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Research Article

ANTIMICROBIAL ACTIVITY OF *APARAJITHA* VAPORIZER AND SPRAY

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ABSTRACT

Infectious diseases are responsible for an immense global burden that impacts public health systems and economies worldwide, disproportionately affecting vulnerable populations. Its control and prevention relies on a thorough understanding of the factors determining transmission. *Aparajitha Choornam* is a classical *Dhoopa yogam* used by traditional Ayurvedic physicians since past for fumigation which prevents the spread of infectious fever and also disinfects the air from pathogens. It is highly effective in disinfecting living spaces during the seasonal spread of flu, chicken guinea, chicken pox, measles etc. *Aparajitha* vaporizer and azep spray are two modified form of *Aparajitha dhoopachoornam* prepared out of same ingredients that offers more user friendly and round the clock action. The present study compares the antibacterial activity of *Aparajitha* vaporizer and surface spray with Streptomycin in gram positive and gram negative bacteria and antifungal activity with Clotrimazole in Aspergillus niger and Candida albicans. The method used was Agar Disc Diffusion Method. The zones of inhibitions were calculated and its triplicate was done. To analyse the statistical data, one-way ANOVA and Dunnets tests were performed. The antibacterial activity of streptomycin and samples using Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus and streptococus mutans were found to be nearly same. Similarly antifungal activity of samples and clotrimazole as standard drug with aspergillus niger and with candida albicans were nearly same. The formulation of vaporizer and spray is purely herbal and thus it is beneficial to replace these with chemicals containing the same. The health benefits of these herbs were also got by using this herbal spray and vaporizer and can confidently use in the rooms of pregnant women, infants, old people etc. Thus this work gives a scientific validation of antimicrobial activity of *Aparajitha* vaporizer and surface spray which is more customer friendly.

INTRODUCTION

Infectious disease has always a adverse effect to human and the transmission of pathogens can occurs in various ways including physical contact, contaminated food, body fluids, objects, airborne inhalation or through vector organisms[1]. Infectious diseases are sometimes called contagious when they are easily transmitted by contact with an ill person or their secretions (e.g., influenza)[2]. Transmission of infectious diseases may also involve a vector.

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A common strategy used to control vector borne infectious diseases is to interrupt the life cycle of a pathogen by killing the vector[3]. One of the ways to prevent or slow down the transmission of infectious diseases is to recognize the different characteristics, distance traveled by victims, and level of contagiousness[4]. Another effective way to decrease the transmission rate of infectious diseases is to recognize the effects of small-world networks. In epidemics, there are often extensive interactions within hubs or groups of infected individuals and other interactions within discrete hubs of susceptible individuals[5]. Despite the low interaction between discrete hubs, the disease can jump to and spread in a susceptible hub via a single or few interactions with an infected hub. Thus, infection rates in small-world networks can be reduced somewhat if interactions between individuals within infected hubs are eliminated[6]. However, infection rates can be

drastically reduced if the main focus is on the prevention of transmission jumps between hubs[7]. Infection control addresses factors related to the spread of infections within the health-care setting including prevention monitoring/investigation of demonstrated or suspected spread of infection within a particular health-care setting and management (interruption of outbreaks)[8].

In Ayurveda, from the ancient times, many medicinal plants and herbal formulations have been used for the benefit of the human healthcare system and curing various diseases[9]. The Ayurvedic formulations against microbes are mostly combination of different plant products, and those plant products themselves contain many constituents which together and collectively act against microbes. *Aparajitha Dhoopa choornam* is mentioned in Ayurvedic texts in the context of *Jwara Chikitsa* for fumigation. The fumigation prevents the spread of infectious fever and also disinfects the air from pathogens. *Aparajitha* vaporizer and surface spray are two modified forms of *Aparajitha dhoopachoorna* which helps to eliminate the cumbersome process of burning.

AIM AND OBJECTIVES

To determine antimicrobial activity of *Aparajitha* vaporizer and surface spray.

MATERIALS AND METHODS

Sample Collection

The herbal raw materials were procured from authentic sources and authenticated by *Dravya guna* experts. Foreign matter in the drugs were removed manually, cut into small pieces and washed, cleaned and subjected to drying under shade.

Sample Preparation

Aparajitha Vaporizer

Table 1: Ingredients of *Aparajitha* Vaporizer

Item no.	Sanskrit name	Botanical name	Quantity (For 10 L)
1.	*Guggulu*	*Commiphora mukul*	2kg
2.	*Dhyamakam- Mayurasikha*	*Actiniopetris dichotoma*	2kg
3.	*Vacha*	*Acorus calamus*	2kg
4.	*Sarja - Ralah*	*Shorea robusta*	2kg
5.	*Nimba*	*Azadirachta indica*	2kg
6.	*Arka*	*Calotropis gigantea*	2kg
7.	*Agaru*	*Ardisia solanacea*	2kg
8.	*Daru*	*Cedrus deodara*	2kg
9.	IPA	IPA	10 litres
10.	Fragrance		

Method of Preparation

1. Ethanolic extract of item no 1-8 was taken and mix it homogeneously.
2. Add the above to item no 9 and mix it homogeneously.
3. Add item no. 10 and mix.
4. Pack in appropriate containers.

Aparajitha Asep Spray

Table 2: Ingredients of *Aparajitha* Asep spray

Item no.	Sanskrit name	Botanical name	Quantity (For 10 L)
1.	*Guggulu*	*Commiphora mukul*	2kg
2.	*Dhyamakam- Mayurasikha*	*Actiniopetris dichotoma*	2kg
3.	*Vacha*	*Acorus calamus*	2kg
4.	*Sarja - Ralah*	*Shorea robusta*	2kg
5.	*Nimba*	*Azadirachta indica*	2kg
6.	*Arka*	*Calotropis gigantea*	2kg
7.	*Agaru*	*Ardisia solanacea*	2kg
8.	*Daru*	*Cedrus deodara*	2kg

9	IPA	IPA	10 litres
10	Fragrance		400ml
11	Camphor		400gm
12	Active aqua		3 liters
13	Glycerin		100ml
14	Propylene glycol		100ml
15	NaOH 10%		50ml
16	Isopropyl alcohol		5.950 litres

Experimental analysis - Antimicrobial Activity

Antibacterial assays- Agar Disc Diffusion Method[10]

Principle

The antimicrobials present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Materials Required

1. Muller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.8gm of the commercially available Muller Hinton Agar Medium (MHI Agar Media) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Nutrient broth (1L)

One litre of nutrient broth was prepared by dissolving 13gm of commercially available nutrient medium (HI Media) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

3. Sterile Whatman paper discs of diameter 10mm
4. Streptomycin (standard antibacterial agent, concentration: 10mg/ml)
5. Culture of test organisms; growth of culture adjusted according to McFarland Standard, 0.5%
   - *Pseudomonas aeruginosa* (ATCC27853)
   - *Staphylococcus aureus* (ATCC 25923)
   - *Enterococcus faecalis* (ATCC 29212)
   - *Streptococcus mutans* (ATCC 25175)

Procedure

Petriplates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture of *Pseudomonas aeroginosa, Enterococcus faecalis, Staphylococcus aureus* and *Streptococcus mutans* (growth of culture adjusted according to McFarlands Standard, 0.5%). Plates were placed with sterile paper discs having respective test samples. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the discs (NCCLS, 1993).

Antifungal assays - Agar Disc Diffusion Method[11]

Principle

In order to access the biological significance and ability of the sample, the antifungal activity was determined by Agar well diffusion method. The anti-fungals present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Materials Required

1) Potato Dextrose Agar Medium (1 L)

The medium was prepared by dissolving 39gm of the commercially available Potato Dextrose Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Clotrimazole (standard antifungal agent, concentration: 10mg/ml)

3. Culture of test organisms; growth of culture adjusted according to McFarland Standard, 0.5%

- *Aspergillusniger* (ATCC 16404*)*
- *Candida albicans* (ATCC 10231)

Procedure

Potato Dextrose agar plates were prepared and overnight grown species of fungus, *Aspergillusniger and Candida albicans* were swabbed. Plates were placed with sterile discs having respective test samples. The zone of inhibition was measured after overnight incubation at room temperature and compared with that of standard antimycotic (Clotrimazole) (NCCLS, 1993).

RESULTS AND DISCUSSION

Results of Antibacterial Assays

Gram Negative

Table 3: Organism: *Pseudomonas aeruginosa*

Concentration	Zone of inhibition (mm)
Streptomycin (100µg)	33
AVSS (100µL)	32
AVSS (100µL)	32

Gram Positive

Table 4*:* Organism: *Enterococcus faecalis*

Concentration	Zone of inhibition (mm)
Streptomycin (100µg)	23
AVSS (100µL)	19
AVSS (100µL)	19

Table 5: Organism: *Staphylococcus aureus*

Concentration	Zone of inhibition (mm)
Streptomycin (100µg)	20
AVSS (100µL)	22
AVSS (100µL)	22

Table 6: Organism: *Streptococcus mutans*

Concentration	Zone of inhibition (mm)
Streptomycin (100µg)	27
AVSS (100µL)	29
AVSS (100µL)	29

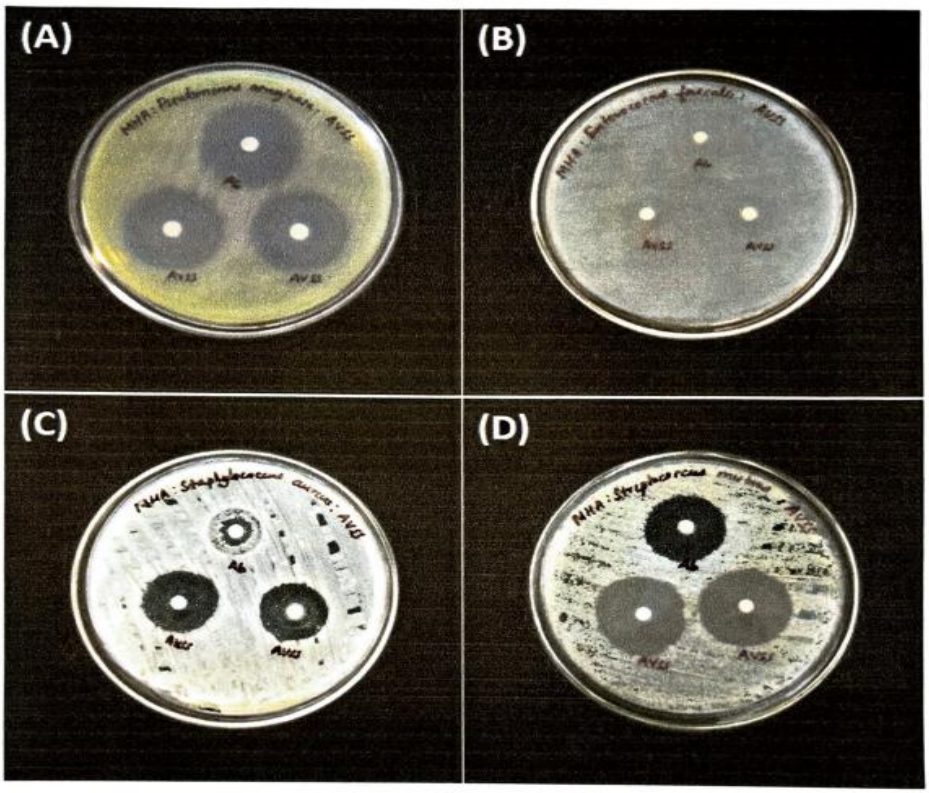

Figure 1: Figure: Photomicrographs portraying the antibacterial activity of AVVS against selected pathogens (A) Psuedomonas aeruginosa (B) Enterococcus faecalis (C) Staphylococcus aureus (D) Streptococcus mutans

Table 7: Statistical Analysis

Pseudomonas aeruginosa						
Concentration	**Zone of inhibition (mm)**	**Zone of inhibition (mm)**	**Zone of inhibition (mm)**	**Average**	**Std Dev**	**Std error**
Streptomycin (100µg)	33	33	33	33	0	0
AVSS (100µL)	32	31	31	31.33333333	0.57735	0.19245
AVSS (100µL)	32	32	31	31.66666667	0.57735	0.19245
Enterococcus faecalis						
Concentration	**Zone of inhibition (mm)**	**Zone of inhibition (mm)**	**Zone of inhibition (mm)**			
Streptomycin (100µg)	23	23	23	23	0	0
AVSS (100µL)	19	19	19	19	0	0
AVSS (100µL)	19	19	19	19	0	0
Staphylococcus aureus						
Concentration	**Zone of inhibition (mm)**	**Zone of inhibition (mm)**	**Zone of inhibition (mm)**			
Streptomycin (100µg)	21	23	23	22.33333333	1.154701	0.3849
AVSS (100µL)	25	25	25	25	0	0
AVSS (100µL)	25	25	25	25	0	0
Streptococcus mutans						
Concentration	**Zone of inhibition (mm)**	**Zone of inhibition (mm)**	**Zone of inhibition (mm)**			
Streptomycin (100µg)	27	27	27	27	0	0
AVSS (100µL)	29	28	28	28.33333333	0.57735	0.19245
AVSS (100µL)	29	29	28	28.66666667	0.57735	0.19245

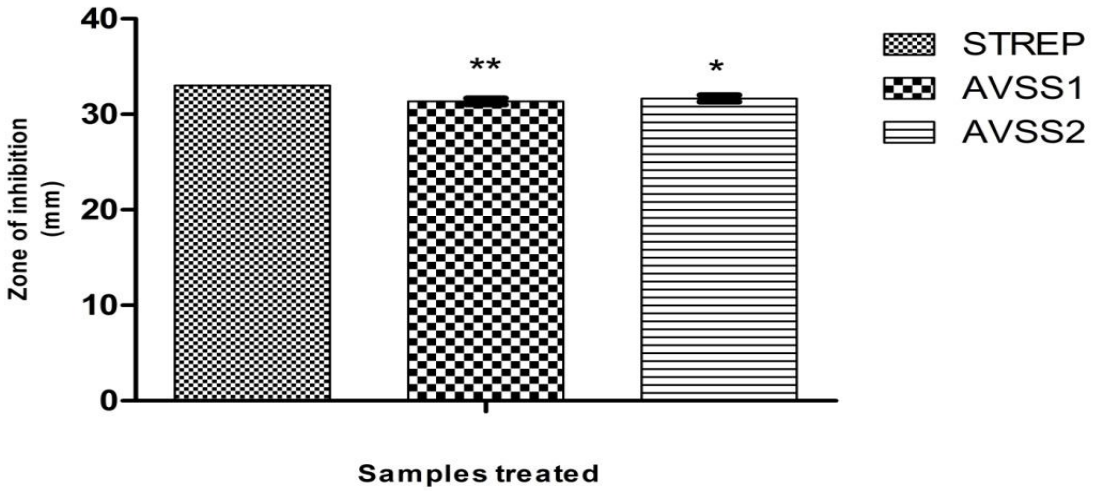

Figure 2: Graphical representation depicting the antibacterial effect of AVSS on *Psuedomonas aeruginosa* by Disc diffusion method. Along Y axis Zone of inhibition, Along X axis samples treated. All experiments were done in triplicates and results represented as Mean+/- SE. One-way ANOVA and Dunnets test were performed to analyse data. **p < 0.01 compared to streptomycin, *p < 0.1 compared to streptomycin

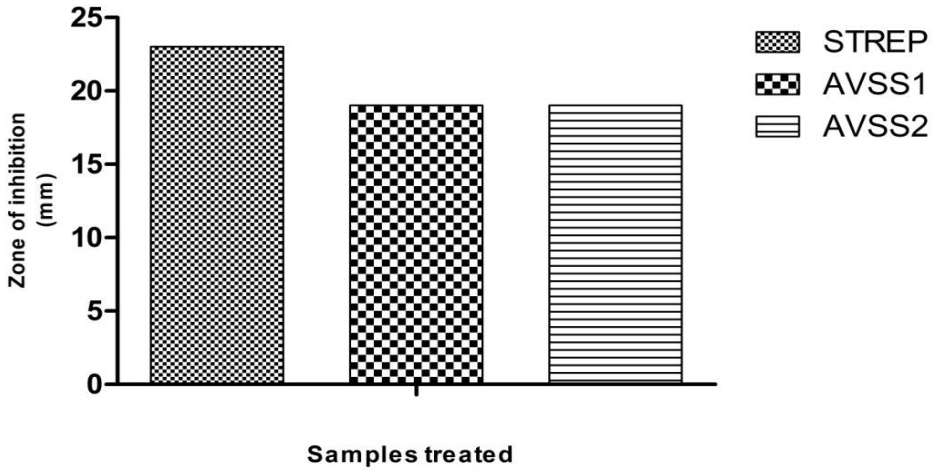

Figure 3: Graphical representation depicting the antibacterial effect of AVSS on Enterococcus faecalis by Disc diffusion method. Along Y axis Zone of inhibition, Along X axis Samples treated. All experiments were done in triplicates and results represented as Mean+/- SE. One-way ANOVA and Dunnets test were performed to analyse data.

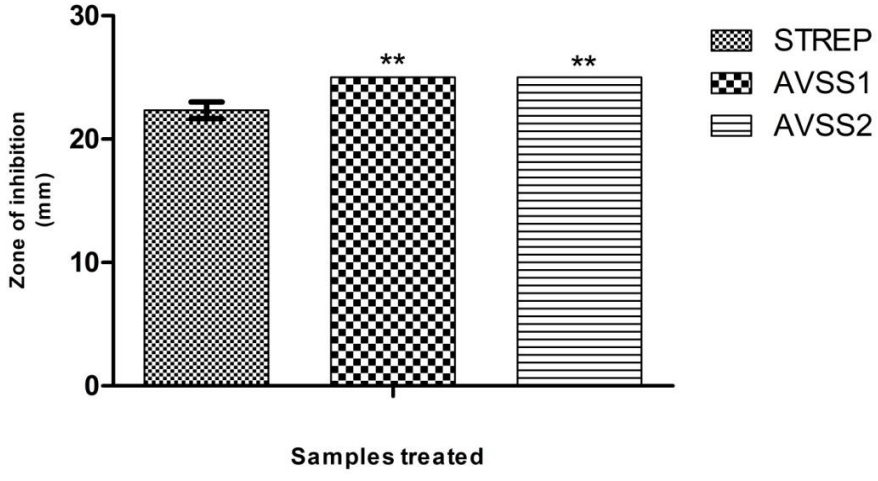

Figure 4: Graphical representation depicting the antibacterial effect of AVSS on Staphylococcus aureus by Disc diffusion method. Along Y axis Zone of inhibition, Along X axis Samples treated. All experiments were done in triplicates and results represented as Mean+/- SE. One-way ANOVA and Dunnets test were performed to analyse data. **p < 0.01 compared to streptomycin

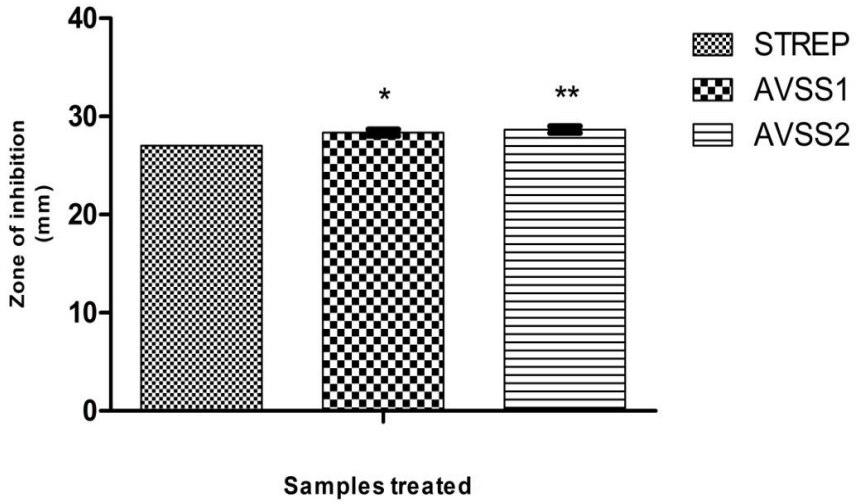

Figure 5: Graphical representation depicting the antibacterial effect of AVSS on Streptococcus mutans by Disc diffusion method. Along Y axis Zone of inhibition, Along X axis Samples treated. All experiments were done in triplicates and results represented as Mean+/- SE. One-way ANOVA and Dunnets test were performed to analyse data. **p < 0.01 compared to streptomycin, *p < 0.1 compared to streptomycin.

Results of Antifungal activity

Organism: *Aspergillus niger*

Table 8: *Aspergillus niger*

Concentration	Zone of inhibition (mm)
Clotrimazole (100µg)	23
AVSS (100µL)	22
AVSS (100µL)	22

Organism: Candida albicans

Table 9: *Candida albicans*

Concentration	Zone of inhibition (mm)
Clotrimazole (100µg)	28
AVSS (100µL)	18
AVSS (100µL)	18

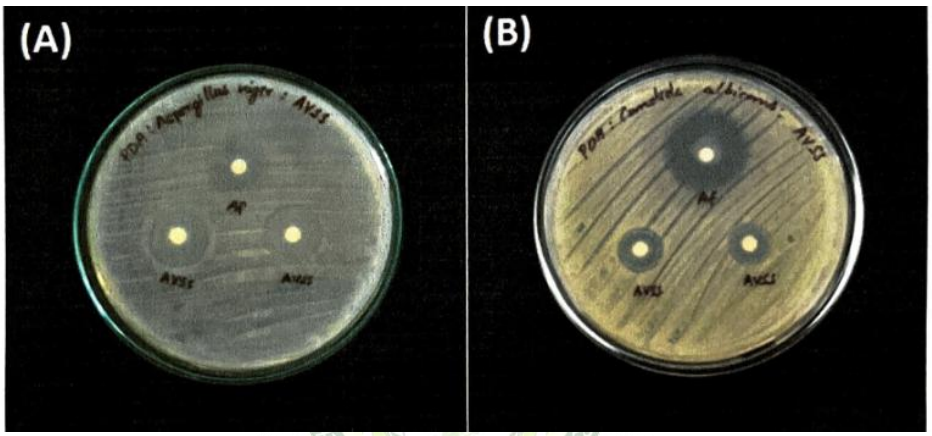

Figure 6: Photomicrographs portraying the antifungal activity of AVVS against selected pathogens (A) Aspergillus niger (B) Candida albicans

Table 10: *Aspergillus niger*

Aspergillus niger						
Concentration	**Zone of inhibition (mm)**	**Zone of inhibition (mm)**	**Zone of inhibition (mm)**	**Average**	**Std Dev**	**Std error**
Clotrimazole (100µg)	23	24	23	23.33333333	0.57735	0.19245
AVSS (100µL)	22	21	21	21.33333333	0.57735	0.19245
AVSS (100µL)	22	21	21	21.33333333	0.57735	0.19245
Candida albicans						
Concentration	**Zone of inhibition (mm)**	**Zone of inhibition (mm)**	**Zone of inhibition (mm)**	**Average**	**Stdev**	**Std error**
Clotrimazole (100µg)	33	26	27	28.66666667	3.785939	1.26198
AVSS (100µL)	18	18	18	18	0	0
AVSS (100µL)	19	19	18	18.66666667	0.57735	0.19245

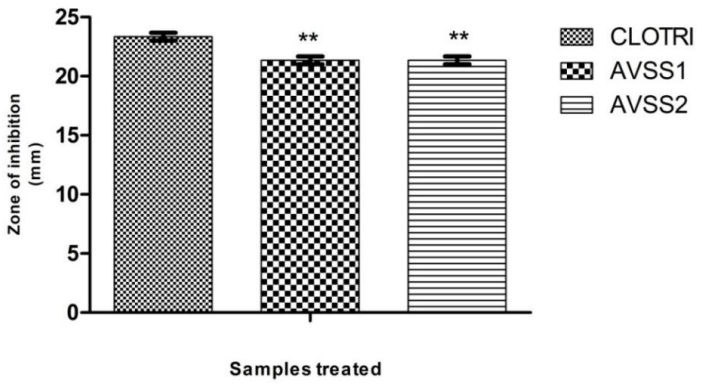

Figure 7: Graphical representation depicting the antifungal effect of AVSS on Aspergillus niger by Disc diffusion method. Along Y axis Zone of inhibition, Along X axis Samples treated. All experiments were done in triplicates and results represented as Mean+/- SE. One-way ANOVA and Dunnets test were performed to analyse data. **p < 0.01 compared to clortrimazole.

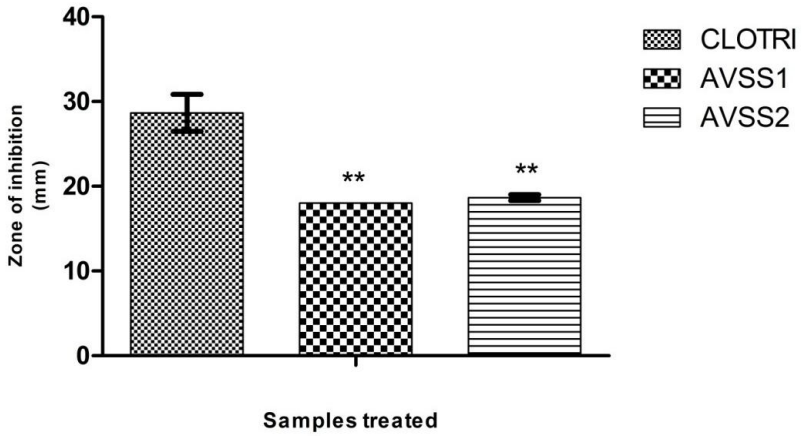

Figure 8: Graphical representation depicting the antifungal effect of AVSS on Candida albicans by Disc diffusion method. Along Y axis Zone of inhibition, Along X axis Samples treated. All experiments were done in triplicates and results represented as Mean+/- SE. One-way ANOVA and Dunnets test were performed to analyse data. **p <0.01 compared to clortrimazole.

DISCUSSION

Infection prevention and control (IP & C) practices are important in maintaining a safe environment for everyone by reducing the risk of the potential spread of diseases[12]. Microorganisms are known to survive on non-antimicrobial inanimate touch surfaces for extended periods of time[13]. This can be especially troublesome in hospital environments where patients with immune deficiencies are at enhanced risk for contracting nosocomial infections[14]. The immune system is an effective barrier against infectious agents. Whenever the pathogens may overwhelm the immune system's ability to fight them off, an infection becomes harmful[15]. Bacteria, viruses, fungi, and parasites are different types of pathogens. Bacterial infections can be treated with antibiotics. The emergence and spread of antibiotic resistance, as well as the evolution of new strains of disease causing agents, are of great concern to the global health community[16]. Effective treatment of a disease entails the development of new pharmaceuticals or some potential source of novel drugs. Commonly used medicinal plants of our community could be an excellent source of drugs to fight off this problem. Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been found *in vitro* to have antimicrobial properties[17]. *Aparajitha Dhoopa choornam* is a widely using *Dhoopayoga* which prevents the spread of infections and also disinfects the air from pathogens. *Aparajitha* vaporizer and surface spray are two modified forms of *Aparajitha dhoopachoorna* which helps to eliminate the cumbersome process of burning. The aim of the present study is to do invitro anti microbial activity of *Aparajitha* vaporizer and surface spray using Agar Disc Diffusion Method and compared with standard drug. In antibacterial assay it was compared with standard drug Streptomycin (standard antibacterial agent, concentration: 100µg) with sample drug in 100µL concentrations. Petriplates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture of *Pseudomonas aeroginosa, Enterococcus*

faecalis, Staphylococcus aureus and *Streptococcus mutans* (growth of culture adjusted according to McFarlands Standard, 0.5%). Plates were placed with sterile paper discs having respective test samples. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the discs (NCCLS, 1993). Pseudomonas aeruginosa is a gram negative bacteria. The zone of inhibition of standard drug streptomycin obtained was 33. In case of sample drug the average zone of inhibition obtained was 31.33333333 and 31.66666667 respectively i.e., for gram negative bacteria the zone of inhibition of sample drugs and standard drug was shown nearly same. Enterococus faecalis is a gram positive bacteria. The zone of inhibition of standard drug is 23 while that of sample drug was found to be 19 each i.e., nearly same. Staphylococcus aureus is a gram positive bacteria. The zone of inhibition of standard drug is 22.333 while that of sample drug is 25 each i.e., in Staphylococus aureus sample drugs show more inhibition than that of standard drug. Streptococcus mutans is a gram positive bacteria. The zone of inhibition of standard drug is found to be 27 while the sample drug shows the zone of inhibition 28.33 and 28.66 respectively ie more than that of standard drug. Thus by analyzing the result of the antibacterial study using Agar Disc Diffusion Method with four bacteria, the sample drug has more zone of inhibition in Staphylococcus areus and streptococcus mutans. Also in cases of Pseudomonas aeruginosa and Enterococus faecalis the zone of inhibition of sample drugs are nearer to that of standard drug. The antifungal study was done with two fungi *Aspergillusniger* and *Candida albicans.* Clotrimazole (100µg) was used as standard drug. Aspergillosis is a group of illnesses caused by Aspergillus fungi. Some types include Allergic bronchopulmonary aspergillosis (ABPA), aspergilloma, chronic pulmonary aspergillosis and invasive aspergillosis. They usually affect people with weakened immune systems or lung conditions. The average zone of inhibition of standard drug was obtained as 23.33333333 while that of sample drug was 21.3333333 respectively which are nearer to that of sample drug. *Candida albicans* is the prevalent trusted source cause of fungal infections in people. It can be found in the GI tract, the mouth, and the vagina. The zone of inhibition of standard drug is 28.66666667 while that of sample drug is 18 and 18.66666667 respectively which is nearer to that of standard drug.

CONCLUSION

The present study proves the antimicrobial activity of both vaporizer and spray. Even though both vaporizer and spray showed significant antibacterial and antifungal activity, the antibacterial activity of samples and standard drug obtained was very much closer. The *Aparajitha dhoopayoga* was found to be very effective for the prevention of spread of Chickenguinea, dengue fever, corona virus etc and also has been using traditionally due to its mosquito and insect repellent properties. Although experiments revealed good results in terms of antimicrobial activity to some specific microorganisms, further studies involving more bacteria species, fungal species, viruses, mosquito repellant activity or analyzing the quality and efficacy of these products by other in vitro or in vivo tests are needed.

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