

# Formulation of Herbal Sanitizers and Determining Their Antimicrobial Activities Against Skin Pathogens

Rutuja Sunil Patankar  
Microbiology Dept., Patkar Varde College  
Mumbai, Maharashtra, India

Dr. Nayna Chandak  
Head of Microbiology Dept.  
Patkar Varde College, Mumbai  
Maharashtra, India

**Abstract:- Medicinal properties of plants is been studied since long back and various research on it was carried out and from that various pharmaceutical, health care, cosmetic products are been manufactured. This research was aim to formulate the herbal sanitizer. Sanitizer formulated from neem and lemon juice. Phytochemical and antioxidant properties of neem extract and lemon juice were analysed. Their MIC was checked against standard culture and hand isolates from lab assistance. MIC of neem extract and lemon juice was found. Three different sanitizers were formulated lemon, lemon-neem and neem sanitizer. Formulated sanitizer was compared with sterillium sanitizer and it was found that sterillium was effective against standard organism only and not against hand isolates while formulated sanitizer was effective against both. Even in comparison with respect to zone of inhibition, formulated sanitizers were showing more zones as compared to sterillium sanitizer. Antibacterial, antifungal and antibiofilm activity of formulated herbal sanitizer was studied. Antibiotic potentiation activity of formulated sanitizer was tested against Methicillin Resistant Staphylococcus aureus and isolate IV to find out change in the sensitivity pattern of antibiotics. Finally lemon, lemon-neem and neem sanitizer efficacy was tested. Result obtained showed reduction in bacterial growth after treatment with sanitizer. As neem sanitizer was tested found to be bitter hence instead of using it as hand sanitizer it can be use as a spray on medical devices or as a floor cleaner.**

**Keywords:- Sanitizer, MIC, Antibacterial.**

## I. INTRODUCTION

Hand hygiene is now regarded as one of the most important element of infection control activities. In the wake of the growing burden of health care associated infections, the increasing severity of illness and complexity of treatment, superimposed by multi-drug resistant pathogen infections, health care practitioners are reversing back to the basics of infection preventions by simple measures like hand hygiene. This is because enough scientific evidence supports the observation that if properly implemented, hand hygiene alone can significantly reduce the risk of cross-transmission of infection in healthcare facilities (7).

Washing hands with soap and water is the best way to reduce the number of microorganisms (germs) on hands.

When soap and water are not readily available, alcohol based hand sanitizers or rubs are acceptable. Hand sanitizers are effective against bacterial and fungal infections, as well as enveloped viruses, such as the common cold and flu viruses and in preventing nosocomial infections caused by different opportunistic microorganisms. Alcohol rub sanitizers containing at least 70% alcohol kill 99.9% of the bacteria on hand 30 seconds after application and 99.999% in 1 minute (10). Cleansing products powered by natural essences like witch hazel, bitter orange peel extract, thyme, lavender (a popular one), and even organic alcohol are on the rise.

Medicinal plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds (WHO survey, 1996). Phytochemicals are secondary metabolites, which are produced by medicinal plant.

So, different studies and sanitizer formulation were carried out. Sanitizer with complex chemicals as well herbal sanitizer was formulated and their efficacy were checked against various groups of organisms. Like, in this research three herbal sanitizers were formulated from neem and lemon. As, Neem *Azadirachta indica* extract is an important source of compounds having anti-microbial, anti-oxidant, anti-tumor, anti-malarial, anti-fungal, anti-inflammatory and anti-viral properties and Lemon *Citrus limon* is an important medicinal plant, antibacterial potential in crude extracts of different parts (viz., leaves, stem, root, fruit and flower) of lemon against clinically significant bacterial strains has been reported (6). Hence in this studies neem leaves and lemon juice is been use their antimicrobial properties were studied and accordingly sanitizers were formulated.

## II. MATERIALS AND METHODS

### A. Preparation of Leaf extract and lemon juice (10):

Tulsi and neem leaves were dried in oven at 50°C and coarsely grinded to get a powder. For methanol extraction, 10g of leaves powder and 90% methanol was added in 250ml flask (maceration) and kept on shaker for 48 hours. After maceration, sample was filtered by whatman no.1 filter paper. This filtrate was placed in water bath at 60°C till thick sticky, dark coloured extract is obtained and lemon were washed, cut into half and squeeze to get a juice. This juice was stored at 4°C for further use.

**B. Antibacterial activity of extract against standard organism:**

Antibacterial activity of extract and juice was carried out by agar well diffusion method against standard organism. After swabbing culture and adding extract the plates were incubated at 37°C for 24 hours.

**C. Phytochemical analysis :**

Test for carbohydrates (Fehlings test), amino acid (Ninhydrin Test), phenols and tannins, Flavonoids, Saponins (Foam test), Terpenoids, steroids and sterols (Salkowski's test) was performed as per given reference (3).

**D. Radical Scavenging Activity by the DPPH Assay:**

DPPH assay was performed to check radical scavenging activity of neem extract and lemon juice. Absorbance of all the samples was taken (9). The percentage radical scavenging activity of each extracts was calculated using the following equation

$$\text{Percentage radical scavenging activity (RSA \%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100\%}{\text{Absorbance of control}}$$

**E. Collection of hand isolates from lab assistance:**

Hand swabs of lab assistants by using sterile cotton swabs were taken. Plated on nutrient medium and incubated. Colony characterization, gram staining and biochemical were performed. Isolates were labeled as isolate I, II, and so on and preserved for further use and to study the effect of herbal sanitizer on hand microflora.

**F. Antibiotic sensitivity test (AST) of pathogens:**

To study the susceptibility of bacteria to various antibiotics AST was performed by referring Kirby Bauer and Koneman's chart.

**G. MIC determination by Macro agar dilution method (8):**

Minimum inhibitory concentration of all three extracts was done by macro agar dilution method. Four different dilutions of extract using DMSO as diluent was prepared. Undiluted, 1:2, 1:4 and 1:8 were the dilutions used, along with control. 1ml of these dilutions was mixed with 19ml of LB butts and was poured on petri plates. Then culture suspension of 0.5 McFarland turbidity was prepared and 5µl of culture were spot inoculated on plates. Plates were kept stand for 5mins and then incubated at 37°C for 24 hours. MIC was determined by inhibition of culture at particular dilution.

**H. Preparation of sanitizer:**

- Lemon sanitizer: Add 19ml of 70% Isopropyl alcohol in flask. In that add 2ml Glycerol and 4ml Rose water. Then add 25ml of Lemon juice.
- Neem-Lemon sanitizer: Add 19ml 70% Isopropyl alcohol in flask. In that add 2ml Glycerol and 4ml Rose water. Then add 12.5g of neem extract and 12.5ml lemon juice.

- Neem sanitizer: Add 18ml of 70% isopropyl alcohol in flask. In that add 2ml Glycerol and 4ml Rose water. Then add 1g of neem extract.

**I. Antimicrobial and Antifungal activity of sanitizer (1):**

For this method, Mueller-Hinton agar plates were used. Agar surface of each plate was streaked by a sterile cotton swab with the reference bacterial strain. Then agar plate was punched with a sterile cork borer of 4 mm size and then poured 100 µL of each sample (sanitizer) with micropipette in the bore. Plates were allowed to stand for 30 min. Then incubated at 37°C for 24 h. Zone of inhibition (mm) of the bacterial strains against three herbal sanitizers was measured.

**J. Effect of sanitizer on biofilm (4):**

Crystal Violet assay: This is a quantitative method for biofilm detection. Loopful of test organisms was inoculated in a 10 mL of trypticase soy broth with 1% glucose in test tubes. Tubes were incubated at 37°C for 24 h. After incubation, decant tubes was wash with sterile saline. Base of tube was Heat fix. Tubes were then stained with crystal violet (0.1%). Excess stain washed with distilled water. Distained with 4ml of ethanol by incubating for 5mins at room temperature. Recorded reading by taking optical density at 660nm.

**K. Efficacy of sanitizer:**

To check efficacy a 200mm size of petriplate were taken. In that 200ml of LB agar were poured. After cooling of agar two plates one for control and other for test were selected. Without applying sanitizer hand of assistant were placed on control plate and with applying sanitizer hand of assistant were placed use as test. Then plates were incubated at 37°C for 24hours. The decrease in number of colony was determined. Same procedure was followed for all three sanitizers.

**L. Antibiotic potentiation:**

This procedure is modification from Kirby-Bauer disc diffusion method. Different dilution of the sanitizers was prepared ranging from 1:10, 1:20, and 1:30 with sterile distilled water. 1ml of each dilution were added to 9ml of MH agar and then poured into petriplate. MH agar plate without sample was taken as control. Within 15mins after adjusting turbidity (0.5 McFarland standards) of inoculum suspension, a sterile cotton swab was dipped into it and was swabbed over entire surface of MH agar medium containing different dilution of sample. The antibiotic disc was placed in agar plate seeded with respective bacteria. Antibiotic disc used were Oxacillin (1mcg) for MRSA and Norfloxacin (10mcg) for Isolate IV. The plates were incubated at 37°C for 24hours. After incubation each inhibition zone diameter (IZD) will be measured and analyzed according to clinical and laboratory standards institute guidelines and the antibacterial activity will be expressed as the mean of IZD (mm).

**III. RESULTS**

**A. Antimicrobial and Antifungal of leaf extract and lemon juice:**

Antimicrobial and antifungal activity of extract against standard organism was performed by Agar well diffusion method. Neem extract was found to be effective against *S. aureus* and MRSA and not against *C. albican* and *P. aeruginosa*. Tulsi extract did not show antibacterial activity against selected organisms. Lemon juice was inhibiting *S. aureus*, MRSA, *P aeruginosa* and not to *C. albican*. Hence neem extract and lemon juice was selected for further study. DMSO was use as diluent for extract which does not show its antibacterial activity against selected organisms (Table 1).

Table 1. Antimicrobial and Antifungal activity of extract by agar well diffusion method

Organism	S.aureus	MRSA	C.albican	P.aeruginosa
	Zone of Inhibition (mm)			
Neem extract	14	10	-	-
Tulsi extract	-	-	-	-
Lemon juice	16	14	-	20
DMSO	-	-	-	-

**B. Phytochemical analysis:**

Presence and absence of phytochemicals in lemon juice and neem extract as shown in (Table 2).

Table 2. Lemon juice and Neem phytochemicals

Test	Result	
	Lemon juice	Neem extract
Saponins	-	+
Tannin	+	+
Steroid	-	-
Phenol	+	+
Flavonoids	+	+
Carbohydrate	-	-
Amino Acid	-	-
Terpenoid	-	+

Key: +: Present / - : Absent

**C. Free radical scavenging assay:**

Free radical scavenging assay of neem extract and lemon juice was performed by DPPH method and absorbance was taken at 530nm. Percentage radical scavenging activity was calculated by formula. Neem extract has more radical scavenging activity than lemon juice. Even diluted sample of neem extract and lemon juice shows a radical scavenging activity (Table 3).

Table 3. DPPH assay of neem extract and lemon juice

Concentration	Absorbance at 530nm			
	Neem extract	RSA%	Lemon juice	RSA%
Undiluted	0.28	47.16	0.42	8.69
1:2	0.06	88.67	0.29	36.95
1:4	0.05	90.56	0.28	39.13
1:8	0.01	98.11	0.26	43.47
Control	0.53	-	0.46	-
Blank	0.0	-	0.0	-

**D. Antibiotic Sensitivity Test:**

Antibiotic sensitivity test of selected culture were performed by referring kirby bauer and koneman's chart (Table 4). *Candida albican* found to be resistant to clotrimazole.

Table 4. AST of selected culture

Culture	Zone Of Inhibition(mm)							
	<i>S.aureus</i>	MRSA	<i>P.aeruginosa</i>	Iso I (Gram positive cocci)	Iso III (Gram positive diplococci)	Iso IV (Gram positive short rods)	Iso VI (Gram positive rods)	Iso VII (Gram positive short rods)
Antibiotic								
Oxacillin	36	-	19	17	24	-	10	-
Norfl oxacin	34	10	-	19	25	12	27	15
Vanc omycin	30	14	21	16	2	23	24	16
Tetra cycline	37	35	-	34	40	39	40	27

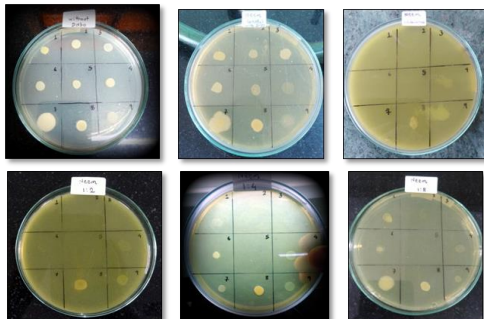
Antibiotic	Zone of inhibition
	Iso V (Gram negative short rods)
Ciprofloxacin	36
Norfloxacin	31
Piperacillin	27
Tetracycline	15

Key: - → No inhibition

**E. MIC determination:**

MIC for isolate I, IV, VII and *S. aureus* were found to be 1:2. While that for isolate III MIC found to be 1:4. For isolate VI MIC found to be 1:8. No inhibition at culture V, MRSA and *P. aeruginosa* is seen, it means further dilutions were needed to be performed. ( Figure 1 a). MIC for isolate I, III, IV, V, VI, VII no growth was seen at any concentration it means further dilutions were needed to be performed. MIC for *S. aureus* and *P. aeruginosa* were found to be 1:2. For MRSA it was found to be at undiluted concentration (Figure 1 b). For *C. albican* no inhibition by neem extract and lemon juice was found at any concentration.

**A. MIC of neem Extract**



**B. MIC of lemon Juice**

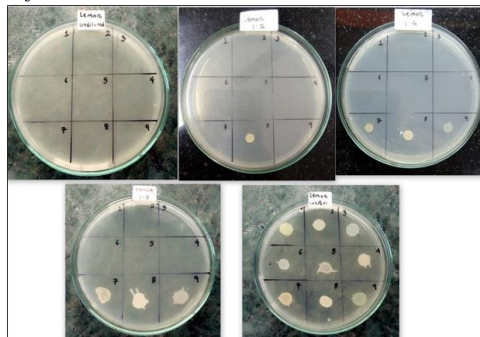


Fig 1:- a,b: MIC of neem extract and lemon juice against selected bacteria was performed by using macro agar dilution method.

**F. Sanitizer:**

Formulation of sanitizers were done by using neem extract and lemon juice with base iso-propyl alcohol, rose water and glycerol. Three different sanitizers were formulated named as lemon, lemon-neem and neem sanitizer (Figure 2 a,b,c).



a. Lemon b. Lemon-neem c. Neem

Fig 2:- a,b,c: Formulated Sanitizer

**G. Antibacterial activity of sanitizer by agar well method:**

Antibacterial activity of all formulated sanitizers with positive control that is Sterillium and other control is the base use to formulate sanitizer was checked. Against isolate III and isolate VI no growth on plate was seen. For all other isolates inhibition was seen. It was found that all three sanitizers were showing more inhibition than sterillium and sanitizer base (Table 5. and Figure 3).

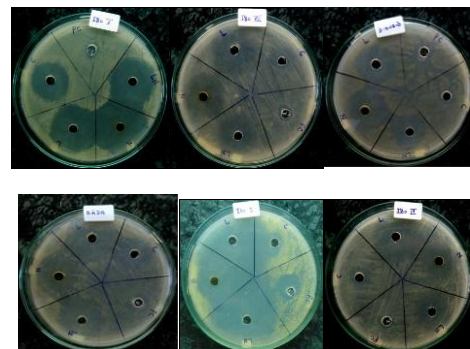


Fig 3:- Antibacterial activity of sanitizers by agar well diffusion method

Table 5. Antibacterial activity by agar well diffusion method

Sanitizer	Lemon	Lemon-Neem	Neem	Positive control - Sterillium	Control-Sanitizer base
	Zone of inhibition (mm)				
Culture	Inhibition	Inhibition	Inhibition		
Isolate I				26	22
Isolate III	-	-	-	-	-
Isolate IV	24 (12)	23 (11)	22 (10)	8	12
Isolate V	32 (8)	34 (10)	28 (4)	8	24
Isolate VI	-	-	-	-	-
Isolate VII	28 (28)	24 (24)	25 (25)	8	-
<i>S. aureus</i>	24 (14)	24 (14)	25 (15)	15	10
MRSA	32 (12)	28 (8)	23 (3)	12	20
<i>P. aeruginosa</i>	-	14 (4)	15 (5)	-	10
<i>C. albican</i>	12 (4)	10 (2)	15 (7)	10	8

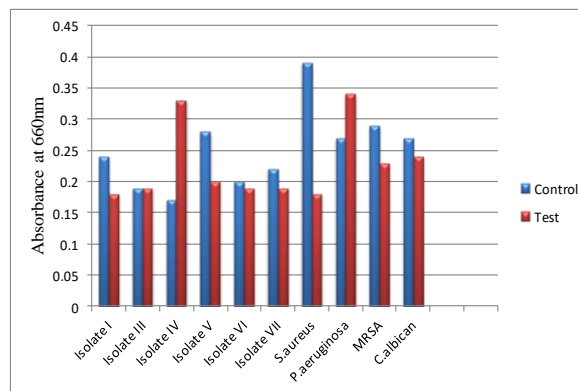
Key: – No growth  
 In bracket ( ) → Difference in zone of inhibition between sanitizer and base.

**H. Anti-biofilm activity: (Crystal violet tube method assay quantitative):**

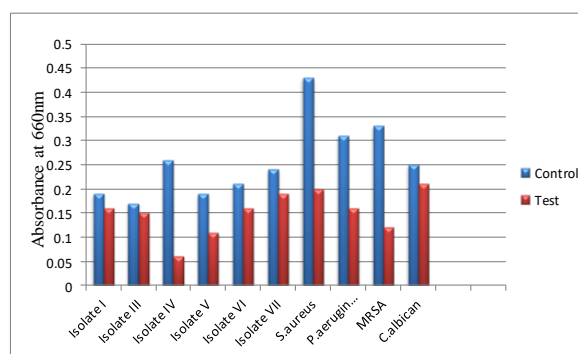
After performing assay the antibiofilm activity was studied by taking absorbance at 660nm and comparison

between test and control tubes was done. Antibiofilm activity was found against all bacteria except for isolate IV and *P. aeruginosa* in presence of lemon sanitizer (Graph 1). In presence of lemon-neem sanitizer antibiofilm activity was found against all bacteria (Graph 2). In presence of neem sanitizer antibiofilm activity was found against all bacteria (Graph 3).

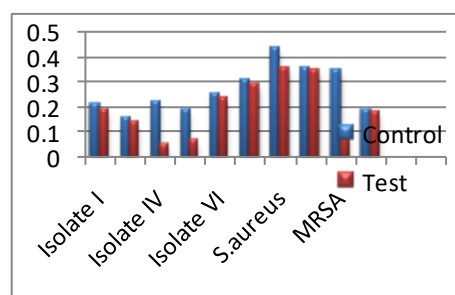
Graph 1. Antibiofilm activity - lemon sanitizer



Graph 2. Antibiofilm activity – lemon-neem Sanitizer



Graph 3. Antibiofilm activity – neem sanitizer



**I. Antimicrobial and Antifungal activity of Sanitizer:**

Antimicrobial and antifungal activity of sanitizer was studied by agar handprint method and all three sanitizers were found to be effective as reduction in growth was observed as making difference in growth of before applying and after applying plate (Figure 4,5,6).



Fig 4:- Antimicrobial activity of lemon sanitizer

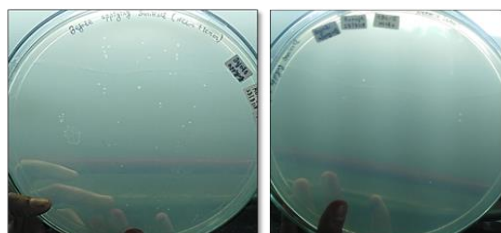


Fig 5:- Antimicrobial activity of lemon-neem sanitizer

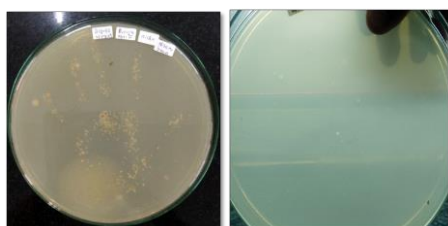


Fig 6:- Antimicrobial activity of neem sanitizer

**J. Antibiotic potentiation:**

Antibiotic potentiation activity assay was performed to study the ability of sanitizers to change sensitivity pattern of antibiotics. It was performed by selecting antibiotics against which organisms are resistant. By referring AST performed earlier, the MRSA and isolate IV was selected which was showing resistance against antibiotic Oxacillin, Vancomycin and Norfloxacin. Different dilutions of sanitizer were prepared. In presence of lemon sanitizer against MRSA no change in sensitivity pattern of Oxacillin were seen. Sensitivity pattern of Vancomycin and Norfloxacin was found to be changed (Table 6) and against isolate IV no change in sensitivity pattern was seen.

In presence of lemon-neem sanitizer, against MRSA no change in sensitivity pattern of Oxacillin was seen. Sensitivity pattern of Vancomycin and Norfloxacin found to be changed at dilution 1:10 (Table 7) and against isolate IV greater change in sensitivity pattern of Norfloxacin was obtained at all three dilutions (Table 8). In presence of neem sanitizer against MRSA, no change in sensitivity pattern of Oxacillin, Vancomycin and Norfloxacin was seen and against isolate IV, change in sensitivity pattern of Norfloxacin was seen at dilution 1:10 (Table 9).

Table 6. Antibiotic potentiation activity of lemon sanitizer on MRSA

MRSA	Lemon sanitizer	Norfloxacin-10
	Control	11
	1:10	14
	1:20	11
	1:30	12

Table 7. Antibiotic Potentiation activity of lemon-neem Sanitizer on Isolate IV

Isolate IV	Lemon-Neem sanitizer	ZONE OF INHIBITION (mm)
		Norfloxacin-10
	Control	13
	1:10	24
	1:20	17
1:30	14	

Table 8. Antibiotic potentiation activities lemon- neem sanitizer on MRSA

MRSA	Lemon-neem sanitizer	Norfloxacin-10
	Control	9
	1:10	18
	1:20	12
	1:30	11

Table 9. Antibiotic potentiation activities of neem sanitizer on isolate IV

Isolate IV	Neem sanitizer	ZONE OF INHIBITION (mm)
		Norfloxacin-10
	Control	16
	1:10	18
	1:20	15
1:30	14	

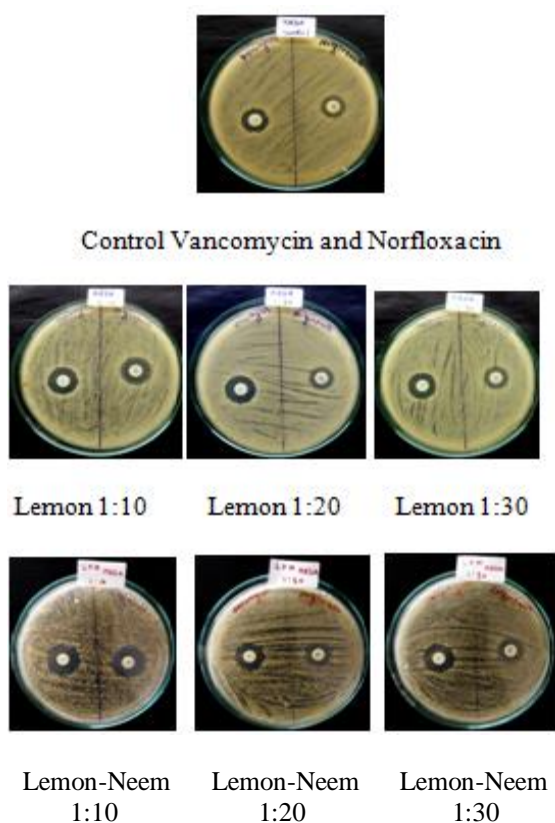


Fig 7:- Antibiotic potentiation of sanitizer against MRSA

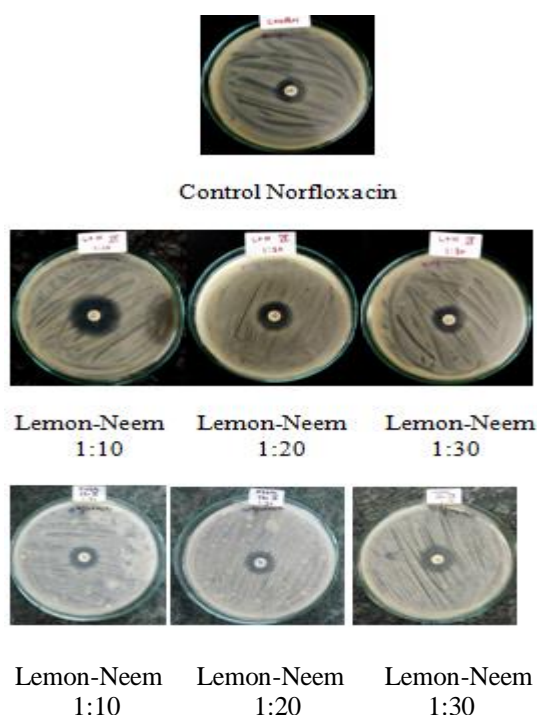


Fig 8:- Antibiotic potentiation of sanitizer against isolate IV

#### IV. DISCUSSION

Different studies and sanitizer formulation were carried out. Sanitizer with complex chemicals as well herbal sanitizer was formulated and their efficacy were checked against various groups of organisms.

Throughout history, human civilizations have kinetically circumvented plants which have influenced a lot to the humanity. Plants have the facility to endanger diverse variety of phytochemical and biochemical compounds which can be acclimated to perform different biological functions. Many of these phytochemicals have salutary effects on long-term health when consumed by the human and can be efficaciously used to treat human diseases.

Neem (*Azadirachta indica*) is widely used in the ayurvedic medicine system for treating malaria and fever. This study was undertaken to prepare crude extracts of neem leaves, same way in this research work, neem extract was prepared by methanol extraction method. Neem phytochemical screening, the total phenol content and free radical scavenging activity were assessed by DPPH method. Biochemical screening of the crude extracts of neem leaves revealed positive results of flavonoids, saponins, steroids, alkaloids, amino acid and tannins(5). Same way phytochemical screening of neem extract was performed in this research. Phytochemical like tannin, phenol, saponin, terpenoid and flavonoid was found to be present. Neem showed radical scavenging activity even at lowest dilution effectively, by DPPH assay. Antioxidant activity at various concentration and RSA% was calculated by using formula. Of neem extract, RSA% found to be at concentration undiluted - 47.16%, 1:2 - 88.67%, 1:4 - 90.56%, 1:8 - 98.11%.

Citric acid was found to be an effective permeabilizer and potentiating agent for VISA/MRSA isolates (11). So lemon juice was also selected for formulation of sanitizer. Phytochemical analysis was carried out as tannin, phenol and flavonoids found to be present. Of lemon juice, RSA% found to be at concentration undiluted - 8.69%, 1:2 - 36.95%, 1:4 - 39.13%, 1:8 - 43.47%.

Bohara et al., concluded that neem leaf extract has a significant antimicrobial effect against *E. faecalis*, *Candida albicans* and mixed culture (2). In this study MIC of neem extract for isolate I, IV, VII and *S.aureus* were found to be 1:2. While that for isolate III MIC found to be 1:4. For isolate VI MIC found to be 1:8. No inhibition at isolate V, MRSA and *P.aeruginosa* is seen, it means further dilutions needed to be performed. MIC of lemon juice for isolate I, III, IV, V, VI, VII no growth was seen at any concentration it means further dilutions were needed to be performed. MIC for *S. aureus* and *P. aeruginosa* were found to be 1:2. For MRSA, MIC was found to be at undiluted concentration.

Other herbal hand sanitizer, incorporating the leaves extracts of *Ocimum sanctum* Linn.(Tulsi) and *Eucalyptus globulus* (Nilgiri), the well-known herbal combination with multidimensional activities was formulated and their respective antimicrobial efficacy were studied. The formulation was evaluated against the specified micro-organism by culture sensitivity test. The significance was found to be more (13). Even in this research combination of neem and lemon was use to formulate sanitizer. First their individual effectivity was checked and after that combinational activity was also studied.

The prepared formulation of herbal hand sanitizer showed significant results against standard cultures as well as against hand isolates. The significance was found to be more in comparison to the commercially available hand sanitizer. Sanitizer with lemon juice and neem extract were formulated along with alcohol, as alcohol have excellent, rapid (within few seconds) germicidal activity against vegetative bacteria, fungi, and many viruses and antimicrobial activity is based on protein denaturation of microorganisms according to FDA. Alcohol based herbal hand sanitizer is more effective against microorganism than commercially available hand sanitizer (8). Herbal hand sanitizer offer numerous advantages over commercially available hand sanitizer onto both hands usually requires only 15 to 30 seconds. Whereas vigorous friction, rinsing with water, and drying with a towel are not needed like hand disinfectants or soaps. Hence intension behind this research was to formulate herbal sanitizer as it is very beneficial and can easily be use by those people living in slum areas where water is a major issue. Secondly as herbal sanitizer preparation is very cost effective hence it can be sold at very low cost as no complex chemicals are use which ultimately increases the cost of sanitizer. As three different sanitizers were formulated lemon, lemon-neem and neem sanitizer, among this neem sanitizer has its colour and also a bitter test hence it cannot be used as a hand sanitizer but it can be use as disinfectant or floor cleaner, toilet cleaner or to clean medical devices and it does not leave back a colour on instrument or floor.

A number of multidrug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. Therefore it is necessary to search the alternatives that can be potentially effective in the treatment of these pathogens. In one of the research the results obtained by combining the antibiotic with the extracts of *A. citratum*, *C. zeylanicum*, *D. psilurus* and *T. tetraptera* indicate that these extracts contain chemical compounds that can modulate the activity of antibiotics against bacteria expressing MDR phenotypes. The methanol extracts of *A. citratum*, *C. zeylanicum* and *D. psilurus* showed a synergistic effect with antibiotics inhibiting bacteria (12). In this research in antibiotic potentiation activity assay it has been seen that at certain dilutions sanitizer was changing the sensitivity pattern of antibiotics. So it means that this lemon juice and neem extract can be use as alternative of antibiotic or any other antimicrobial product can be formulated.

Formulated sanitizer was compared with sterillium hand sanitizer and it was found that sterillium was effective against standard organism only and not against hand isolates while formulated sanitizer was effective against both. Hence it is also one of the major valuable factor.

## V. CONCLUSION

Medicinal Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Plant extracts or secondary metabolites have served as antioxidants in phytotherapeutic medicines to protect against various diseases for centuries.

Pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds. Phytochemicals are secondary metabolites, which are produced by medicinal plant.

In this research Phytochemicals was found to be present in neem extract and lemon juice. Radical scavenging activity was also found to be present in both. MIC concentration of neem extract and lemon juice was checked by using different dilutions against standard culture and hand isolates. Three different sanitizers were formulated named as lemon, lemon-neem and neem sanitizer. Their antibacterial activity was checked against culture and also compare with sterillium. Formulated sanitizer was found to be more effective than sterillium. Hence it can be use in hospitals. They also showed anti-biofilm activity. Antibiotic potentiation activity of sanitizer against pathogens is changing the sensitivity pattern hence can be use in combination with antibiotic in some formulations.

As more effective is the formulated sanitizer, it can be brought into market. Neem sanitizer due to its bitter taste it cannot be use as hand sanitizer but can be use as floor cleaner, as disinfectant, toilet spray or to clean medical devices as it do not leave any colour traces. Lemon and lemon-neem sanitizer can be use as hand sanitizers. As neem and lemon possesses bioactive molecules it can also be use to formulate cream for wound healing and also for treating other skin diseases. Due to its antibiofilm activity, neem extract and lemon juice both can be use in toothpaste or in mouth wash liquid for treatment of dental plaque.

## REFERENCES

- [1]. Abbas, S. Z., Hussain, K., Hussain, Z., Ali, R., Abbas, T. (2016) Anti-Bacterial Activity of Different Soaps Available in Local Market of Rawalpindi (Pakistan) against Daily Encountered Bacteria, vol 7(11): pg.711-713.
- [2]. Bohora, A., Hegde, V., Kokate, S. (2010) Comparison of antibacterial efficiency of neem leaf extract and 2% sodium hypochlorite against *E. faecalis*, *C. Albicans* and mixed culture. Endodontology. Vol22(10): pg. 3-10.
- [3]. Choudhari, S., Sutar, M., Chavan, M. (2016) Formulation, evaluation and antibacterial efficiency of herbal hand wash. Indo Ame. J. Pharma. Res., vol 6(4): pg.5202-5209.
- [4]. Christina, W., Lukowicz, R., Merchant, S., Valquier, H., Caballero, J., Sandoval, J., Okuom, M., Huber, C., Brooks, T. D., Wilson, E., Clement, B., Wentworth, C. D., Holmes, A. E. (2017) Quantitative and Qualitative Assessment Methods for Biofilm Growth: A Mini-review. Research & Reviews: J. of Engi. Techno., vol 8(250): pg. 5578-5581.
- [5]. Hashemi, Z. S., Hossain, A. (2016) Biological activities of different neem leaf crude extracts used locally in Ayurvedic medicine. Pacific Science Review A: Natural Science and Engineering, Vol 18(2): pg. 128-131.
- [6]. Kawaii, S., Yasuhiko, T., Eriko, K., Kazunori, O.,



- Masamichi, Y., Meisaku, K., Chihiroito, Hiroshi, F. (2000) Quantitative study of flavonoids in leaves of Citrus plants. *J. Agri. Food Chem.*, vol 48: pg.3865-3871.
- [7]. Mathur, P. (2011) Hand hygiene: Back to the basics of infection control. *Ind. J. Med. research*, vol 134(5): pg. 611–620.
- [8]. NCCLS (2002). Performance standard for antimicrobial susceptibility testing: Twelfth information supplement M100-S-12. Villanova, PA, USA.
- [9]. Sahaa, M. R., Hasana, S. M., Aktera, R., Hossaina, M. M., Alamb, M. S., Alam, M. A., Mazumderc, M. E. (2008) In vitro free radical scavenging activity of methanol extract of the leaves of *Mimusops elengi* linn. *Bangl. J. Vet. Med.*, Vol 6(2): pg. 197-202.
- [10]. Thombare, M. A., Udugade, B. V., Hol, T. P., Mulik, M. B, Pawade, D. A. (2015) Formulation and evaluation of novel herbal hand sanitizer. *Indo American J. Pharma. Research*, vol 5(1): pg.483-488.
- [11]. Thool, V. U., Wadher, B. J., Bhoosereddy, G. L. (2014) Original Research Article Citric acid: A prospective permeabilizer for treatment of VISA infections . *Int. J. Curr. Microbiol. App. Sci.*, Vol 3(9): pg. 177-183.
- [12]. Voukeng, I. K., Author, V. K., Dzoyem J. P., Fankam, A. G., Noumedem, A. K., Kuate, J. R. (2012) Antibacterial and antibiotic-potential activities of the methanol extract of some cameroonian spices against Gram-negative multi-drug resistant phenotypes . *BMC Research Notes* Vol 5(299) : pg. 480-491.
- [13]. Wani, N. S., Bhalerao, A. K., Ranaware, V. P., Zanje, R. (2013) Formulation and Evaluation of Herbal Sanitizer. *Int. J. of PharmTech Research*, vol 5(1): pg. 40-43.