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EVALUATION AND COMPARISON OF DISINFECTANT ACTIVITY OF SOME COMMERCIAL BRANDS BY USING STANDARD METHODS

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ABSTRACT

The aim of this study was to evaluate and compare some commercially available disinfectant for their efficacy at laboratory level. The commercially available brands of disinfectant i.e. Harpic and Patanjali was evaluated and compared for their efficacy. The three microorganisms namely *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* were used to check the activity of the disinfectant depending upon the procedures. The efficacy of disinfectant were determined by standard procedures such as Phenol coefficient test, Rideal Walker test, Chick Martin test, Kelsey Skyes test, Disk Diffusion test, Serial Dilution test, Viable Count test. The effectiveness of the disinfectant were determined by their ability to reduce the microbial growth and activity. On the basis of results from the standard procedures the Harpic was found to be more effective than Patanjali as per our findings on laboratory scale.

KEYWORDS: Disinfectant, Microbes, Efficacy, E.coli, Phenol coefficient test, Kelsey Skyes test.

INTRODUCTION

A disinfectant is a chemical agent, which destroys or inhibits growth of pathogenic microorganisms in the non-sporing or vegetative state.[1] The term disinfection is generally used for a process in which micro organisms present on non living or inanimate objects and surfaces are killed by using chemical substances.[2] They are used to sterilise and clean the bacteria on non-living surfaces such as ceramic, wood, stone, or metal instruments and surfaces to control and prevent infection.[3] They may also be used to disinfect skin and other

tissues prior to surgery.[1] Disinfection in hospital practice is mainly achieved either by surface disinfection (e.g., disinfection of surfaces of the tables, trolleys, instruments, walls and floors, etc.) or immersing the contaminated objects in the disinfectant solution.[4] Disinfectant users should be aware of their short-term and long-term toxicity since they may have general biocidal activity and may accumulate in the environment or in the patient's or caregiver's body.[5]

There are three main purposes for which disinfectants are used

a. Decontamination of objects before disposal or reuse
b. Reduction of microbial contamination of the inanimate environment
c. Disinfection of the skin or hands.[6]

Ideal Disinfectant should be

a. Capable of killing all disease-causing organisms.
b. Cleans and disinfects in a single operation.[7]
c. Inexpensive.
d. Non-corrosive and nontoxic.
e. Effective at room temperature.[5]
f. It should be stable upon storage and should not undergo any chemical change.
g. It should have a wide spectrum of antimicrobial activity.[8]

Classification

Based on mechanism of action

a. Action on membrane (E.g. Alcohol, detergent)
b. Denaturation of cellular proteins (E.g., Alcohol, Phenol)
c. Oxidation of essential sulphydryl groups of enzymes (E.g., H_2O_2, Halogens)
d. Alkylation of amino, carboxyl-and hydroxyl group (E.g., Ethylene Oxide, Formaldehyde)
e. Damage to nucleic acids (Ethylene Oxide, Formaldehyde).[9]

Evaluation of Disinfectant

Disinfectants used in hospitals and laboratories must be tested periodically to ascertain its potency and efficacy. As certain disinfectants lose potency on standing and addition of organic matter, their efficacy must be tested. While certain methods help in selecting the right dilution of disinfectant for use others test the efficacy of disinfectant already in use. Some methods compare the performance with that of phenol whereas other methods simply state if

the disinfectant is effective or not. There are several methods of testing disinfectants, with their own advantages and disadvantages. All these tests can be allocated to one of the following disinfectant tests: carrier test, suspension test, capacity test, practical test, field test or in-use test.[10] For evaluating the activity of disinfectant the methods used are.

A) The Bactericidal tests include

a. The Phenol Coefficient Test

b. The Rideal Walker Test

c. The Chick Martin Test

B) The Bacteriostatic tests include

a. Serial Dilution Test

b. Disk Diffusion Test

c. Viable Count Test

d. Kelsey Sykes Test.[11]

MATERIAL AND METHODS

Disinfectant Sample

The samples were purchased from local shops and were stored as instructed/ directed before examination.

Table No. 1: Names of brands of disinfectant for evaluation.

Sr. No	Disinfectant Brands	Manufactured and Marketed by
1.	Harpic	Reckitt Benckiser (India) Pvt. Ltd. Unit-II, B-96, Eldeco SIDCUL, Industrial Park, Sitargani, Uttarakhand 262405.
2.	Patanjali	PATANJALI GRAMODHYOG (NYAS) Village BhadaurpurSaini. P.O.DaultapurBahadrabad, Haridwar-249405 Uttrakhand, India.
3.	Liquophin phenyle*	BDN PHARMACEUTICALSW-4, MIDC Industrial Area, Nagpur- 440028.

(*This Brand considered as a standard for study)

Fig. 1. Commercial Brands of Disinfectants.

Equipments

Table No. 2: List of Equipments.

Sr. No.	Equipment	Company Name
1.	Sterile test tube	Borosil, Mumbai
2.	Sterile beaker	Asco, Mumbai
3.	Sterile petri plate	Borosil, Mumbai
4.	Sterile conical flask	Asco, Mumbai
5.	Incubator	Remi Instruments Ltd. Mumbai-India-400053.

Chemicals

Table No. 3: List of Chemicals.

Sr. No.	Chemicals	Company Name
1.	Nutrient Broth	HIMEDIA, MUMBAI
2.	Agar Agar Powder	LOBA Chemie, MUMBAI
3.	Peptone Granular	LOBA Chemie, MUMBAI
4.	Beef Extract	LOBA Chemie, MUMBAI
5.	Sodium Chloride	LOBA Chemie, MUMBAI
6.	Distilled Water	

Composition of Medium

a. Nutrient Agar Medium

Table No. 4: Formulation Table.

Sr. No.	Ingredients	Quantity Taken
1.	Beef Extract	1g
2.	Peptone	1g
3.	Sodium Chloride	0.5g
4.	Agar	2g
5.	Distilled Water	100ml

All the ingredients are weighed as the above mentioned formula. Ingredients were then dissolved with the aid of heat and sterilized in autoclave at about 50 lb. pressure and temperature of 121°C for 15 mins.[12]

b. Nutrient broth media

Table No. 5: Formulation Table.

Sr. No.	Ingredients	Quantity Taken
1.	Nutrient Broth Media	1.3g
2.	Distilled Water	100ml

All the ingredients are weighed as the above mentioned formula. Ingredients were then dissolved with the aid of heat and sterilized in autoclave at about 50 lb. pressure and temperature of 121°C for 15 mins.[12]

Evaluation Methods

a. Phenol Coefficient Test[13]

Procedure

- Nutrient broth media was prepared and sterilized. The 10ml of media was then transferred to each sterile test tube.
- Different dilutions of test disinfectants (1:150, 1:175, 1:200, 1:222, 1:250) and phenol (1:50, 1:60, 1:70, 1:80, 1:90) was prepared.
- The loopful culture of bacteria i.e. Staphylococcus aureus was inoculated to each dilutions.
- All the dilutions were agitated to ensure contact between the disinfectant and microbes.
- Using sterile technique, at interval of 2, 5, 7 and 10 minutes one loop full was transferred from each dilutions into the sterile test tubes of nutrient broth.
- All the tubes were incubated for 48hrs at 37°C.
- Agents with a phenol coefficient >1 are more effective than phenol. The agents with phenol coefficient 1 means show equal activity as standard and < 1 means its activity is worse than phenol.

It was calculated by;

$$\textbf{Phenol coefficient} = \frac{\text{Highest dil. of disinfectant that kill microbes in 10min not in 5min}}{\text{Highest dil. of phenol that kill microbes in 10min not in 5min}}$$

b. Rideal Walker Test[2,14]

Procedure

- The dilutions of the test disinfectant and phenol were first prepared and quantities of each 5ml were measured and transferred to sterile test tubes.
- The nutrient broth media was prepared and 5ml of media was transferred to each sterile test tube.
- To the 5ml of dilution a loop full culture of E.coli was inoculated. At intervals of $2^{1/2}$ min, 5 min, $7^{1/2}$ min and 10 min, subcultures were made into 5ml of nutrient broth, using a standard wire loop.
- The test tubes were then incubated for not less than 48hrs and not more than 72hrs at 37°C.
- The presence and absence of growth in each test tube were recorded.

It was calculated by,

$$\textbf{Rideal Walker coefficient} = \frac{\text{Dil. of disinfectant that kills microbes in } 7^{1/2}\text{min not in 5min}}{\text{Dil. of phenol that kills microbes in } 7^{1/2}\text{min not in 5min}}$$

c. CHICK Martin Test[4]

Procedure

- A small quantity of yeast was collected and dried in the sun for 5 days.
- Thereafter 3g of dry yeast was then dissolved in 100ml of distilled water to give a 3% w/v organic suspension. The 2ml of microbial culture of E.coli was mixed with 48ml of 3% organic suspension.
- The 2.5ml of each phenol and disinfectant dilutions was separately mixed with 2.5ml organic suspension.
- After 30min of contact time, a standard loop full of the disinfectant- culture- organic suspension was transferred to the 10ml of nutrient broth media and incubated for 48hrs at 37°C.
- Thereafter the presence and absence of growth was recorded.
- The Chick Martin Coefficient was calculated by dividing the mean of the highest concentration of the phenol permitting growth and the lowest concentration showing absence of growth to the corresponding mean concentration of disinfectant.

It is calculated by,

$$\text{Chick Martin Coefficient} = \frac{\text{Mean of highest conc. showing growth and the lowest conc. showing absence of growth of phenol}}{\text{Mean of highest conc. showing growth and the lowest conc. showing absence of growth of test disinfectant}}$$

d. Disk Diffusion Test[15]

Procedure

- The nutrient agar was prepared and sterilized.
- The 30ml of media was then transferred to test sterile test tubes. The media was then inoculated with microbial subculture i.e. E.coli, B.subtilis, S.aureus.
- This media was then transferred to the sterile petri plates. The bores were made with the borer and filled with the disinfectant samples.
- The plates were incubated for 48hrs at 37°C. The zone of inhibition of microbial growth was then measured.

e. Kelsey Skyes Method[4,14]

Procedure

- The nutrient broth media was prepared and sterilized.
- The different dilutions of disinfectant were prepared. A loop full bacterial culture of E.coli was then inoculated in the dilutions.
- At the time intervals of 0 min, 10 min, 20 min and 30 min the culture and dilutions were made into the nutrient broth media using standard wire loop.
- The test tubes were incubated for 48hrs at 37°C. The presence and absence of microbial growth was recorded.

f. Serial Dilution Method[16]

Procedure

- A stock solution was prepared by taking 10ml of disinfectant and 1ml of E. coli culture.
- 1ml of solution was pipette out and diluted in 9ml of distilled water (10^{-1}) and labelled as test tube 1.
- From test tube 1, 1ml of solution was pipette out and diluted in 9ml of distilled water (10^{-2}) and labeled as test tube 2.

- Further dilutions were prepared of concentration 10^{-3} and 10^{-4}.The nutrient agar media was prepared and sterilized.
- The dilutions were spread on the petri plate containing solidified nutrient agar media respectively and incubated for 48 hrs at 37°C.
- The number of colonies were counted.

g. Viable Count Method[17,12]

Procedure

- The nutrient agar was prepared and sterilized.
- The 30ml of media was then transferred to sterile petri plates.
- The dilution of each disinfectants was prepared by taking 1ml of disinfectant, 9ml of distilled water and 1ml of bacterial culture of E.coli.
- At the interval of 0min, 10min, 20min and 30min the plates were streaked by streak plate technique.
- The plates were incubated for 48 hrs at 37°C.
- The number of colonies were counted.

RESULT AND DISCUSSION

a. Phenol Coefficient Test: The phenol coefficient test was performed on the of disinfectant brands making dilutions and inoculating it with bacteria and the results were observed.

1. Harpic: The phenol coefficient test was performed and the result was found to be.

Table No. 6: Observation of Phenol coefficient test of Harpic.

Sr. No.	Conc/Time	2min	5min	7min	10min
1.	1:150	-	-	-	-
2.	1:175	+	+	-	-
3.	1:200	+	-	-	-
4.	1:222	+	+	-	-
5.	1:250	+	+	+	-

$$\textbf{Phenol coefficient} = \frac{\text{Highest dil. of disinfectant that kill microbes in 10min not in 5min}}{\text{Highest dil. of standard that kill microbes in 10min not in 5min}}$$

= 222/80

= 2.77

2. Patanjali: The phenol coefficient test was performed and the result was found to be.

Table No. 7: Observation of Phenol coefficient test of Patanjali.

Sr. No.	Conc/Time	2min	5min	7min	10min
1.	1:150	-	-	-	-
2.	1:175	+	-	-	-
3.	1:200	+	+	-	-
4.	1:222	+	+	+	+
5.	1:250	+	+	+	+

$$\textbf{Phenol coefficient} = \frac{\text{Highest dil. of disinfectant that kill microbes in 10min not in 5min}}{\text{Highest dil. of standard that kill microbes in 10min not in 5min}}$$

= 200/80

= 2.5

On the basis of phenol coefficient test the Harpic was found to have the highest phenol coefficient of 2.77 and Patanjali was found to have the phenol coefficient of 2.5.

b. Rideal Walker Test: The rideal walker test was performed on various brands of disinfectant by making dilutions and inoculating it with bacteria and the results were observed.

1. Harpic: The rideal walker test was performed and the result was found to be.

Table No. 8: Observation of Rideal Walker Test of Harpic.

Sr. No.	Conc/Time	2.5min	5min	7.5min	10min
1.	1:95	+	-	-	-
2.	1:105	+	+	-	-
3.	1:115	+	-	-	-
4.	1:125	+	+	-	-
5.	1:135	+	+	+	-

$$\textbf{Rideal Walker coefficient} = \frac{\text{Dil. of disinfectant that kills microbes in } 7^{1/2}\text{min not in 5min}}{\text{Dil. of standard that kills microbes in } 7^{1/2}\text{min not in 5min}}$$

= 125/80

= 1.56

2. Patanjali: The rideal walker test was performed and the result was found to be.

Table No. 9: Observation of Rideal Walker Test of Patanjali.

Sr. No.	Conc/Time	2.5min	5min	7.5min	10min
1.	1:95	-	-	-	-
2.	1:105	+	-	-	-
3.	1:115	+	+	-	-
4.	1:125	+	+	+	-
5.	1:135	+	+	+	+

Rideal Walker coefficient = $\frac{\text{Dil. of disinfectant that kills microbes in } 7^{1/2}\text{min not in 5min}}{\text{Dil. of standard that kills microbes in } 7^{1/2}\text{min not in 5min}}$

= 115/80

= 1.43

On the basis of Rideal Walker test the Harpic was found to have rideal walker coefficient of 1.56 and Patanjali was found to have rideal walker coefficient of 1.43.

c. Disk Diffusion Test: The disk diffusion test was performed on various brands of disinfectant making dilutions and inoculating it with bacteria and the results were observed.

Table No. 10: Observation of Disk Diffusion Test.

Sr. No.	Brands	Average of Diameter of Zone of Inhibition (cm)		
		E. coli	S. aureus	B. subtalis
1.	Harpic	4.3	4.9	4.7
2.	Patanjali	1.5	1.5	1.5

On the basis of disk diffusion test the Harpic showed highest zone of inhibition than Patanjali and showed better disinfectant activity.

d. Kelsey Sykes Test: The kelsey skyes test was performed on various brands of disinfectant making dilutions and inoculating it with bacteria and the results were observed.

1. Harpic: The kelsey skyes test was performed and the result was found to be.

Table No. 11: Observation of Kelsey Skyes Test of Harpic.

Sr. No.	Conc	0 min	10 min	20 min	30 min	Result
1.	1:25	-	-	-	-	-
2.	1:50	-	-	-	+	-
3.	1:75	-	-	+	+	+
4.	1:100	+	+	+	-	+

2. Patanjali: The kelsey skyes test was performed and the result was found to be.

Table No. 12: Observation of Kelsey Skyes Test of Patanjali.

Sr. No.	Conc	0 min	10 min	20 min	30 min	Result
1.	1:25	-	-	-	-	-
2.	1:50	-	-	-	+	-
3.	1:75	+	+	-	+	-
4.	1:100	+	+	+	-	+

On the basis of observation from Kelsey Skyes test the disinfectant Harpic and Patanjali was found to be effective against the bacterial culture E.coli at the same concentration of 1:50.

e. Viable Count Method: The viable count method was performed on disinfectants and the colonies were counted.

Table No. 13: Observation of Viable Count Method.

Sr. No.	Brands	Time in Min./ No. of colonies			
		0	10	20	30
1.	Harpic	20	12	10	5
2.	Patanjali	25	11	2	0

On the basis of viable count method the disinfectant that showed lowest number of colonies at all the time intervals was Patanjali as compared to Harpic.

f. Chick Martin Test: The chick martin test was performed on various brands of disinfectant by using different dilutions and the growth of microbes were recorded.

Table No. 14: Observation of Chick Martin Test.

Sr. No.	Brands	Concentration				Chick Martin coefficient
		1:25	1:50	1:75	1:100	
1.	Harpic	-	-	-	+	1
2.	Patanjali	+	+	+	+	0

The chick martin coefficient of Harpic was found to be 1 and that of Patanjali was found to be 0 which concludes that Harpic was effective against the microbial culture.

g. Serial Dilution Method: The dilutions of disinfectant of various concentration was prepared and the number of colonies were recorded.

Table No. 15: Observation of Serial Dilution Test.

Sr. No.	Brands	Concentration/ No. of colonies			
		10^{-1}	10^{-2}	10^{-3}	10^{-4}
1.	Harpic	10	14	7	0
2.	Patanjali	24	18	13	6

The lowest number of colonies by serial dilution method was found in Harpic than Patanjal.

CONCLUSION

A wide range of disinfectants are available commercially that undergo extensive testing in controlled environment before market release. However, the products and procedures as described may not be adequately disinfect or decontaminate items when the surfaces have been contaminated with highly resisitant or unusal organisms. So it is necessary to check the activity of disinfectant and the concentration at which it is effective. The 2 different commercially available brands of disinfectant were evaluated, analyzed and compared for its microbial activity according to the standard procedures.

Table No. 16: Most Effective Brand from Standard Procedures.

Sr. No.	Standard Procedures	Most Effective Brand
1.	Phenol Coefficient Test	Harpic
2.	Rideal Walker Test	Harpic
3.	Chick Martin Test	Harpic
4.	Disk Diffusion Test	Harpic
5.	Viable Count Method	Harpic
6.	Kelsey Skyes Test	Harpic & Patanjali
7.	Serial Dilution Method	Harpic

According to the standard procedures and our finding on laboratory scale the Harpic was found to have good microbial activity against E. coli, S.aureus and B.subtalis as compared to Patanjali. Only in Kelsey Skyes test the Harpic and Patanjali showed same disinfectant activity.

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REFERENCES

1. archives.who.int/eml/wmf/2004/English/Disinfectants%20and%20antiseptics.pdf.
2. Kumar B., Ashok Y., Srinivasan R., “Evaluate the Disinfectant Activity of Some Commercial Preparation by Rideal Walker Test”, Int. J. Pha. Ana. Res., 2012; 2(1): 1-7.

3. http://www.broschdirect.com/blog/how-disinfectants-and-antiseptics-work.
4. Eliasa E., Samulb O., Emmanuela N., Abraham O., "Evalution of Efficacy of Disinfectant Using Standard Methods in Healthcare Facilites in Kogi State, Northcentral Nigeria", Asian J. Biomed. & Phar. Sci., 2013; 3(27): 34-38.
5. http://cden.tu.edu.iq/images/New/2016/Lectures/DR.husam/2017/7.pdf.
6. Ononugbol C., Reward E., Anthony C. Ike, " The Effect of pH and Temperature on Phenol Coefficient of Two Common Disinfectant Using Clinical Isolates of Escherichia coli & Staphylococcus aureus", Int. J. Adv. Mic., 2018; 10(2): 1-7.
7. https://www.researchgate.net/publication/282049548_Basic_Principle_of_Disinfectant_For_Postgraduate_Students_2015.
8. https://www.brainkart.com/article/Properties-of-Ideal-Disinfectant_17843/.
9. http://www.srmuniv.ac.in/sites/default/files/downloads/DISINFECTANTS.pdf.
10. https://www.microrao.com/micronotes/pg/testing_of_disinfectants.pdf.
11. Kale V., Bhusari K., "Applied Microbiology" Himalaya Publising House, Third edition, 2015; 198-202.
12. Kokare C., "Pharmaceutical Microbiology Experiments and Techniques", Career Publication, First edition, 2005; 53-56: 62-63.
13. http://niazi-pharmaceuticalinfo.blogspot.com/2009/06/determination-of-phenol-coefficient.html.
14. https://audauda.files.wordpress.com/2017/01/phct-401_disinfectant-evalution_dec-2016.pdf.
15. https://repository.seafdec.org.ph/bitstream/handle/10862/1635/Chapter2-Disk-Diffusion-Method.pdf?sequence=1&isAllowed=y.
16. https://nptel.ac.in/courses/102103015/module5/lec1/5.html.
17. https://microbeonline.com/pour-plate-method-principle-procedure-uses-dis-advantages/.