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Antimicrobial Efficacy of Citrofresh - A Newer Organic Disinfectant

Dr. Sheshaprasad R[1], Dr. Anuradha Pai[2], Dr. Anisha Yaji[3]

[1]Senior Lecturer, Department of oral medicine and radiology, The Oxford Dental College and hospital, Hosur Road, bommanhalli, Bangalore 560068

[2]Prof and Head, Department of Oral Medicine and Radiology, The Oxford Dental College and Hospital Hosur Road, Bommanhalli, Bangalore 560068

[3]Consultant Oral Medicine and Radiology, Sri Krishna Sevashrama Hospital, Jayanagar 5th Block, Bangalore-560041

Abstract: ***Background: Disinfectants are an important part of infection control programs and are widely used in hospitals, households, markets, and other places for a variety of topical and surface applications. A widespread, indiscriminate use of contemporary antiseptic and disinfectant products has given rise to the development of microbial resistance and also cross-resistance to antibiotics. Recently disinfectant products derived from plant sources have become popular. Citrofresh is an herbal, eco-friendly disinfectant effective against bacteria, virus, fungus and spores with a long lasting residual effect. Methods: To determine the efficacy of Citrofresh, different concentrations of Citrofresh were tested for antibacterial, sporicidal, antiviral and antifungal activity by using Kelsey Maurer capacity test, Kelsey Sykes capacity test, retro screen virology test, viral load reduction tests, pre-fumigation and post-fumigation agar culture plates test, minimal bactericidal concentration test, minimal inhibitory concentration test, potable water test, and direct skin contact by using Citrofresh wipes. Result: As per Kelsey Sykes and Kelsey Maurer capacity tests, Citrofresh exhibited bactericidal activity at concentrations between 2%-8% for an exposure duration of 8-18 minutes against several bacteria such as Bacillus cereus, Staphylococcus aureus, salmonella cholerasius, Pseudomonas aeroginosa, Methicillin-resistant Staphylococcus aureus (MRSA), and vancomycin-resistant Enterococcus faecium (VREF). The sporicidal activity was observed at 20%. Citrofresh Minimum Bactericidal Concentration (MBC) testing record sheet showed no growth of Staphylococcus pneumoniae, It was effective on Corynebacterium species, Serratia marcescens, Morganella morganii, Enterobacter aerogenes at higher concentrations (100%, 50%, 25%). The growth of fungus such as Chrysosporium species, Rhizopus, Penicillium species was inhibited at concentrations of 6.25%-100%. Retroscreen virology studies have shown that Human influenza A virus and Human rhino virus exhibited the most significant viral load reduction at 1-2% concentration. Citrofresh Superconcentrate and 75% Ethyl Alcohol impregnated Baby Wipes inhibited viable aerobic bacteria from the skin surface. Conclusion: The above test results show that Citrofresh has a significant disinfectant activity on par with chemical disinfectant with an added advantage of being a certified natural, organic product. (316 WORDS)***

Keywords: Disinfectant, Organic, Citrofresh, MBC, Kelsey Sykes test

1. Introduction

Disinfection means killing or removing pathogenic microorganisms from the inanimate surfaces through chemical or nonchemical methods. Antiseptics and disinfectants are used widely in hospitals, households, markets and other places for a variety of topical and surface applications. A wide variety of chemical disinfectants is important components of infection control practices that aid in the prevention of nosocomial infections. The widespread, indiscriminate use of antiseptic and disinfectant products has given rise to the development of microbial resistance including cross-resistance to antibiotics. Apart from their bactericidal activity most of the chemical disinfectants are sporostatic rather than sporicidal (1).

Every year more than 30,000,000 foodborne infections are estimated to occur, resulting in more than 9,000 deaths. More than 2 million nosocomial infections are estimated to occur each year, contributing to more than 75,000 deaths. Nosocomial infections are estimated to cause more than 19,000 deaths per year and contribute to another 58,000 deaths (2). About1.4 million patients worldwide in developed and developing countries are affected at any time by nosocomial infection (3).

Nosocomial infections, foodborne infections like diarrhea, food poisoning are emerging infectious diseases that cause mild to serious life-threatening illness. Several pathogenic organisms such as P aeruginosa in food products, salmonella in poultry products, E coli in meat, presence of E coli, Klebsiella pneumoniae, Citrobacter, and Enterobacter extensively in kitchen sinks and drains, nosocomial pathogens, including methicillin-resistant Staphylococcus aureus, vancomycin-resistant Enterococcus species, and Clostridium difficile in hospital environment are responsible for increasing infections despite use of antiseptics and antibiotics. Food borne pathogens are acquired through ingestion of contaminated raw fruits and vegetables, direct contact with contaminated surfaces and by the inadequate cooking of contaminated food. Many human pathogenic viruses and bacteria may survive in a sufficient dose and for an appropriate duration to serve as a potential source of human exposure (2).

Potential sites for antibacterial action in Gram-positive or Gram-negative bacteria are the cell wall or outer membrane, the cytoplasmic membrane, functional and structural proteins, DNA, RNA and other cell components (1, 4). Disinfection treatments are used in nosocomial, industrial, domestic or food processing environments to control the contamination of surfaces from microorganisms. Antimicrobial agents may be of different types such as

physical or chemical or a combination of the two. Commonly used antimicrobial agents for disinfection of hospital equipment, surfaces, wards are chlorine and its derivatives compounds, iodophors, alcohols, nitrogen compounds such as formaldehyde compounds, peroxide compounds, phenols, quaternary ammonium compounds, acid-anionic surfactant derivatives and chlorhexidine (5).

Newer disinfection techniques use steam vapors or hydrogen peroxide mist/vapors/plasma, UV light, thermal and non-thermal gas plasma, irradiation, ozone, and nitrogen dioxide chambers, optimizing chemical antimicrobial agents (2) and several naturally occurring agents such as phenols, alkaloids, flavonoids (6, 7).

The commonly used herbal disinfectants are Eucalyptus robusta, Senecio scandens, Callicarpa nudiflora, Rhodomyrtus tomentosa, Loropetalum chinense, and Mosla chinensis, etc. The skin and mucosa disinfectants made from medicinal herbs are also referred to as plant antiseptics which possess bacteriostatic property. Herbs usually used for herbal fumigants include Chinese atractylodes, argy wormwood leaf, Mosla chinensis oil, fir wood, pine wood (8). Neem, garlic, and green tea are equally efficacious as chlorhexidine and these herbal products can be used as potent alternatives to chlorhexidine as a disinfectant for toothbrushes (9, 10). An important characteristic of plant extracts and their components is their hydrophobicity, which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death

Most of the chemical disinfectants used for antibacterial activity generate various unwanted chemicals byproducts known as disinfection by-products in water that is hazardous. Indiscriminate use of disinfectants and antiseptics result in the development of multiple drug resistance. Adverse effects on the host, that is implicated to disinfectants include hypersensitivity, immune-suppression, and allergic reactions. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. Hence in the recent times, herbs are studied for their antiseptic and disinfectant properties since they are economical, safe and easily available. The study aims to assess the antimicrobial action of Citrofresh against potential human pathogens.

2. Methodology

Disinfection activity was tested by in-house standardized procedure. For the various tests, Citrofresh was used in concentrations ranging from 0.05 – 100%. Different concentrations of Citrofresh are tested for antibacterial, antiviral and antifungal activity by using Kelsey Maurer capacity test, Kelsey Sykes capacity test, retro screen virology test, viral load reduction tests, the effect of Citrofresh on pre-fumigation and post-fumigation agar culture plates, minimal bactericidal concentration, minimal inhibitory concentration. These set of tests were used to tests disinfectant effect of Citrofresh against bacteria such as Bacillus cereus, Bacillus subtilis, Pseudomonas aeroginosa, E.coli, Salmonella cholerasius, MRSA, VRSA, Clostridium perfringes, listeria monocytogenes, listeria innocua, chrysosporium species, Streptococcus pneumonia, Serratia marcercens, Proteus vulgaris, propionibacterium acne and virus such as influenza virus, fungus-like Aspergillus niger, Rhizopus, Penicillium species . The invitro tests were conducted at Analytical microlabs, Douglas Hocking Research Institute, The Geelong Hospital, Barwon Health, North Geelong, Australia. Retroscreen virology testing and report was conducted by Citrofresh international Ltd, North Geelong, Victoria, Australia. Efficacy of Citrofresh in direct contact assay against Avian influenza virus was conducted by virology department, Onderstepoort Veterinary Institute, South Africa. Australian Rickettsial reference laboratory Foundation limited for testing efficacy of disinfectant against several viral phenotypes. The swabs from operation theatre, OT trolleys, ventilators, floors, mattress, AC duct have been used as pre fumigation / post-fumigation swabs for culture and sensitivity testing at several hospitals in South India.Microbiology report for pre-fumigation and post-fumigation culture plates were conducted at Raghav's diagnostic and research Pvt Ltd. The test product, citrofresh has been manufactured in Australia by GDM Technologies Pvt Ltd and imported into India.

Kelsey Sykes capacity test: This capacity test determines the appropriate use of dilutions of the disinfectants.Kelsey-Sykes test is a triple challenge test, designed to determine concentrations of disinfectant that will be effective in clean and dirty conditions. The initial working concentration of Citrofresh 14P of 10% and 12% in dirty and clean condition was determined by agar and broth dilution, which can be extrapolated to MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) under certain conditions. The dirty condition was obtained by adding 1% Bovine Serum Albumin to the bacterial suspension. The clean condition was obtained by adding 0.03% bovine serum albumin to the bacterial suspension. The bacterial suspension was prepared in Standard Hard Water. Muller Hinton Broth and Horse Blood Agar were used for detecting bacterial growth. Incubated at 32o C for 48 hours and growth is assessed by turbidity. The disinfectant is evaluated on its ability to kill microorganisms or lack of it and the result is reported as a pass or a fail and not as a coefficient.
For testing sporicidal activity, for induction of spore formation, PCA agar slope culture was used to produce initial inoculums concentration of $0.2\text{-}2X10^8$ spores after incubation at 37^oC for 48 hours in nutrient broth.

Kelsey Maurer capacity test: The 'in use test' is a test that determines whether the chosen disinfectant is effective in actual use like hospital practice and also for the period of its use. The effectiveness of the disinfectant is determined by its ability to inactivate a known number of the standard strain of pathogenic organisms on a given surface with a certain given time. MBC and MIC are assessed during these serial dilutions.

Retroscreen virology testing: Citrofresh was tested in four different concentrations against the above-noted viruses;

0.25%, 0.5%, 2%, and 5%. Each Citrofresh concentration was tested for toxicity on each of the four cell lines.

Retroscreen Virology used three different adherent cell lines; C1008, MDCK and MRC-5 for the detection of cytopathic effect (CPE) of the viruses after exposure to Citrofresh for 1 and 5 minutes. Following the above contact times, cells were incubated at 37 ºC and CPE's were checked and recorded every day for up to 7 days. Prior to the viral testings, an Acute Toxicity Assay was carried out to determine the adherent cells viability against Citrofresh® and each cell line exhibited >80% viability, which was the minimum requirement for further studies.

The pH versus Citrofresh concentration was also determined, including the titer of viral log reduction, to evaluate the effect of pH on viral growth inhibition. Evaluation of the virucidal efficacy of Citrofresh against avian influenza virus in a direct contact assay was performed in standard 96 well flat-bottomed tissue culture plates. Provision was made of virus, cell and disinfectant controls, keeping to a 4% and 6% concentration.

Citrofresh was prepared as a 10% solution in DMSO and diluted further in tissue culture medium to 4% and 6%. Virus and disinfectant were allowed to interact at room temperature for an arbitrary 10 mins. 10 fold dilutions were placed on the plate in duplicate form. Titrations were carried out across the plate excluding the cell controls (row 5 & 6). Tests were performed on a preformed monolayer and incubated at 37°C in 4, 5% CO2 for 6 days. Plates were examined daily for cytopathic effect.

3. Result

Citrofresh at 12% concentration with an exposure time of 10 minutes eliminates Bacillus cereus. Kelsey sykes capacity test (Table 1) showed that Citrofresh at a concentration of 2-8% inhibited the growth of bacteria such as Staphylococcus aureus, salmonella cholerasius, Pseudomonas aeroginosa and methicillin-resistant Staphylococcus aureus on exposure for 8-20 minutes. Following exposure for 8-18 minutes to Citrofresh 20% concentration showed no growth for Bacillus cereus spores in a PCA agar slope culture.

Kelsey Maurer capacity test (Table 2) also showed no growth for bacteria such as Bacillus cereus, Bacillus subtilis, Clostridium perfringes at 8% concentration following exposure for 8-18 minutes, listeria monocytogenes and listeria innocua, Pseudomonas aeroginosa, Gentamicin resistant Pseudomonas aeroginosa, Proteus vulgaris and E.coli and Acinetobacter at Citrofresh concentration of 8-10% after exposure for 8-18 minutes.

Citrofresh MBC (Table 3) testing record sheet shows no growth of Staphylococcus pneumoniae, Corynebacterium species, Serratia marcescens, morganella morganii, Enterobacter aerogenes at higher concentrations of Citrofresh (100%, 50%, 25%) on Muller Hinton broth and horse blood agar. There is also no growth of Colletotrichum acutatum observed at 100, 25 and 12.5% concentration of Citrofresh. Spores of Bacillus cereus, Bacillus subtilis, and Clostridium perfringes showed no growth at 8% concentration with an exposure time of 8-18minutes.

The growth of chrysosporium species, Rhizopus, Penicillium species is inhibited at concentrations of 100, 50, 25, 12.5 and 6.25% of Citrofresh. MIC for Rhizopus species is 12.5%, Penicillium is 25% and Propionibacterium acne was 1.56% concentration.

A 0.125% concentration of Citrofresh after 5 minutes of exposure eliminates bacterial contamination in potable water. At concentrations of 4%, Citrofresh inhibited MRSA and VREF on day 5 after 18minutes of exposure. Serratia marcescens is inhibited at 0.5%concentration by broth dilution technique and at 0.75 % in horse blood agar. Citrofresh has a MIC of 0.5% and MBC of 0.75% against Serratia marcescens. Aspergillus niger is also inhibited at 10% Citrofresh concentration.

Prefumigation agar culture plates showed no growth of Streptococcus viridans, aerobic pyogenic pathogens, coagulase negative staphylococcus from floor swabs, Proteus species from spittoon swab after 48 hours of incubation on Mac Conkey's medium at 37^0C. Post-fumigation with citroshield plates for culture yields no growth of aerobic pyogenic pathogens from the swabs obtained from the floor, dental chair and spittoon on incubation in Mac Conkey's agar at 37^0C for 48 hours.

Retroscreen virology studies(Table 4) have shown that out of 4 viruses, Human influenza A virus exhibited the most significant viral load reduction of log 10 2.8 at 1% Citrofresh concentration, log 10 2.5 reductions for human rhinovirus at 2% concentration. Citrofresh also exhibited concentration-dependent viral load reduction against SARS, influenza A, and human rhinovirus. 4% and 6% Citrofresh reduced the H5N2 avian influenza virus load to greater than or equal to > 3 logs.

4. Discussion

The above study shows that at a concentration of 2-10%, Citrofresh inhibited growth of several bacteria namely Staphylococcus aureus, salmonella cholerasius, Pseudomonas aeroginosa, Neisseria gonorrhea, Proteus vulgaris, Bacillus subtilis, Clostridium perfringes, Bacillus cereus, Serratia marcescens and E.coli and spores of Bacillus cereus, Bacillus subtilis, Clostridium perfringes following exposure for 8-18minutes. The growth of several antibiotic-resistant strains of bacteria has also been inhibited by Citrofresh. This has been illustrated in case of inhibition of gentamicin-resistant Pseudomonas aeroginosa, gentamicin resistant Acinetobacter at 10% concentration and inhibition of methicillin-resistant Staphylococcus aureus and vancomycin resistant Enterococcus faecium at 4% concentration. These resistant strains have been responsible for various serious nosocomial infections which can increase the morbidity and mortality rates during hospitalization.

Citrofresh minimum inhibitory concentration testing record sheet showed that Propionibacterium acne was inhibited at 1.56% concentration.

Sterilization of culture medium yielding coagulase-negative Staphylococcus, aerobic pyogenic pathogenic isolated from the dental chair, spittoon, floor swabs post-fumigation also substantiates the disinfectant property of Citrofresh. The minimum inhibitory concentration of Citrofresh for Rhizopus is 12.5 and that for Penicillium species is 25% thus exhibiting fungicidal action. Citrofresh also has inhibitory action against the growth of influenza virus at 1% concentration while against SARS and Human rhinovirus, Citrofresh show concentration-dependent inhibition. Citrofresh also inhibits the growth of H5N2 avian influenza virus at a lower Citrofresh concentration of 4-6%. 4% concentration of Citrofresh has a killing rate of >99.999% after 10minute exposure or after 45 minutes contact time at 2% concentration. Citrofresh also inhibits the growth of bacteria in potable water.

The above test results substantiate the broad spectrum activity of Citrofresh as bactericidal, sporicidal, fungicidal, virucidal disinfectant with inhibitory action against resistant strains of bacteria as well.

Bacteria, viruses, and bacteria spores are frequently shed from infected and/or colonized patients or staff into the hospital environment, especially in the vicinity of patients and surfaces frequently touched by hospital staff. Some bacterial species, including C. difficile spores, VRE, MRSA and Acinetobacter species, can survive for 4–5 months or more on dry surfaces, and norovirus can survive for up to one week. There is a risk of transmission even at low concentrations as seen with a high rate of transmission with just one virus particle. The importance of surface contamination is also shown by the reduction in the rate of healthcare-associated infections when effective measures of environmental hygiene are implemented (11).

Several disinfectants such as phenolic compounds, alcohols, quaternary ammonium compounds, hypochlorites, iodines and iodophors, hydrogen peroxide, glutaraldehyde, formaldehyde etc., are used to inhibit the microorganisms on inanimate objects at several places like hospital ICU, operation theaters, wards, offices, houses. These chemical disinfectants have a selective action against gram positive/negative bacteria, capsulated/ nonencapsulated bacteria but may not exhibit activity against spores, virus, and fungus.

Plants are rich in a wide variety of antimicrobial phytochemicals such as flavonoids, tannins, alkaloids, terpenoids, phenolic compounds etc (12). The commonly used herbal disinfectants are Eucalyptus robusta, Senecio scandens, Callicarpa nudiflora, Rhodomyrtus tomentosa, Loropetalum chinense, and Mosla chinensis, H. suaveolens (L.) etc. These compounds have proven their efficacy against Staphylococcus aureus, E.coli, anthrax bacillus, beta-hemolytic streptococcus, Corynebacterium diphtheria, Salmonella typhi, Pseudomonas aeroginosa and Shigella dysentriae (13, 14). Plant medicines have the property of bacteriostatic effects at low concentration while demonstrating the bactericidal effect at high concentration or extending the disposal time.

The antibacterial activity of the Citrofresh is due to a synergistic activity of the citrus fruit bioflavonoid complex and certain other naturally occurring organic acids. The active ingredients in the Citrofresh act by destroying the cellular membrane of the microorganisms (15). The bitter orange extract (bioflavonoid complex) kills bacteria (Gram positive & Gram negative), virus (RNA & DNA), mould and fungus - including MRSA, VRE, SARS, GRGNB, H1N1, Salmonella, E.Coli, Pseudomonas, Clostridium Perfringens, Listeria Monocytogenes and other multi-resistant strains. The kill rate is 99.999%. A 20% solution destroys even the spores.

The disinfectant activity of Citrofresh has been evaluated against various organisms and strains on different sites and materials such as skin, surgical instruments, hospital wards, sprays, fumigation etc. Citrofresh has been used in several hospitals, nursing homes, schools in Bangalore, Hyderabad, Secunderabad, Vijayawada, Kuppam, Tirupati, and Vishakapatnam. The product was certified by Australian Therapeutic Goods Administration (Australian Health Ministry) as a hospital grade disinfectant, New Zealand Food Safety Authority for use in export registered establishments, Food Standards Australia & New Zealand as a food ingredient and food additive, Biological Farmers of Australia as a Certified Organic and the ingredients are approved by FDA of USA and Generally Regarded as Safe (GRAS).

But still, there is a need for more studies to evaluate the disinfectant role of Citrofresh on several other surfaces. Molecular mechanism of action of Citrofresh also needs to be evaluated.

Many alternative herbal disinfectants exhibit comparable disinfection qualities to traditional disinfectants and sanitizers, such as accelerated hydrogen peroxide, quaternary ammonium compounds (QUATs), and chlorine-based disinfectants (bleach) (16).

The herbal disinfectants are less toxic, environmentally friendly, and natural, easier application and preparation, little damage to items to be disinfected and less drug-resistance.

5. Conclusion

Based on the results above it can be concluded that Citrofresh has significant antibacterial, antiviral, sporicidal and antifungal activity that makes it equal in efficacy to contemporary chemical disinfectant and can be used as an alternative to these disinfectants. There is a need for a more extensive study on this product to determine the molecular mechanism of action to enhance the use of this environmentally friendly product. (2,870 WORDS)

References

[1] McDonnell G, Russell AD. Antiseptics and Disinfectants: Activity, Action, and Resistance. Clinical Microbiology Reviews. 1999;12(1):147-79.
[2] Rutala WA, Weber DJ. Disinfection and sterilization in health care facilities: what clinicians need to know.

Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2004;39(5):702-9.
[3] WHO Guidelines on Hand Hygiene in Health Care: First Global Patient Safety Challenge Clean Care Is Safer Care. Geneva: World Health Organization; 2009. 4 Hpohh. https://www.ncbi.nlm.nih.gov/books/NBK144030/.
[4] Spentzouris N. Comparative study on desinfection efficacy of Thymus Vulgaris and Aloe Vera extracts with commercial disinfectants, on bacteria isolated in nosocomial environmental. In: Sweden TiaMtfSDoFS, editor. http://www.uppsatser.se/uppsats/43cc054a30/2015.
[5] (2008) GfDaSiHF. https://www.cdc.gov/infectioncontrol/guidelines/disinfection/authors.html. William A. Rutala, Ph.D., M.P.H; David J. Weber, M.D., M.P.H.
[6] Cowan MM. Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews. 1999;12(4):564-82.
[7] Boyce JM. Modern technologies for improving cleaning and disinfection of environmental surfaces in hospitals. Antimicrobial Resistance & Infection Control. 2016;5(1):10.
[8] Chen ZB. Study and application of herbal disinfectants in China. Biomedical and environmental sciences : BES. 2004;17(4):492-8.
[9] Anand PJS, Athira S, Chandramohan S, Ranjith K, Raj VV, Manjula VD. Comparison of efficacy of herbal disinfectants with chlorhexidine mouthwash on decontamination of toothbrushes: An experimental trial. Journal of International Society of Preventive & Community Dentistry. 2016;6(1):22-7.
[10] Anand PJ, Athira S, Chandramohan S, Ranjith K, Raj VV, Manjula VD. Comparison of efficacy of herbal disinfectants with chlorhexidine mouthwash on decontamination of toothbrushes: An experimental trial. J Int Soc Prev Community Dent. 2016;6(1):22-7.
[11] Gebel J, Exner M, French G, Chartier Y, Christiansen B, Gemein S, et al. The role of surface disinfection in infection prevention. GMS hygiene and infection control. 2013;8(1):Doc10.
[12] Mujeeb F, Bajpai P, Pathak N. Phytochemical Evaluation, Antimicrobial Activity, and Determination of Bioactive Components from Leaves of Aegle marmelos. BioMed Research International. 2014;2014:497606.
[13] Harikumar PS, Manjusha CM. Study on the antibacterial activity of selected natural herbs and their application in water treatment. Drink Water Eng Sci Discuss. 2013;2013:199-231.
[14] Chandran P, Ig Barry A, Shaji R, Vineetha J, Unnikrishnan N, S A. Analysis of phytochemical, antimicrobial and disinfectant properties of leaf extracts of H. suaveolens (L.) Poit2016. 746-52 p.
[15] Thawatchai Phaechamud JC, Wanpen Saengthongpinit, Aruni Chuekaew. Antimicrobial Activities of Some Substances Used in Oral Cavity Spray. Research Journal of Pharmaceutical, Biological and Chemical
[16] Sciences. ISSN: 0975-8585.
[17] Fong D, Gaulin C, Lê M-L, Shum M. Effectiveness of Alternative Antimicrobial Agents for Disinfection of Hard Surfaces2017.

Table 1: Kelsey Sykes capacity test:

Organisms	Effective Citrofresh concentration	Duration of exposure
Bacterial suspension	12%	10 min
Staphycoccus aureus NCTC 6571	2-4%, 6%	10 min, 20 min, 30 min
Salmonella Cholersuis ATCC 14028	4%, 6%	10 min, 20 min, 30 min
Pseudomonas aeroginosa NCTC 10332	4%, 6%	10 min, 20 min, 30 min
MRSA ATCC 43300	2%, 4%, 6%	10 min, 20 min, 30 min

Table 2: Kelsey Maurer capacity test:

Organisms	Effective Citrofresh concentration	Duration of exposure
Bacillus cereus spores ATCC 10876	20%	8 min, 18 min
Bacillus subtilis spores(Gordon 122)	20%	8 min
Bacillus subtilis spores (Gordon 124)	20%	8 min
Bacillus subtilis (environmental isolate)	10%	8 min, 18min
Clostridium perfringes ATCC	10%	8 min, 18min
Listeria monocytogenes NCTC 11994	8%	8 min
Listeria ivanoii NCTC 11846	8%	8 min
Listeria innocua NCTC 11288	8%	8 min
Pseudomonas aeroginosa NCTC 6749	10%	8 min, 18 min
Pseudomonas aeroginosa (Gentamicin resistant)	10%	8 min, 18 min
Acenitobacter species	10%	8 min, 18 min
Proteus vulgaris NCTC 4635	6%, 8%	8 min, 18 min
Escherichia coli NCTC 8196	6%, 8%	8 min, 18 min
Staphylococcus aureus NCTC 4163	4%, 6%, 8%	8 min, 18 min

Effective citrofresh concentration against certain bacteria:

Streptococcus pneumonia	0.39%
Corynebacterium species	0.39%
Serratia marcescens	1.56%
Morganella morganii	1.56%
Enterobacter aerogens	0.78%

Table 3: MIC & MBC

Organisms	Minimum inhibitory concentration(MIC)	Minimum bactericidal concentration(MBC)
Pseudomonas aeroginosa NCTC 6749	4%	8%
Pseudomonas aeroginosa (Gentamicin resistant)	4%	8%
Acinetobacter sp. (Gentamicin Resistant)	4-6%	8%
Serratia marcescens	0.5%	0.75%
Listeria monocytogenes NCTC 11994	2.5%	2.5%

MIC with different citrofresh dilutions:

Citrofresh SC 10 times diluted Organism: Colletotrichum Acutatum	6.25-100%
Citrofresh SC 15 times diluted Organism: Colletotrichum Acutatum	12.5-100%
Citrofresh SC 20 times diluted Organism: Colletotrichum acutatum	50-100%
Chrysosporium sp.	6.25-100%
Rhizopus species	12.5-100%
Pencillium species	25-100%
Propionibacterium species	1.56%-100%

Table 4: Retroscreen viral testing report

Organisms	Effective Citrofresh concentration	Duration of exposure	Reduction in viral load
Human influenza virus	1%	1 min & 5 min	Log_{10} 2.8
Human rhino virus	2%	1 min & 5 min	Log_{10}2.5
H5N2 Avian influenza virus	4% and 6%		>3 log reduction of viral load