion of the incubation were re-incubated to measure the effectiveness of steaming.

4. Results was recorded in log reduction with observed fungal and bacterial growth.

MSME report reveals that there is a significant inhibition in bacteria and fungus in time-dependent manner.

ULR No.: TC61582000001440

Description of sample: Pressurized Steam Disinfection Chamber  
Identification: Model: PSDC-6

Name of the Microorganism(s)	Escherichia coli (DMFCC-643)	Staphylococcus aureus (DMFCC-99)	Enterococcus faecalis (DMFCC-439)	Aspergillus brasiliensis (DMFCC-1349)
N <sub>0</sub>	6.37	6.84	6.44	7.5
(Values in Log)				
Contact time	20 sec / 40 sec	20 sec / 40 sec	20 sec / 40 sec	20 sec / 40 sec
N <sub>t</sub>	5.30 / 4.99	5.30 / 4.99	5.32 / 5.08	7.18 / 7.13
(Values in Log)				
R %	90.8 / 95.2	90.7 / 95.5	92.5 / 95.6	83.1 / 84.8
Log R	1.02 / 1.42	1.04 / 1.35	1.12 / 1.86	0.72 / 0.77

Note:  
N<sub>0</sub>: Initial population (In Log value)  
N<sub>t</sub>: Number of cells at the end of the contact time (Time: 20 sec and 40 sec).  
R%: Percentage Reduction =  $[(N_0 - N_t) / N_0] \times 100$ .  
Log R: Log Reduction (where  $R = 10^{N_0 - N_t}$ )

Date of test: 09.06.2020 to 20.06.2020  
Environmental condition: Humidity: 22.7  
Place of Testing: At Site and Laboratory.  
Test Procedure followed: Test has been carried as per test method provided by customer.  
Insulated plates of Fungus and Bacteria mentioned above were exposed to the environment of Steam Chamber for the time mentioned and results are provided.

Authorised Signatory: [Signature]

### Conclusion

Based upon our conducted study we conclude that essential phytotherapeutic oils with steam could be used as effective disinfectants in any public and private sector to control respiratory tract infection and improve the relief of symptomatic patients, due to their anti-bacterial and anti-viral activity, which was observed at optimized vapour phase condition in the pressurized disinfectants.

### Conflict of interests

The authors declare that there is no conflict of interest.

### Acknowledgments

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### References

- Cascella M, Rajnik M, Cuomo A, et al. 2020. Features, Evaluation, and Treatment of Coronavirus. Stat Pearls. NCBI.
- Sunlight, Heat And Humidity Weaken Coronavirus: US Official <https://www.businessleague.in/2020/04/24/sunlight-heat-and-humidity-weaken-coronavirus-us-official>.
- Coronavirus in Mumbai: Study shows positive results via steam therapy on asymptomatic and symptomatic patients By Swapnil Mishra.
- Steam Sterilization Guideline for Disinfection and Sterilization in Healthcare Facilities (2008)
- Enforcement Policy for Sterilizers, Disinfectant Devices, and Air Purifiers During the Coronavirus Disease 2019 (COVID-19) Public Health Emergency.
- Horvath G and Ács K. ‘ Essential oils in the treatment of respiratory tract diseases highlighting their role in bacterial infections and their anti-inflammatory action: a review’ Flavour Frag. J.2014; 30 : 331-341. <https://doi.org/10.1002/ffj.3252>.
- Pirtle E.C. and Beran G.W., ‘Virus survival in the environment’ Rev. Sci. Tech. 1991; 10 (3) : 733-748. <https://doi.org/10.20506/rst.10.3.570>.
- Moghadamtousi S. Z., Khadir H. A., Hassandarvish P., Tajik H., Abubakar S. ‘A Review on Antibacterial, Antiviral, and Antifungal Activity of Curcumin’. Bio. Med . Res. Int., 2014; 64 : 1-12. <https://doi.org/10.1155/2014/186864>.
- Ong G.H. Syamsiah A.S., Hasrul A.H., Maizatul Z., Jihan R., ‘Antiviral Effect Of Aqueous Neem Extract From Branches Of Neem Tree On New Castle disease virus. Mal. J. Veter. Res., 2014; 5(2): 5-9.

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