Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(3): 1171-1175
Received: 11-03-2020
Accepted: 15-04-2020

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Herbal hand Sanitizer for management of Indoor bioaerosol and touch surfaces

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Abstract
Objective: The present research paper is aimed at formulate effective herbal biocide which can disinfect Indoor air, hands and touch surfaces using the herbal extracts from plants of *Curcuma longa* rhizome and *Azadirachta indica*.
Methodology: The extracts of dried *Curcuma longa* rhizome and dried *Azadirachta indica* leaves was extracted using ethanol followed by formulation of herbal sanitizer. The efficacy of sanitizer was evaluated by enumerating the bioaerosol before and after spraying in indoor air and wiping of touch surfaces for its antibacterial activity
Results: The results recorded showed that HS-3 is antibacterial in nature with 97% of impact on bacterial aerosol in Indoor air and on touch surfaces.
Conclusion: The author concludes the possible reason for killing or inactivation of bacterial bioaerosol may be due to presence of Curcumin which contains aromatic phenol ring along with carboxylic functional group and Azadirachtin contains highly oxidized tetranor triterpenoid chemical.

Keywords: Bioaerosol, herbal sanitizer, antibacterial effect, contact time, biocide, touch surfaces

1. Introduction
Human Covid-19 has become global health concern spreading from human to human. Humans are facing biggest threat by many airborne pathogens such as Novel corona virus-19 SARS-2. The confirmed cases of COVID -19 as for now are 70,756 in India (MoHFW, 2020) [1]. A people infected with Novel corona virus may not show symptoms initially and incubation time is between 2-10days (WHO, 2020) [2]. The spread of infection is by droplets carrying infectious organisms from sneezing or coughing by a carrier or infected person. A corona virus was seen in the feces (Clarke *et al*., 1979) [3]. When the corona virus infected person uses toilets they remain on toilet surfaces and may get aerosolized during flushing activities.
These droplets remain in the air as bioaerosol carrying corona virus for few minutes or deposited on the surface as infectious agent. If a person touches the surface and touches their face or nose they get infected. Human corona virus can remain infectious on inanimate surfaces of room temporally for up to 9 days at 30˚C. Touch surfaces are very critical and potential source of viral transmission. The WHO recommends 0.1% sodium hypochlorite (NaOCl), 62 to 71% ethanol/min or 0.5% hydrogen peroxide to ensure effective surface cleaning and disinfection procedure. Calcium and Sodium hypochlorite are salts of hypochlorous acid (HOCl) as biocide agent (WHO, 2020) [4]. They are broad-spectrum biocides and are effective against all bacteria, fungi, algae and virus. They act by oxidizing proteins, interfering with respiration and metabolism, and destroying cell walls after ell wall destruction or virus capsule destruction the virus becomes avirulent (Sykes, 1939) [5] During the process of rupture of cell or virus the cell originals and genetic materials remains on the surface leaving behind endotoxins.(Hurley, 1992) [6].
For disinfection of water and water carrying pipes, salts of hypochlorous acid (HOCl) can be used as Biocides. They are most effective when the pH of the water is between 6.5 and 7.5, requiring a short (one hour) contact time at this pH range. Hypochlorite salts are easier to handle than chlorine gas, but have the same limitations. They are also more expensive than chlorine gas. They are often alternated with polymeric quaternary ammonium compounds. (Khayum *et al*., 2011) [7] Other types of biocides found in nature are medicinal plants which are in practice from thousands of years. The medicinal value of drug plants is due to the presence of certain phytochemicals in the plant tissues which have a definite physiological effect on the human body. These chemicals include alkaloids, flavanoids, glycosides, tannins, gums, resins, essential oils, fatty oils, carbon compounds, and hydrogen, oxygen and nitrogen salts of some chemicals which are ultimately ecofriendly. The rich resource is decreasing at an alarming rate as a result of over-exploitation.

In response to the World Health Organization directives, the practice of complementary and alternative medicine is now growing in developing countries, ultimately resulting in several pre-clinical and clinical studies that have provided the scientific basis for the usefulness of many plants used in folk medicine to treat infections. (Vijaya & Ananthan, 1997; Dilhuydy & Patients, 2003) [8, 9]. Azadirachta indica (Neem) is a widely respected tree with many beneficial effects and applications, particularly known amongst diverse medicinal treasures for its extraordinary therapeutic and ethno-medicinal values for humanity. Neem is considered to be "India's free flower," "wonder flower," "Nature's drug store," "Divine tree," "heal everything," "Materia medica" and "Panacea of all diseases." Several parts of Neem tree has medicinal properties and hence can be used in many ways (Tiwari, 2014) [10].

Plants and plant products have been used extensively in the past to treat medical problems. Numerous studies have been performed to collect different natural products for antimicrobial screening but research has not been intensively focused on the combinations of these items for their enhanced antimicrobial activity. The common practice is the use of herbs and medicinal plants as the first medicines. Every culture on Earth has relied on the vast variety of natural chemistry found in plants for their medicinal properties, through written or oral tradition. All herbal medicines are substances derived from plants with a specific therapeutic function. The use of herbal plants as traditional health remedies is the most common in Asia, Latin America and Africa, majority world's population has reported to have minimal side effects (Khatoon *et al*., 2017) [11].

The plant extracts *Azadirachta indica*, *Vitex negundo*, *Gingiber officinale* and *curcuma longa* was examined with experimental organisms *Desuflovibrio desulfuricans* and *Pseudomonas aeruginosa* in situ conditions. With these primary results of earlier study, present study opted for *Azadirachta indica* and Curcuma longa extracts for formulation of herbal sanitizer. Therefore, this study is to find Herbal sanitizer with combination of herbal extracts which are alcohol based. The herbal extracts of selected plant species were tested with different concentration by varying volume of herbal extract and ethanol. To optimize herbal sanitizer formulation for optimum action against bacterial aerosol and touch surfaces in the laboratory condition.

2. Methodology

The present study is to develop a cost effective, Ecofriendly and easily available herbal sanitizer which can be used as Herbal Hand sanitizer, surface cleaner and air spray to inactivate infectious Bioaerosols.

2.1 Collection of Plant Material

Fresh leaves of *Neem* (Azadirachta *indica*) free from disease were collected from Biopark, of Jnanabharathi campus, Bangalore University. Rhizomes of *Turmeric* (*Curcuma longa)* rhizome were collected from the model agroecology farm "Amrita Bhoomi" situated in Chamarajanagar- Biligiri Rangana Hills Road, Hondarabalu in Karnataka state, India. The leaves and rhizomes were thoroughly washed 2-3 times with running water and once with sterile distilled water and rhizomes and leaves were then air dried in shade on sterile blotting paper for a week and then powdered with the help of blender and sewed with 2mm mesh size. And fine powder is stored for further use.

2.2 Solvent Extraction: extraction of Curcumin content from *Curcuma longa and Azadirachtin from Azadirachta indica* was carried out by following method prescribed by Harborne, (1998) [12] with little modification. Thoroughly washed dried rhizome of *Turmeric* (*Curcuma longa)*, leaves of *Neem, (Azadirachta indica*). 1g of shade dried powder of each plant was accurately weighed separately and to which 100ml of 80% ethanol was added and sonicated for 60 minute for the extraction and filtered using what's man filter paper. The solvent extract was preserved at 5^0C in air tight bottle until further use.

2.3 Formulation of herbal sanitizer: The herbal sanitizer was formulated by mixing 5ml of *Curcuma longa rhizome* extract (CLE), 5ml of *Azadirachta indica* leaf extract (AIE) and 10ml of 80% ethanol to 80ml of distilled water. The obtained solution was used to disinfect touch surfaces and as air sanitizer. The different concentration herbal sanitizer was prepared by varying volume of herbal extract and ethanol. The different concentration were 3ml,5ml,7ml and 9ml of each herbal extract and volume of 80% ethanol and volume of Distilled water were kept contestant.

Table 1: Herbal sanitizer formulation with different concentration

Sr. No	Herbal sanitizer concentration	Abbreviation spray
1	10ml -80% ethanol added to 80ml of distilled water (Blank)	HS-B
2	3ml- CLE+3ml- AIE+10ml 80% ethanol +80ml of distilled water	HS-1
3	5ml- CLE+5ml- AIE+10ml 80% ethanol +80ml of distilled water	HS-2
4	7ml- CLE+7ml- AIE+10ml 80% ethanol +80ml of distilled water	HS-3
5	9ml- CLE+9ml- AIE+10ml 80% ethanol +80ml of distilled water	HS-4

2.4 Evaluation of Biocide efficiency of herbal sanitizer: The Herbal sanitizer was tested by following methods of US EPA (1980) [13], the touch surfaces were identified and listed according to usage like office tables switch board, toilet handles, doors and mobile screens were tested before and after application of herbal sanitizer. The herbal sanitizer is sprayed using spray bottle and tested indoor of various dimension during working hours at all sampling sites which were under consideration. Before carrying out the herbal sanitizer spray test, all ventilations of the sampling sites were closed. Bacterial bioaerosol sampling was carried out. The sampler was set at a height of 147cm above the ground level to collect samples of representative of the breathing zone. Herbal sanitizer spray are sprayed in the corner and center of the rooms with nozzle pointing upward the number of spray were determined according to the room dimension and care was taken for uniform dispersion of herbal sanitizer all along the breathing zone in the sampling site. Air samples were taken before spraying and 5 minutes time gap was given for reaction and contact time was given for effective sanitization of air, again sampling was carried out after 5 and 30 minutes for bacterial aerosol.

2.5 Measurement of Indoor environmental conditions

Indoor Air Sampling was carried out following, ASTM (2014) E1370-14 [14] Preliminary examination of sampling sites was carried out and standard temperature, relative humidity, carbon dioxide and carbon monoxide concentration

was measured using wet and dry bulb thermometer and expressed in degree Celsius, relative humidity was expressed in percentage.

2.6 Sampling and analysis of indoor bacterial aerosol

Indoor bacterial aerosols sampling was carried out following methods prescribed by Anderson, (1958) and Lindsey *et al*., (2017) [15, 16]. Anderson single stage Microbial air Sampler HiMedia (LA474) was used to collect indoor air sample of bacteria by adjusting the flow rate to 28.7 L/min and sampled for 1 min on sterile nutrient agar plates. Bacterial plates were incubated at 37°C and colonies were counted after 24 hrs and 48 hrs.

Table 2: Sampling Site Room Dimension Number of Spray

Sr. No	Sampling sites	Room dimension	Number of spray
1	Toilet	6ftX 9ft	25
2	Seminar Hall	10ftX24ft	35
3	Office Room	10ftX8ft	10

2.7 Enumeration of bacterial organisms

Enumerations of bacterial organisms were carried out following APHA, (2012) [17] by Standard plate count (viable count) method. A viable cell is described as a cell capable of dividing and constituting a population (or colony). Using Digital microbial colony counter (Model DCC100) is used for viable cell count. Total numbers of viable cells are reported as colony forming units (CFUs). Estimation of microbial numbers by CFU assumes that every colony is separate and founded by a single viable microbial cell (Goldman *et al*., 2008) [18]. Colony forming unit (CFU) represents a rough estimate of the amount of viable bacteria or fungal cells in a sample.

3. Results

3.1 Formulation of herbal sanitizer

The herbal sanitizer fig-1 has not shown any distinct smell or odour of Curcumin or Azadirachtin. The colour of the prepared herbal sanitizer was pale yellow.

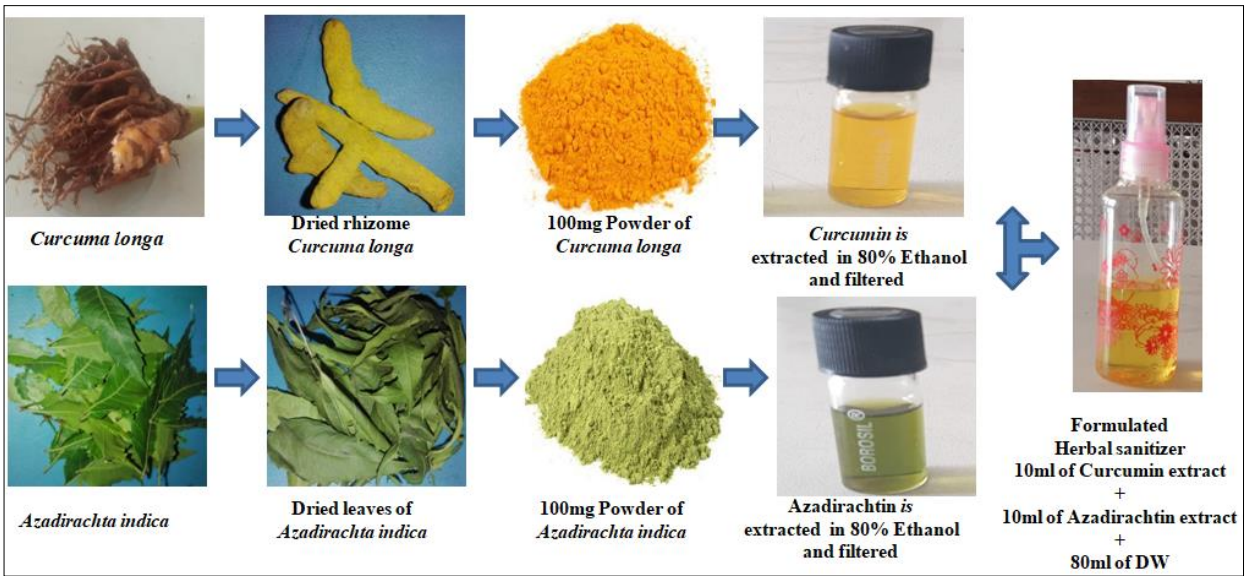

Fig 1: Efficiency of herbal sanitizer in indoor air against bacterial aerosol

3.2 Indoor environmental factors

The important indoor thermal comfort factors influencing indoor environment are temperature, relative humidity and air speed. ASHRAE (2013) [19] guidelines recommends indoor temperature range as minimum 20°C and maximum 26.6 °C. The results (Table-2) of indoor thermal comfort factors of different sampling sites before and after spray of herbal sanitizer is discussed in detail. The average temperature and Relative Humidity was recorded in toilet before spray was 26.5 °C and 54.8% and after spray was 26.0 °C and 56% respectively. In Seminar hall the average temperature and Relative Humidity was recorded before spray was 27 °C and 44.5% further after spray was 26.2 °C and 46%. Similarly average temperature in office room before spray was recorded as 26.5 °C and after spray 25.8 °C, relative humidity recorded was 50% and after spray 52% respectively. Environmental factors within the dwelling that could influence the air temperature include the temperature of the surrounding surfaces, air movement, relative humidity and the rate of air exchange (ventilation). Temperature and humidity have a strong and significant impact on the indoor air quality; at a constant pollution level, the perceived air quality decreases with increasing air temperature and humidity. Similar findings have been reported by Arundel *et al*., (1986) [20] of the health effects of relative humidity in indoor environments suggests that, relative humidity increases the incidence of respiratory infections and allergies. Unless the relative humidity exceeds 60%, most species of fungi cannot grow. Relative humidity also affects the rate of formaldehyde off-gassing from building materials in the interior, the rate of sulfur and nitrogen dioxide formation of acids and salts, and the rate of ozone formation.

Table 3: Indoor environmental factors at different herbal test sites

Sr. No	Sampling Site	Average Temperature ASHRAE standard 2013 20-26.6°C		Average Relative Humidity ASHRAE standard 2013 30-60%	
		Before	After	Before	After
1	Toilet	26.5	26.0	54.8	56
2	Seminar hall	27	26.2	44.5	46
3	Office Room	26.5	25.8	50	52

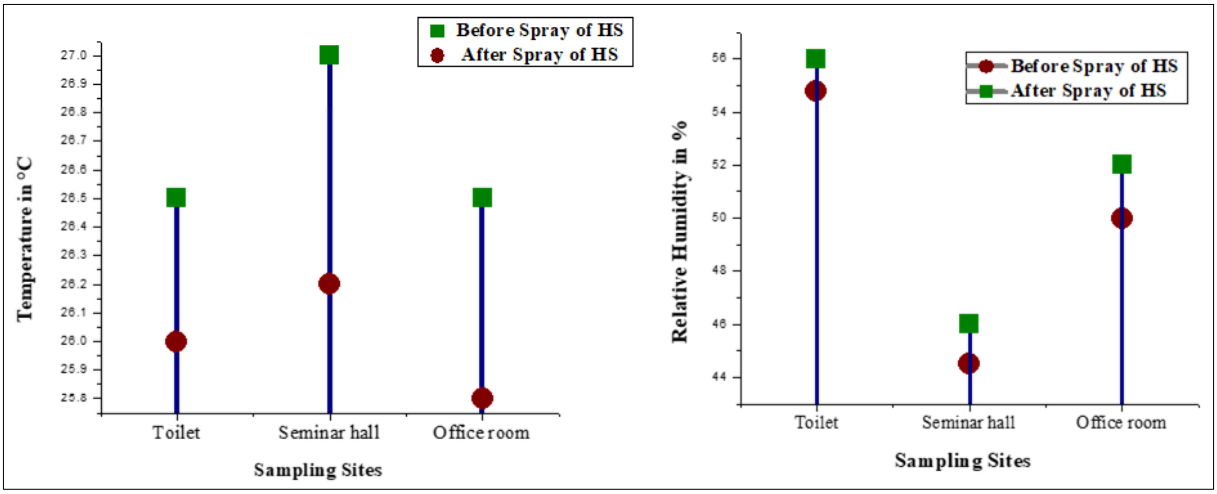

Fig 2: Indoor environmental factors at sampling sites

3.4 The efficacy study of herbal sanitizer

Efficiency of herbal sanitizer spray was carried out at sampling sites (table-4) against bacterial aerosol. The average airborne bacterial aerosol in toilets ranged between 540-610 CFU/m^3, similarly the average airborne bacterial aerosol ranged between 280-400 CFU/m^3 in seminar hall. In office room airborne bacterial aerosol was recorded 240 to 450 CFU/m^3. The possibility of occurrence of pathogenic bacteria in toilets *Micrococcus sp., Staphylococcus sp. and Bacillus sp Pseudomonas sp., Staphylococcus sp. Legionella sp.,* and *Klebsiella sp.* have been investigated in early studies by Shilpashree Mayachar and Nandini, (2020) [21]. For estimating optimum concentration for herbal sanitizer to show highest efficiency against bacterial aerosol HS-B, HS-1, HS-2 and HS-3 was evaluated Table -4. The bacterial aerosol reduced to 26% after spraying HS-B which contain 10% Ethanol were as the bacterial aerosol reduced by 68%, 76% and 80% after 5minutes. The bacterial aerosol reduced by 90%, 94% and 97% after 30 minutes by HS-1, HS-2 and HS-3 respectively. Therefore the optimum concentration of CLE and AIE in the herbal sanitiser formulation was 9ml CLE and 9ml AIE along with 10ml Ethanol and 80ml Distilled water i.e. HS-3. The overall Efficiency of HS-3 spray has shown highest reduction of bacterial aerosol in indoor environment.

Table 4: Reduction of Bacterial aerosol after spraying of HS of different concentration and its Efficiency

Sr. No	Sampling sites	HS-B	HS-1	HS-2	HS-3	HS-B	HS-1	HS-2	HS-3	HS-B	HS-1	HS-2	HS-3
		Bacterial aerosol Count (CFU/m^3) Before Spray of HS				Bacterial aerosol Count (CFU/m^3) After 5minutes				Bacterial aerosol Count (CFU/m^3) After 30minutes			
1	Toilets	600	540	610	600	400	150	130	80	380	25	26	12
2	Seminar Hall	350	280	400	290	240	82	75	50	200	30	20	8
3	Office Room	450	360	410	240	382	130	125	70	350	45	25	10
4	Average Efficiency of HS					26%	68%	76%	80%	33%	90%	94%	97%

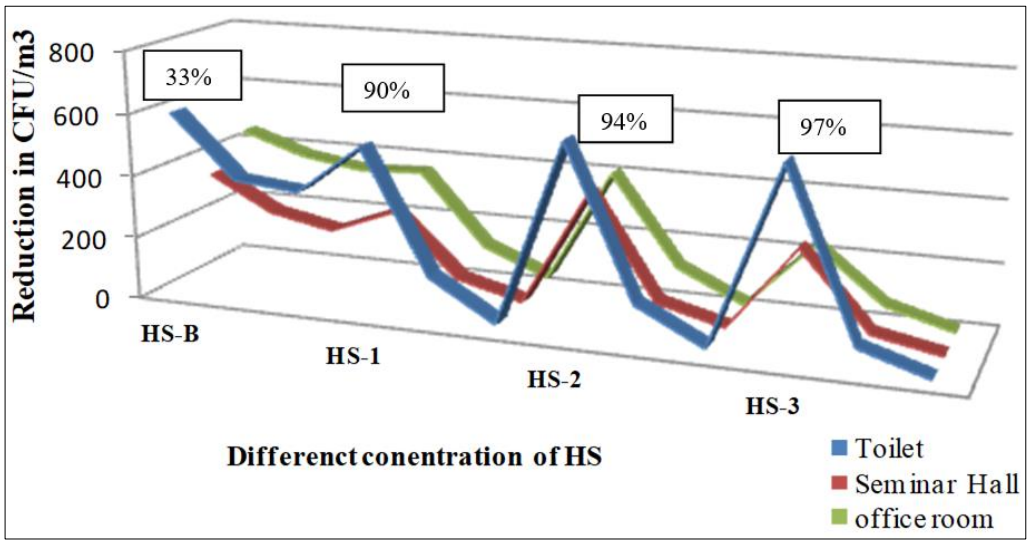

Fig 3: Reduction of Bacterial aerosol after spraying of HS of different concentration and its Efficiency

3.4 Efficiency of herbal sanitizer on touch surfaces

The efficiency of herbal sanitizer before and after application is carried out by swabbing the surface using sterile cotton swabs on to sterile nutrient agar plates under aseptic condition. The touch surface of the tables were found to be 95% sterilized after application 0.25 ml of herbal air sanitizer, the switch board surface was cleaned 90% after application of 0.25ml of herbal air sanitizer, similarly, mobile screen 100% sanitization was achieved after application of 0.25ml of herbal sanitizer and on door Handle surfaces 93% sanitization was

achieved after 1minute of application, necessary precaution was taken applying alcohol sanitizer. The possible reason for redaction of bacterial concentration in indoor air and on touch surfaces is due to the presence Azadiractine chemical in the Herbal sanitizer. Medicinal plants have become a major source of new chemical entities to develop novel therapeutic agent. Plant like *Azadiractine indica* has shown antiviral activity against several viruses and bacteria of Dhawan, (2012) [22].

4. Conclusion

The present world is facing problems raised due to contagious diseases caused by pathogenic microbes and viruses. These organisms find their way into the human body through air by getting attached to airborne particles and inert surfaces. They are viable on these surfaces for few minutes to many hours. Therefore, it becomes very important for humans to repeatedly perform sanitization of Indoor air and surfaces which they frequently come in contact. Many sanitizers are available which are made up of harmful chemicals, which can bring skin problems.

The herbal sanitizers with the easily available herbal extracts were found to be highly efficient as air spray, hand sanitizer and for surface sterilization. The possible reason for killing or inactivation of bacterial bioaerosol may be due to presence of Curcumin which contains aromatic phenol ring along with carboxylic functional group and Azadirachtin contains highly oxidized tetranor triterpenoid chemical together with Ethanol which is proven to be anti viral, preventing them from spreading to cells and as antibacterial resulted in efficient inactivation of touch surfaces on airborne bacterial Bioaerosol In the past. Experimental evidences with small pox, chicken pox, fowl pox and Herpes virus have proven that Neem is quite effective for preventing by absorbing viruses, inhibiting its multiplication and prevent them from spreading. Therefore, it is recommended that this formulation can be used for the present situation to prevent spreading of covid-19, as this is an herbal, green formulation it has no negative environmental and health impacts.

5. Acknowledgement

The author acknowledges Bangalore University and Department of environmental science to prove opportunity to carry out this work.

6. References

1. MoHFW. Ministry of health and family welfare: Home Page Government of India, 2020. URL:https://www.mohfw.gov.in/index.php (Accessed 05.13.2020)
2. World Health Organization. (2020). Corona virus disease (COVID-19): situation report, 2019, 67.
3. Clarke SK, Caul EO, Egglestone SI. The human enteric corona viruses. Postgraduate medical journal. 1979; 55(640):135-142.
4. World Health Organization. Infection prevention and control guidance for long-term care facilities in the context of COVID-19: interim guidance, 2020. (No. WHO/2019-nCoV/IPC_long_term_care/2020.1). World Health Organization.
5. Sykes G. The influence of germicides on the dehydrogenases of Bact. Coli: Part I. The succinic acid dehydrogenase of Bact. Coli. Epidemiology & Infection. 1939; 39(4):463-469.
6. Hurley JC. Antibiotic-induced release of endotoxin: a reappraisal. Clinical Infectious Diseases. 1992; 15(5):840-854.
7. Khayum A, Nandini N, Chandrashekar JS, Durgesh R. Assessment of Drinking Water Quality of Bangalore West Zone, India-a case Study. Environ. We. Int. J Sci. Tech. 2011; 6:113-122.
8. Vijaya K, Ananthan S. Microbiological screening of Indian medicinal plants with special reference to enteropathogens. The Journal of Alternative and Complementary Medicine. 1997; 3(1):13-20.
9. Dilhuydy JM. Patients' propensity for complementary and alternative medicine (CAM): a reality which physicians can neither ignore nor deny. Bulletin du cancer. 2003; 90(7):623-628.
10. Tiwari R, Verma AK, Chakraborty S, Dhama K, Singh SV. Neem (*Azadirachta indica*) and its potential for safeguarding health of animals and humans: A review. Journal of Biological Sciences. 2014; 14(2):110-123.
11. Khatoon AKHTARI, Arzoo ATIA, Mohapatra ASHIRBAD, Bihari K. Studies on *in vitro* evaluation of antibacterial activities of Eucalyptus Globulus labill leaf. Int J Curr Pharm Res. 2017; 9(4):140-142.
12. Harborne AJ. Phytochemicals methods a guide to modern techniques of plant analysis. Springer science & business media, 1998.
13. EPA U. Innovative and alternative technology assessment manual, EPA US Environmental Protection Agency, Washington, DC. 1980; 430/9–78–009.
14. ASTM. E1370-14 Standard guide for air sampling strategies for worker and workplace protection. West Conshohocken, PA: ASTM International, 2014a.
15. Andersen AA. New sampler for the collection, sizing, and enumeration of viable airborne particles. Journal of Bacteriology. 1958; 76(5):471.
16. Lindsey WG, Green BJ, Blachere FM, Martin SB, Law BF, Jensen PA, Schafer M. Sampling and characterization of bioaerosols. NIOSH manual of analytical methods. 5th ed. Cincinnati (OH): National Institute for Occupational Safety and Health, 2017.
17. APHA. Standard methods for examination of water and waste-water, 22nd edn. American Public Health Association, Washington, DC, 2012.
18. Goldman JL. U.S. Patent Application No. 2008; 11:586-562.
19. American Society of Heating, Refrigerating and Air Conditioning Engineers, Inc (ASHRAE). ANSI/ASHRAE Standard Ventilation for Acceptable Indoor Air Quality, 2013, 62(1).
20. Arundel AV, Sterling EM, Biggin JH, Sterling TD. Indirect health effects of relative humidity in indoor environments. Environmental Health Perspectives. 1986; 65:351.
21. Shilpashree Mayachar K, Prof. Nandini N. Qualitative and quantitative evaluation of bioaerosol in selected public spaces International Journal of Biotech Trends and Technology. 2020; 10(1):60-66.
22. Dhawan BN. Anti-Viral Activity of Indian Plants. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. 2012; 82(1):209-224.