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ANTIMICROBIAL SCREENING OF HAND WASH SAMPLES BASED ON NOVEL POLYMER DERIVED FROM POLYETHYLENE GLYCOL, SORBITOL AND CITRIC ACID

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ABSTRACT

Various hand wash samples were formulated using a novel polymer based on sorbitol, polyethylene glycol, citric acid has been synthesized. A combination of maleic, and citric acid has been used for esterification. The experimental conditions, order of addition of ingredients, ratio of ingredients, catalyst, time and temperature have been standardized to get desired molecular weight, HLB ratio and viscosity characteristics. Spectroscopic studies of I.R. and N.M.R. of polymers have been undertaken which reveal presence of ester, ether, free Hydroxyl and free acid groups in the polymer. The selected polymer has been used as replacement of conventional linear alkylbenzenesulphonate in liquid detergent formulations. The compositions are ecofriendly and economical as they depend upon vegetable source polymers. Biodegradation study of novel polymers was studied. The hand wash samples were prepared by using prepared polymers. The antimicrobial activity of hand wash samples for certain bacteria such as, E. coli, S. aureus and were studied.

***Keyword**: Synthesis, condensation, antimicrobial screening, biodegradation.*

1. INTRODUCTION

Starch, sorbitol and sugar are globally available commodities of vegetable origin. These materials are produced in huge amount and can be used as key ingredients in detergent compositions. In our earlier efforts, we have used malenized oils, rosin, starch and sorbitol to develop novel polymers which can be used in powder[1], liquid[2-3] and cake detergents. In this present piece of research work cheaper vegetable products like sugar[4] starch[5]and sorbitol[5]have been used to synthesis polymers which can be used as a replacement of acid slurry of petroleum origin. The hand wash samples available in India are very costly. This is an attempt to develop cost effective ecofriendly and technically superior hand wash samples.

There is a noteworthy demand to synthesize eco-friendly polymers having some biological activities like antifungal and antibacterial. The invasion of polymers by fungi, bacteria and other organism is manifested by loss of mechanical properties, surface degradation, discoloration, staining and other deteriorations[6-9]Polymers are backbone, and the activities can be introduced. In terms of their

biological activity, these polymers are more effective than their monomers. Such polymers are known for their biocidal activity against some bacterial, fungal and viral strains[10].The present paper deals with the synthesis, characterization, and biodegradation studies antibacterial activities of hand wash samples[11-14]

2. MATERIALS AND METHODS

2.1 Synthesis of polymer

In the experimental work novel polymer has been synthesized. The mole ratio, order of addition of ingredients, time of reaction and catalyst has been standardized to get desired HLB ratio, viscosity.

A two-liter glass reactor fitted with stirrer, thermometer and condenser has been used. The heating was affected by an electric heating mantle with temperature regulator. A temperature control of ± 3oC can be achieved by regulator. Calculated quantity of ingredients and catalysts are introduced step by step in the reactor. The temperature is raised slowly and steadily to 130oC in about one hour. The heating is continued for 3-3.5 hrs. till the desired viscosity and characteristics are achieved. At the end of heating period the sample is withdrawn at 50-60 ^{0} C, filtered, weighed and stored in air tight bottles. The molecular weight of polymer is calculated from mass spectra of polymer.

2.2 Preparation of Hand wash based on sorbitol and Polyethylene glycol polymer

Step 1: The amount of polymer was first weighed and mixed with S.L.E.S (Sodium lauryl ether sulphate) and acid slurry, the mixture was allowed to settle down in a glass container (Mixture A).

Step 2: Then weighed amount of sodium carbonate and S.L.S (sodium lauryl sulphate) were thoroughly mixed in another glass container (Mixture B).

Step 3: Mixture A and B were added to a homogenizer pot and mixed thoroughly. Thereafter, urea EDTA, perfume, acetic acid and distilled water in required quantity were added. All this material was stirred together for 20 min to get a homogenous mixture which was then allowed to settle down for 24Hr.

Step 4: The above prepared mixture was then packed in air closed transparent plastic

The various peaks of IR spectra observed for novel polymer are given in the Table -3

The peak at 3398cm^{-1} is due to O-H stretching. This shows broad intermolecular hydrogen bonding of O-H group. The peak at 3398 shows the presence of OH groups in the polymer molecule.

The peak at 2939cm^{-1} is due to =C-H stretching. We have two peaks at 1731cm^{-1} and 1213cm^{-1} which are characteristics peaks for ester group. These two peaks i.e. confirm the presence of –C=O group in the polymer. The peak at 1086cm^{-1} shows symmetric C-O-C stretching mode and 1213 is for asymmetric C-O-C stretching vibration confirms the presence of ether group in the molecule.

The various peaks of NMR spectra observed for polymer P1 are given in Table-4, the value 2-2.9ppm shows the presence of H-COO proton. The NMR peak in the range 3.37-4.04 ppm shows the presence of H-C-OR proton.

2.3 Experimental Analysis of Antimicrobial Activity

2.3.1 Antimicrobial Screening[15-17]

Samples have been synthesized and agar diffusion method was employed to study their antibacterial activity. Test bacterial pathogens used in this study includes, E. coli, and S. aureus. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 hold cultures (100 µl, 104 cfu) and spread evenly on the plate. After 20 min, the wells were filled with different concentrations of samples. All the plates were incubated at 37ºC for24 h and the diameter of inhibition zones were noted.

2.3.2 Biological Activity[18-20]

It depends upon a comparison of the inhibition of growth of microorganism by measuring the concentration of the sample to be examined with the known concentration of standard antibiotic. For the copolymer, the agar diffusion method was employed. During the course of time, the test solution diffuses and the Growth of the inoculated microorganisms found to be affected. The activity developed on the plate was measured by measuring the diameter of the inhibited zone in millimeters. The lowest concentration of the soap sample resulting in no growth after 18 - 24 hrs of incubation was the MIC. The antimicrobial activity of the product is affected by the concentration of the active ingredients and the dwell time.

Concentrations screened: 0.0625, 0.125, 0.25, 0.5, 1.0 and 2.0 mg for all samples.

3. RESULTS AND DISCUSSION

Hand wash samples based on prepared polymer (Table-4) have excellent performance characteristics of foaming, lowering of surface tension and cleaning capacity (Table-5) the samples match with commercial product simultaneously tested.

The minimum Inhibitory concentration of our samples based on polymer P1 and P5 against *E. coli* and S. aureus is 0.5mg. This is also parallel to commercial sample tested simultaneously. So, these concentrations need to be taken into consideration for antimicrobial activity. The results of present antimicrobial assay revealed that the Hand wash samples HD4 and HD5 based on Polymers P4 and P5 showed inhibitory activity against S. aureus pathogen only and all other samples showed inhibitory activity against E. coli. Suggesting that the presence of SO3Na group enhances antibacterial activity. Condensation polymerization the crystalline structures of monomers lost into amorphous nature in copolymer resin,

When the efficacy of the antibacterial Hand soap was compared using the disc agar diffusion method, HD4 was found to be most effective against the bacteria strains tested having the highest zone of inhibition (23 mm) against *Staphylococcus aureus* and 18 mm against *Escherichia coli* at the highest dilution used.

The result of the minimum inhibitory showed that HD4 had better MIC 0.25 mg/ml on *S. aureus*. For *E. coli*, HD1 the MIC were 2 mg/ml. This means that this soap is needed in higher concentrations to kill or inhibit the growth of these pathogens as against Hand soap. Lifebuoy (control) had MIC 1 mg/ml against *S. aureus* and MIC 0.5 mg/ml respectively against *E. coli*. These values are lower than that recorded against any of the medicated soaps.

It was clearly seen from this study that Gram positive bacterium (*S. aureus*) was killed at low concentrations of soaps than Gram negative bacterium (*E. coli*). This observation according to Rama Bhat *et al* (2011) may be explained by the fact that triclosan exhibits particular activity against gram positive bacteria (Bhargava and Leonard, 1996) due to differences in the cell wall composition. In a similar work, Nwambete and Lyombe (2001) reported that Dettol, Lifebuoy and Tetmosol had inhibitory activities against *E. coli* and *S. aureus* at lower concentrations than that tested in this work. Lifebuoy was also reported to have inhibitory effects against *E. coli* and *S. aureus* (Feroze *et al.,* 2014)

Thus, it is routine practices to wash hands prior to eating, after examining a patient and before surgery, in order to remove some potentially harmful transient flora as well as reduce a number of resident flora, which might cause opportunities infections (Saba Riaz *et al.,* 2009)

Table 1: Composition of polymer containing polyethylene glycol (400) and sorbitol as main ingredients (% by weight)

Ingredients	%
Polyethylene glycol (400)	10
Sorbitol (70% solids)	65
Maleic Anhydride	-
Citric acid	18
Oxalic acid	-
Phthalic Anhydride	-
Benzoic acid	-
Sodium bisulphate ($NaHSO_4$)	3.5
Sodium metabisulphite ($Na_2S_2O_5$)	3.5

Table 2: Physicochemical analysis polymers containing polyethylene glycol (400) and sorbitol as main ingredients

Sr. No.	Polymer Property	Observation
1	Acid value of the polymer	83.48
2	pH of 1% solution	4
3	% Solids	89.00
4	Solubility of polymer (i) in water (ii) in Xylene (iii) in 50% alcohol + 50% water (iv) inNaOH solution (60%)	 Soluble Insoluble Partly soluble Soluble
5	Hydrophilic Lipophilic Balance Ratio of polymer (Based on saponification value)	15.9
6	Viscosity by Ford cup No. 4 at 30^0C in seconds.	272
7	Foam height (Cm^3) by cylinder method (For combination of 90% polymer + 10% Acid slurry)	1000
8	Surface tension (dyne/cm) (By stalagnometer)	27.07
9	% oxirane oxygen (By HBr method)	4.2

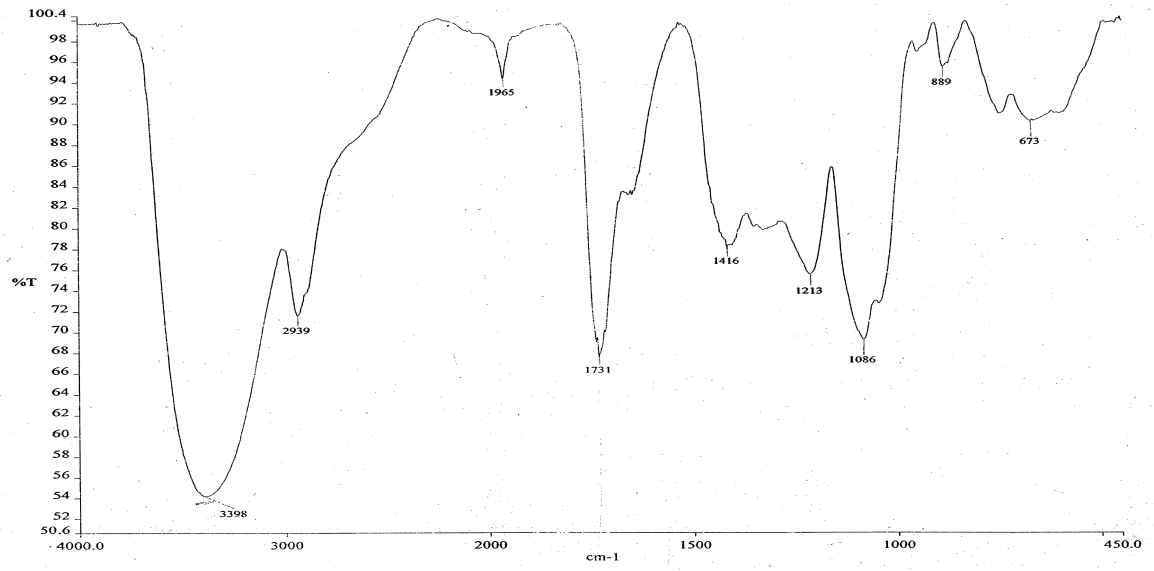

Fig. 1: IR spectra of polymer-P1

Table 3: The Prominent Peaks of the IR spectra of polymer-P1

Wave No.(cm^-)	Functional group	Literature value(cm^-)
3398	-OH stretching	3450-3200
2939	-COOH stretching	2500-3000
1731	-COO stretching	1740-1710
1086	C-O-C stretching	1050-1250
1213	C-O stretching	1200-1400

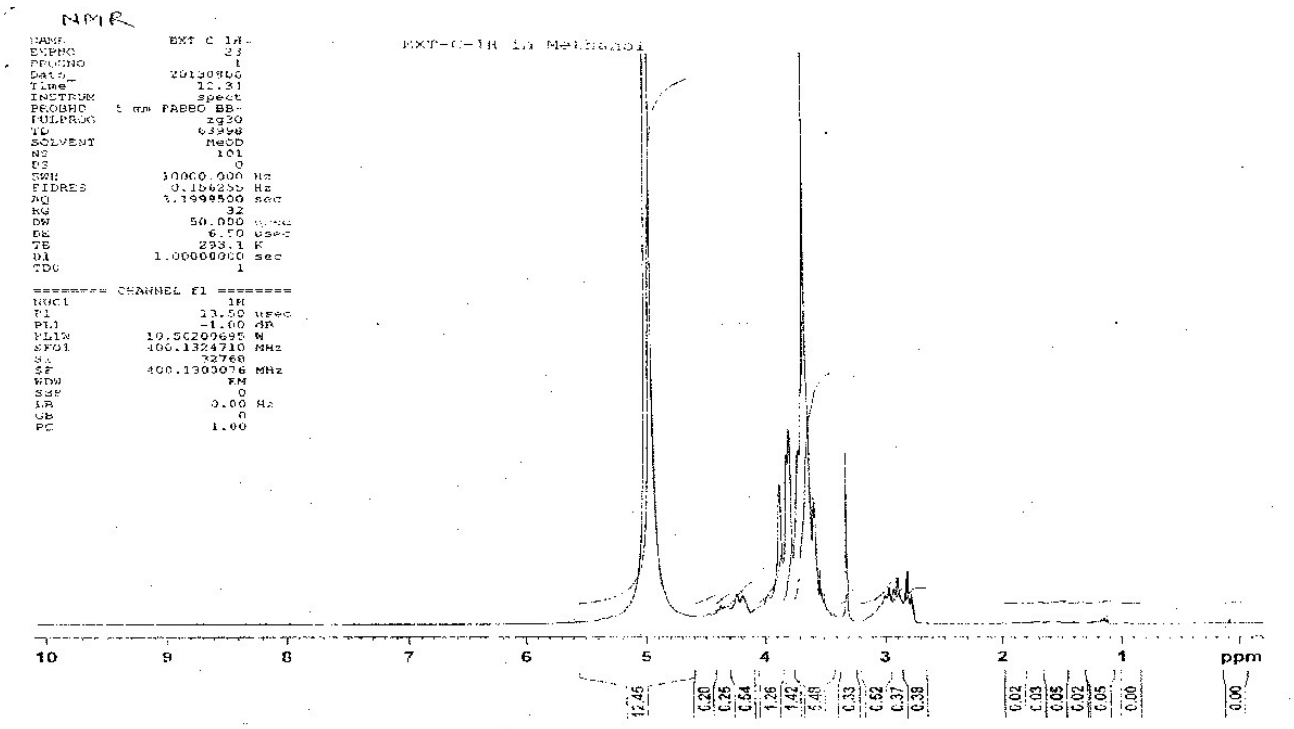

Fig. 2 NMR spectra of polymer-P1

Table 4: The prominent Peaks of the NMR spectra of Novel polymer

Range δ(ppm)	Type of proton	Literature value(ppm)
3.37-4.04	H-C-O-R(ether)	3.3-4.0
1-4	R-OH(Hydroxyl)	1-5.5
2-2.9	H-COO(ester)	2-2.2

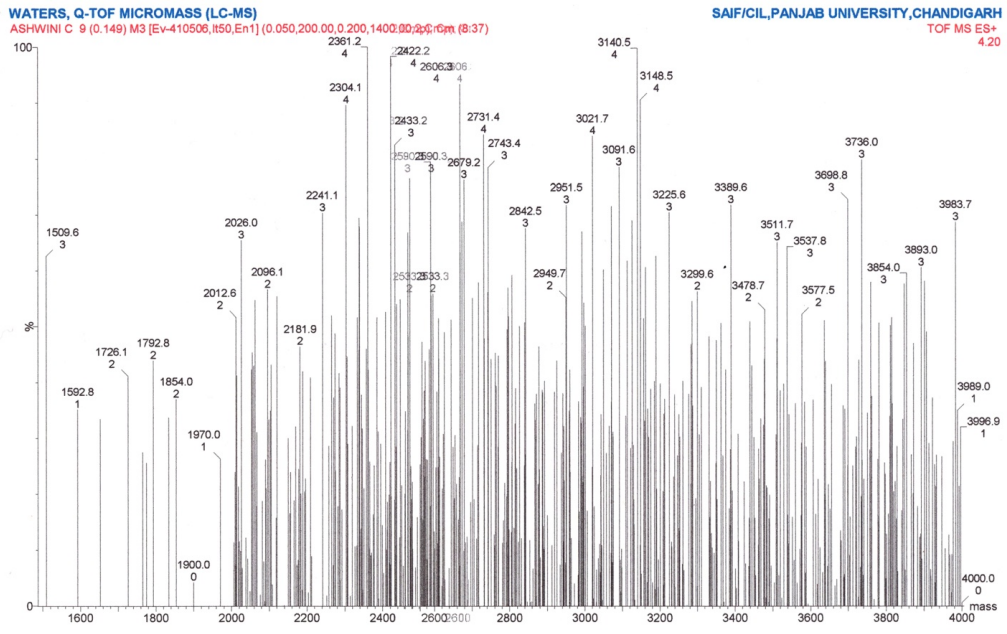

Fig.3: Mass spectra of polymer

Table 5: Number average molecular weight of polymers[3]

Sr.no	Polymer	Number average molecular weight
1	P1	2768

Table 6: Composition of hand wash based on novel polymers - P1, P2, P3, P4, P5

S. No.	Ingredients	Concentration (%)				
		H1	H2	H3	H4	H5
1	Acid slurry (90%)	7.5	7.0	6.5	6.0	5.5
2	Polymer-P1 (89%)	7.5	-	-	-	-
3	Polymer-P2 (82%)	-	8.0	-	-	-
4	Polymer-P3 (86%)	-	-	8.5	-	-
5	Polymer-P4 (85%)	-	-	-	9.0	-
6	Polymer-P5 (74%)	-	-	-	-	9.5
7	Sodiumcarbonate (50%)	04	04	04	04	04
8	Sodium sulphate	02	02	02	02	02
9	SLS (30%)	02	02	02	02	02
10	SLES (70%)	15	15	15	15	15
7	Urea	01	01	01	01	01
8	Sorbitol (70%)	02	02	02	02	02
9	EDTA	0.5	0.5	0.5	0.5	0.5
10	Acetic acid	01	01	01	01	01
11	Water	57.5	57.5	57.5	57.5	57.5
	Total	100	100	100	100	100

Note: H1, H2, H3, H4, H5 are the hand wash based on polymerP1. SLS-sodium lauryl sulphate, SLES- sodium lauryl ether sulphate EDTA-ethylene diamine tetra acetic acid.

Table 7: Analysis of hand wash based on polymer–P1, P2, P3, P4, P5

S.no.	Sample	Density	Surface tension	PH	Foam volume	Acid value	Viscosity
1	H1	0.9743	32.62	6	950	4.30	380
2	H2	0.9660	31.58	6	800	4.52	385
3	H3	0.9721	30.38	6	830	4.49	390
4	H4	0.9473	31.72	6	880	4.41	334
5	H5	0.9908	33.18	5	800	4.47	380
6	Commercial handwash-1	0.9741	32.12	6	700	4.95	398
7	Commercial handwash-2	0.9661	31.57	6	700	4.57	372

Table 8: Analysis of antimicrobial activity of hand wash samples based on sorbitol and polyethylene glycol for (S. aureus).

Sample	0.0625 mg	0.125 mg	0.25 mg	0.5 mg	1.0 mg	2.0 mg	MIC mg
HD1	0	0	0	0	0	0	NF
HD2	0	0	0	0	0	0	NF
HD3	0	0	0	0	0	0	NF
HD4	0	0	8	16	20	23	0.25
HD5	6	6	6	7	13	21	0.0625
CHD1	0	0	0	0	2	9	1.0 mg

Note: 1. NF- MIC not found in the concentrations screened
MIC- Minimum inhibitory concentration.

Table 9: Analysis of antimicrobial activity of hand wash samples based on sorbitol and polyethylene glycol for (E.coli).

Sample	0.0625 mg	0.125 mg	0.25 mg	0.5 mg	1.0 mg	2.0 mg	MIC mg
HD1	0	0	6	7	12	18	0.25
HD2	4	5	7	10	11	12	0.0625
HD3	0	0	0	0	0	11	2
HD4	0	0	4	4	5	9	0.25
HD5	0	0	0	4	8	10	0.5
CHD1	0	0	0	8	9	11	0.5 mg

Note: 1. NF- MIC not found in the concentrations screened
MIC- Minimum inhibitory concentration

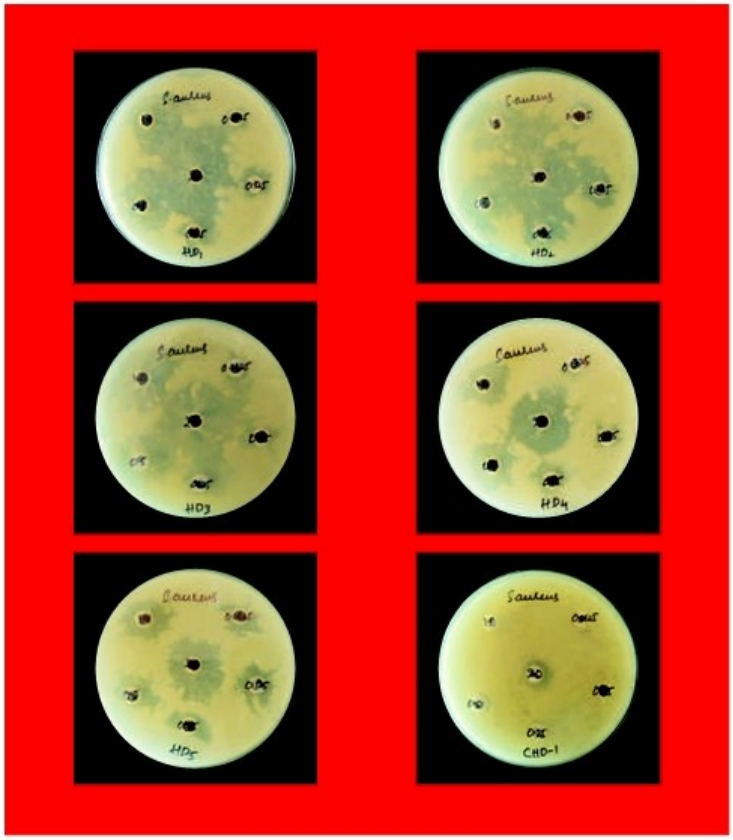

Fig. 4: Photographs of Antimicrobial activity *of S.aureus.*

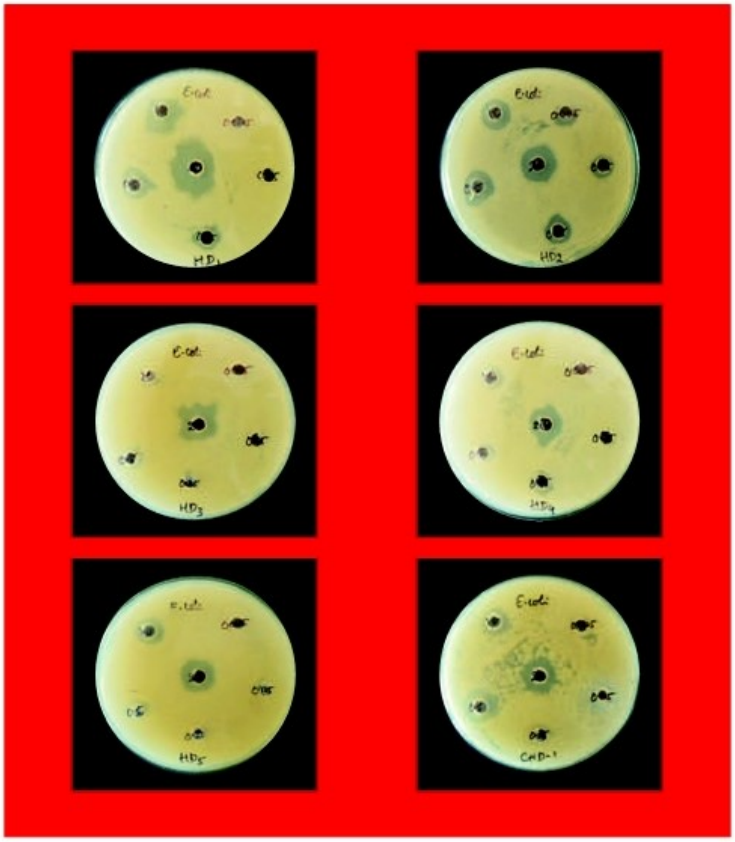

Fig. 5 Photographs of Antimicrobial activity of *E.coli.*

4. CONCLUSION

Hand wash samples based on prepared polymers containing 8-9.5 % polymer (Table-4) are not only comparable but superior to commercial products. They are ecofriendly as they are using smaller proportion of acid slurry. Use of 10 to 20% organic acids gives desirable properties of viscosity, surface tension reduction and water solubility. The HLB ratio, viscosity, foaming and surface tension reducing capacity suggest its use in hand wash samples. The liquid Hand wash tested in this work showed varied levels of effectiveness against the test isolates. The results of present antimicrobial assay revealed that the HD4 and HD5 showed inhibitory activity against S. aureus pathogen only and all other polymers showed inhibitory activity against E. coli. Suggesting that the presence of SO3Na group enhances antibacterial activity

5. ACKNOWLEDGEMENT

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