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ORIGINAL ARTICLE

Evaluation of the Antimicrobial Effectiveness and Cytotoxicity of Wound Care Solution

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ABSTRAK

Proses penyembuhan luka bergantung pada sel tisu yang sihat dan bebas daripada jangkitan bakteria. Produk larutan rawatan luka Dermacyn™ yang dihasilkan dengan teknologi Microcyn berpaten, adalah larutan super-oksida yang digunakan untuk menyembuh ulser, luka potongan dan luka lecur. Penyelidikan ini dijalankan untuk menguji sitotoksisiti dan keberkesanan antimikrob Dermacyn™ yang dijual secara komersial dengan tarikh luput yang berbeza melalui ujian in-vitro. Tiga produk Dermacyn™ dengan tarikh luput yang berbeza diuji dalam penyelidikan ini. Penilaian sitotoksisitas dijalankan diatas sel Medical Research Council 5 (MRC-5). Selepas sel dirawati oleh produk, peratusan sel dikira dengan kit Cell Viability LIVE/DEAD *bersama* flow cytometry*. Selain daripada itu, keberkesanan antimikrob terhadap patogen luka juga diuji melalui kaedah* (suspension test plate count)*. Penemuan menunjukkan bahawa produk menunjukkan sitotoksisiti yang rendah, dan hanya menunjukkan kurang daripada 1% kematian sel pada sel MRC-5 selepas rawatan produk. Selain daripada itu, produk ini juga menunjukkan keberkesanan antimikrob yang signifikan, dengan mencapai pengurangan log 3 'colony-forming units' (CFU) setelah didedahkan selama lima minit kepada 20 jenis bakteria yang diuji. Walau bagaimanapun, kedua-dua sitotoksisiti dan keberkesanan antimikrob mengalami penurunan apabila produk mendekati tarikh luputnya. Kesimpulannya, produk Dermacyn™ adalah pilihan yang selamat untuk sel manusia, dan memberi keberkesanan antimikrob yang tinggi. Ia memenuhi keperluan asas untuk pengurusan luka dan menggalakkan penyembuhan luka secara optimum.*

Kata Kunci: Keberkesanan antimikrob; larutan rawatan luka; larutan super-oksida; sel MRC-5; sitotoksisiti

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ABSTRACT

The process of wound healing relies heavily on the continual viability of healthy tissues and the prevention of bacterial infection. Dermacyn™ wound care solution, featuring a patented Microcyn technology, stands out as a super-oxidised disinfectant solution designed to address ulcers, wounds, cuts and burns. This research undertook an assessment of the cytotoxicity and antimicrobial effectiveness of commercially available Dermacyn™ with varying shelf lives through in vitro assays. Three different Dermacyn™ products, each with distinct shelf lives, were utilised and subjected to testing. The assessment of cytotoxicity was executed on the Medical Research Council cell strain 5 (MRC-5) cell line using flow cytometry alongside LIVE/DEAD cell viability assays. Simultaneously, the antimicrobial efficacy against wound pathogens was evaluated through the suspension test plate count method. The findings revealed that the products exhibited remarkably low cytotoxicity, registering less than 1% of cell death on MRC-5 cells. Moreover, they exhibited significant antimicrobial efficacy, achieving a notable 3-log reduction in colony-forming units (CFU) following a five-minute exposure to 20 challenge strains. Nevertheless, it was observed that both cytotoxicity and antimicrobial efficacy experienced a decline as the product approached its expiry date. In conclusion, Dermacyn™ wound care solution emerges as a remarkably safe option for human cells, boasting a high level of antimicrobial efficacy. It effectively met the fundamental requirements for wound care solutions and management, providing a promising avenue for promoting optimal healing conditions.

Keywords: Antimicrobial efficacy; cytotoxicity; MRC-5 cells; super-oxidised water; wound care solution

INTRODUCTION

Wound healing is a natural physiological reaction to tissue injury and it is a complex biological process. Since wounds remain open and body tissue loses its continuity, they are prone to bacterial infections. The wound site will be an optimum environment for the growth of harmful bacteria. Bacterial infection is one of the major problems associated with impaired wound healing. Slow healing wounds are mainly caused by necrosis and infection. One of the hallmarks of diabetes mellitus is chronic wound due to the neuropathy and high blood glucose (Ambrogi et al. 2020). There are many symptoms of an infected wound such as aches and pains, warm skin around the wound, swelling, yellow or green discharge coming from the wound and unpleasant odor (Hayley 2020). Notably, common bacterial pathogens are known to colonise the wound including *Staphylococcus aureus, Streptococcus* spp*., Escherichia coli, Pseudomonas*

spp., *Klebsiella* spp., *Proteus* spp. and *Enterobacter* spp. (Tom et al. 2019). Wound dressings provide the optimum conditions for wound healing and protect the wound against infiltration by pathogenic microorganisms. The ideal dressing should keep the wound moist and limit bacterial overgrowth (Britto et al. 2022).

Dermacyn™ wound care is based on patented Microcyn™ technology which has obtained 13 Food and Drug Administration (FDA) and 20 Conformité Européennes (CEs) since 2009. It contains highly stable small molecule oxychlorine compounds including hypochlorous acid (HClO) which can eliminate foreign pathogens that attack our body without harming our body cell. Dermacyn™ is considered safe, stable and consistent in wound care management. Dermacyn™ (Oculus Innovative Sciences, Petaluma, CA, USA) with Microcyn™ technology contains super-oxidised water (SOW) which is widely used to moisten, debride and irrigate both acute as well as chronic wounds, burns and ulcers. It helps the body to perform its own healing process through reducing the microorganism growth and maintaining the moist environment (Dermacyn 2018).

The SOW produced from purified water passes through anode and cathode chambers that are separated from a sodium chloride (NaCl) chamber by ionic membranes in REDOX equipment. It is made up of 99.99% water with additional ions. During the process, reactive chlorine and oxygen are formed by molecules dissociation. The sole input material for the production of SOW is purified water and sodium chloride (NaCl) (Sonoma pharma Microcyn 2016). Oxygen free radicals such as superoxide (·O_2-) and the hydroxyl radical (·OH) could cross link or react directly with proteins and other macromolecules and also lipid peroxidation (de Grey 2005).

The oxygen containing molecules of hydrogen peroxide (H_2O_2) and hypochlorite (OCI-) in SOW can facilitate free-radical formation (Suzuki et al. 2002). Since these molecules can potentially damage nucleic acids, proteins or lipids, SOW was tested for in vitro antimicrobial activities. This neutral-pH SOW can eliminate *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Candida albicans* in pure cultures. A study conducted by Landa-Solis et al. in 2005 showed that in 1 ml of 10^8 colony-forming units/ml of each microorganism that was subjected to 9 ml Microcyn or sterile water at room temperature for 30 seconds, there was a $\log_{10}$ reduction factor of 8 in Colony Forming Unit (CFU) of the microorganisms treated with Microcyn. The study also showed that *Bacillus atrophaeus* spores exposed to SOW for five min demonstrated complete inactivation of the spores within two to three minutes ($\log_{10}$ reduction factor >4).

Various studies proved that SOW exerts distinct microbicidal activity against *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Salmonella enteritiditis, Mycobacterium avium intracellulare, Mycobacterium tuberculosis, Listeria monocytogenes and Candida albicans*

(Park et al. 2002; Venkitanarayanan et al. 1999). In addition, SOW has been investigated as disinfectants for instruments and hard inanimate surfaces in hospitals (Rutala et al. 2001).

The literature also describes the use of SOW on humans for the treatment of infectious skin defects, wound care or ulcers (Sekiya et al. 1997). SOW can denature proteins and burst the membrane of single-cell organisms by internal and external differential pressures. Multi-cellular organisms are immune to this reaction because their clumping behaviour leaves less membrane exposed. A study conducted by Gutierrez in 2006 showed that SOW did not induce skin sensitisation or irritation in animals despite its high oxidation reduction potential. Alberto Piaggesi, a consultant diabetologist at the University Hospital of Pisa, Italy, has been using SOW on diabetic foot wounds (Bryant 2005).

As mentioned in the product leaflet, Dermacyn™ has the ability to treat a wide range of pathogens including Gram-positive and Gram-negative bacteria and is non-cytotoxic for granulating tissue. This study was conducted to determine the cytotoxicity and antimicrobial efficacy of Dermacyn™ with in vitro assay. We also compared the cytotoxicity and antimicrobial efficacy of Dermacyn™ products with different expiry dates.

MATERIALS AND METHODS

Test Product Sample

Three Dermacyn™ Wound Care Solutions with different expiry dates were evaluated i.e. Sample 1 (04-04-2020); Sample 2 (10-09-2020); and Sample 3 (20-02-2021). Product samples were obtained from the manufacturer (Dyamed Sdn Bhd. Malaysia).

Cell Culture

MRC-5 human lung fibroblast cell line (American Type Culture Collection; Rockville, MD, USA were cultured in Eagle's minimum essential medium (EMEM) (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA), 2% penicillin streptomycin (Gibco, USA) and 1.6% Fungizone (amphotericin B) (Gibco, USA) and were incubated at 37°C in 5% CO_2 incubator. The cell cultures were examined under an inverted microscope daily, until there was a confluent growth. MRC-5 cell lines were passaged again prior to initiating the assays. Briefly, the old medium was removed and cells were washed twice with 5 ml pre-warmed phosphate-buffered saline (PBS). Then 1 ml of pre-warmed 0.25% trypsin-EDTA (Gibco, USA) was added to detach cells. New medium was added and transferred into a 6-well plate (SPL, Korea) for flow cytometry assay. The plate was incubated at 37°C in a humidified incubator with 5% CO_2.

Cytotoxicity Assays

Cytotoxicity assays with cultured cells are common used for testing the chemicals and drug. This in-vitro cytotoxicity assay was performed

using method as described by Li et al. (2015) and Gillissen et al. (2016). MRC-5 is fibroblasts cell and originally developed from the human lung tissue. In order to reach an optimum environment for human fibroblasts MRC-5, each sample of product was mixed with Dulbecco's Modified Eagle Medium (DMEM) at a ratio 1:5. The cells were harvested from each well of the 6-well plate by adding 300 µl of 0.25% trypsin-EDTA. The detached cells were supplemented with 1 ml of DMEM, No phenol red (Gibco, USA) and were transferred into tubes for cell isolation, flow cytometry and fluorescence-activated cell sorting (FACS). Each tube with cell suspension (1.2 x 10^6 cells) was mixed with 200 µl of product sample 1-3 and incubated at 37˚C in 5% CO_2 for five minutes. In parallel to tubes set up for measurements of cytotoxicity, a tube of cell suspension without any materials was used as an untreated arm (negative control) and a tube of cell suspension pre-heated at 60˚C for 5 minutes was used as a treated arm (positive control). After the incubation, the cell suspension in tubes were centrifuged at 1500 rpm for five minutes, supernatant discarded and washed with 3 ml pre-warmed PBS. Again, 1 ml of DMEM was added to each tube followed by cells labeling with LIVE/DEAD Viability/Cytotoxicity Kit (Invitrogen, Carlsbad, CA). A mixture of 2 µl of 50 µM calcein AM working solution and 4 µl of the 2 mM ethidium homodimer-1 stock was added to each tube and incubated at room temperature for 15-20 minutes in the dark. Stained cells were analysed by FACScan flow cytometer (Becton Dickinson Immunocytometry Systems) after the incubation. During cell acquisition, 488 nm excitation wavelength was used to measure green fluorescence emission for calcein from viable cells and red fluorescence for ethidium homodimer-1 from dead cells.

Antimicrobial Efficacy Assays

(a) Bacterial and fungal strain preparation

The Antimicrobial Effectiveness Test (AET) is a suspension test for microbial killing. In this design, control inocula of the challenge organisms were placed in suspension with the sample to be tested, and then the number of survivors was determined at different time points (USP 2013). For this study we used a variety of bacteria and fungal strains to test the product. There are Gram-positive bacterial that cause the wound infection including *Streptococcus pyogenes*, *Staphylococcus epidermidis* and *Staphylococcus aureus.* Gram-negative bacterial such as *Klebsiella* spp., E*scherichia coli*, and *Pseudomonas aeruginosa* are also present in wound.

In total, there were sixteen bacterial strains and four yeast strains were obtained from the American Type Culture Collection (American Type Culture Collection, Manassas, VA, USA) and clinically isolated organism. All bacterial and fungal strains were grown and maintained with columbia sheep blood agar,chocolate agar, MacConkey agar or Sabouraud dextrose agar for 24-48 hours before

being used in the experiment. Bacterial and yeast colonies were transferred to 3 ml normal saline 0.9% (Oxoid, USA) and harvested by centrifugation at 5,000 rpm for five minutes. The cell pellets were washed twice with normal saline 0.9% and re-suspended with 3 ml normal saline 0.9%. The inocula were assessed by Optical Density (OD)600 measurement to determine the concentration of $1.5x10^8$ CFU/ml.

(b) Bactericidal and fungicidal activity with suspension test

Three product samples with different expiry dates were tested. The bacterial suspensions were adjusted to a concentration of $1.5x10^8$ CFU/ml in normal saline for each strain in a test tube. A pellet of bacterial strain was collected by centrifugation at 5000 rpm for five minutes. The bacterial pellet was added with 1 ml of product sample, mixed thoroughly, and left at room temperature for a specified exposure time of five minutes. Following exposure, the reaction mixture (bacterial cells and product sample) was centrifuged with 5000 rpm for five minutes to remove the supernatant. The collected bacterial pellet was again reconstituted with 1 ml of normal saline and serially diluted for five folds. After dilution, 100 µl of the mixture was spread immediately onto columbia sheep blood agar, chocolate agar, MacConkey agar or Sabouraud dextrose agar and incubated for 24 hours at 37°C. The number of colonies surviving on each plate was counted and cell survival rates were calculated. Each assay was performed for three product samples in triplicates and the average number of colonies was considered for analysis. Data were reported as the $\log_{10}$ reduction and percentage reduction in colony counts based on five minutes of exposure time.

RESULTS

Cytotoxicity

In order to determine the cytotoxicity of the sample product, we assessed each sample by measuring the percentage of surviving cells relative to untreated controls and treated control. For data obtained from the flowcytometry, a scatter plot against the experimentally determined percentage of live cells was generated from the proportion of live cells and the adjusted dye ratio. The results were obtained by applying gating of two regions to count calcein and ethidium bromide (EtBr) positive and negative cells, respectively. All dot plots were gated and show the percentages of live cells and dead cells. In the green dots region, viable cells were located as measured by positive calcein staining. In orange dots region, dead cells were located as measured by positive EtBr staining (Figure 1). During acquisition, statistics were calculated based on the number of currently displayed events representing total number of events, in the whole population. We only focused on % Parent which represented percentage of defined population in total number of events.

By using the LIVE/DEAD cell viability kit, the percentages of dead cells

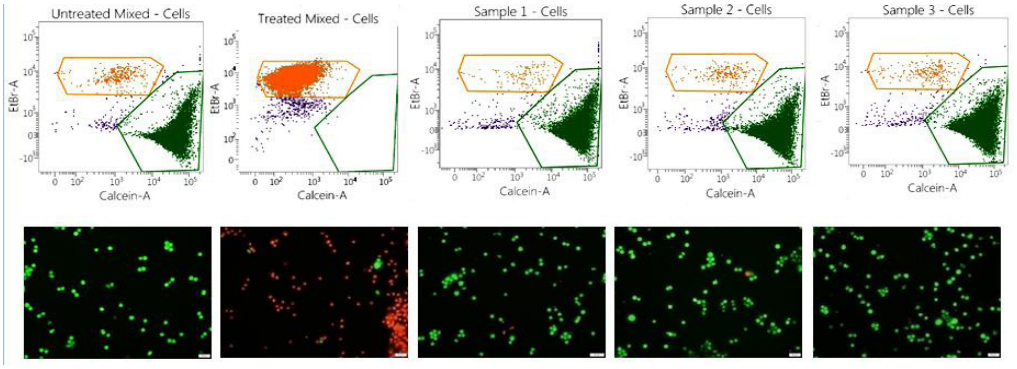

FIGURE 1: Gating for flow cytometric determination of cell viability from scatter plots. Viable cells were denoted by green dots, while dead cells were indicated by orange dots

(red fluorescence) were as followed: heat-treated mixed, 97.3%; untreated mixed, 1.3%; product sample 1, 1.8%; product sample 2, 2.0% and product sample 3, 2.2% (Table 1). The collected data showed the percentage of the cytotoxicity increased 0.4% compare to Sample 1 (04-04-2020) to Sample 3 (20-02-2021). LIVE/DEAD assay results suggested all samples showed very low cytotoxicity against MRC-5 cells and almost similar with untreated cells. Dermacyn™ was non-cytotoxic, deemed safe and caused to harm on human cells.

Bactericidal and Fungicidal Activities

Under the conditions of the study, bactericidal and fungicidal activity with the AET suspension tests showed that exposure time of five minutes was enough to inactivate all tested pathogens. The numbers of colonies of the tested pathogens decreased compared to the tested pathogens without exposure to the product (Figure 2). The study evaluated bactericidal and fungicidal activities for each product sample based on the Initial

TABLE 1: Percentage of dead cells among the viable cells

Name	Events	% Parent	% Grandparent
Treated Mixed : EtBr+	14,594	97.29	96.56
Treated Mixed : Calcein+	4	0.03	0.03
Untreated Mixed : EtBr+	188	1.26	1.20
Untreated Mixed : Calcein+	14,718	98.30	98.51
Sample 1 : EtBr+	265	1.81	1.78
Sample 1 : Calcein+	14,281	97.75	96.31
Sample 2 : EtBr+	287	1.99	1.95
Sample 2 : Calcein+	14,074	97.54	95.81
Sample 3 : EtBr+	321	2.23	2.18
Sample 3 : Calcein+	14,008	97.18	94.97

*EtBr+ = Dead cells *Calcein+ = Viable cells

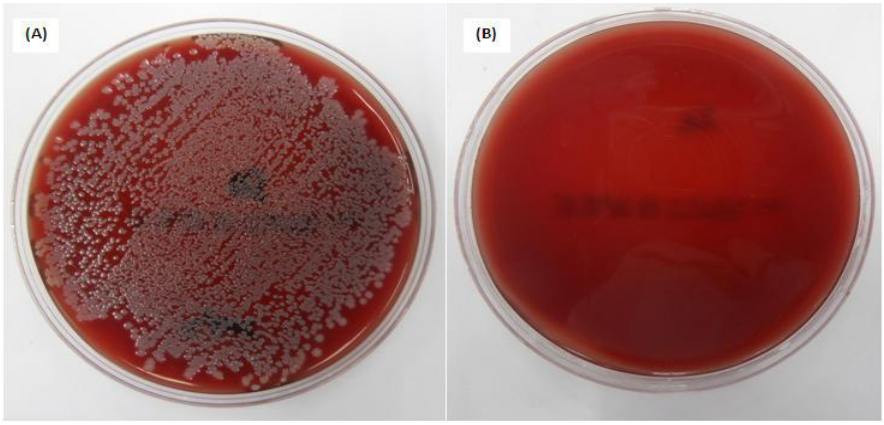

FIGURE 2: Colonies reduction on Columbia sheep Blood agar for Antimicrobial Effectiveness Test (AET). (A) Staphylococcus aureus colonies count on Columbia sheep Blood agar without exposure to product; (B) Staphylococcus aureus colonies count on Columbia sheep Blood agar after five minutes exposure to product

Populations (CFU/mL) and the Post-Exposure Populations (CFU/mL). The $\log_{10}$ and percent reductions which showed that all 20 tested bacterial and fungal strains achieved 1.097-3.000 and 92.7-99.9% within 5 minutes of contact time, respectively (Table 2). Overall, all three samples demonstrated a decrease of initial population $1.5X10^8$ (CFU/ml) to $<1.5x10^5$ (CFU/ml) of the challenge organisms. We emphasised the high performance of samples against *Pseudomonas aeruginosa and Staphylococcus aureus* because these two organisms were commonly implicated in acute and chronic wound infections. Among the various fungal strains, the percentage reduction for *Candida glabrata* (99.1%), *Candida parapsilosis* (98.8%) and *Candida krusei* (96.8%) were relatively low compare to bacterial strains. Interestingly, when considering the effects of different samples, there were increased bactericidal and fungicidal activities of about 0.1-7.0% between samples with different expiry dates. The sample product with later expiry date had better bactericidal and fungicidal effectiveness. However, this was not statistically significant (P=0.162) when we used One Way ANOVA analysis to determine whether there were any significant differences for percentage of colonies reduction on sample 1, 2 and 3.

DISCUSSION

Dermacyn™ is a superoxidised aqueous solution with active compounds; sodium hypochlorite (NaOCl) and HOCl, and the inactive compounds; hydrogen peroxide (H_2O_2), ozone (O_3), chlorine dioxide (ClO_2), sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3) and sodium chloride (NaCl). Super oxidised water (SOW) is helpful in wound management. A study reported that once SOW was exposed to the environment, its stability and shelf-life only ranged from a few hours to days

TABLE 2: Result of log_{10} reduction for challenge strains upon initial and post exposed populations

No	Microorganism species	Sample	Initial population (CFU/ml)	Post-exposure population (CFU/ml)	log_{10} reduction	Percentage reduction
1	*Streptococcus agalactiae* ATCC 13813	1	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		2	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		3	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
2	*Streptococcus pyogenes* ATCC 19615	1	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		2	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		3	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
3	*Staphylococcus aureus* ATCC 25923	1	$1.5x10^8$	$6.3x10^6$	1.396	96.0%
		2	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		3	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
4	*Staphylococcus epidermidis* ATCC 12228	1	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		2	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		3	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
5	*Enterococcus faecalis* ATCC 29212	1	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		2	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		3	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
6	*Enterobacter aerogenes* ATCC 13048	1	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		2	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		3	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
7	*Corynebacterium diphtheriae* ATCC 13812	1	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		2	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		3	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
8	*Escherichia coli* ATCC 25922	1	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		2	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		3	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
9	*Klebsiella pneumoniae* ATCC 13883	1	$1.5x10^8$	$1.0x10^6$	2.170	99.4%
		2	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		3	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
10	*Pseudomonas aeruginosa* ATCC 27853	1	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		2	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		3	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
11	*Proteus mirabilis* ATCC 35659	1	$1.5x10^8$	$2.0x10^6$	1.876	98.7%
		2	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		3	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
12	*Aeromonas hydrophilia* ATCC 35654	1	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		2	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		3	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%

13	*Stenotrophomonas maltophilia* ATCC17666	1	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		2	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		3	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
14	*Acinetobacter baumannii* (Clinical isolate)	1	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		2	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		3	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
15	*Methicillin-resistant Staphylococcus aureus* (MRSA) (Clinical isolate)	1	$1.5x10^8$	$1.2x10^7$	1.097	92.7%
		2	$1.5x10^8$	$8.0x10^6$	1.213	93.9%
		3	$1.5x10^8$	$4.8x10^5$	2.495	99.7%
16	*Enterococcus faecium* (VRE) (Clinical isolate)	1	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		2	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		3	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
17	*Candida albicans* ATCC10231	1	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		2	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		3	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
18	*Candida glabrata* ATCC64677	1	$1.5x10^8$	$1.4x10^6$	2.030	99.1%
		2	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
		3	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%
19	*Candida parapsilosis* ATCC22019	1	$1.5x10^8$	$1.8x10^6$	1.921	98.8%
		2	$1.5x10^8$	$1.4x10^6$	2.030	99.1%
		3	$1.5x10^8$	$1.1x10^6$	2.135	99.7%
20	*Candida krusei* ATCC6258	1	$1.5x10^8$	$4.9x10^6$	1.486	96.8%
		2	$1.5x10^8$	$3.4x10^6$	1.645	97.8%
		3	$1.5x10^8$	$<1.5x10^5$	3.000	99.9%

(Bryant 2005). In a study conducted by Chittoria et al. 2007, they used SOW in 20 patients and showed that 40% of patients had complete wound healing while 60% of patients showed reduced infection and promoted granulation. Another study which compared patients with lower extremity ulcers treated with Dermacyn found that the ulcers healed in 98% of the Dermacyn group, and avoided amputation (Allie 2006). Investigators from many parts of the world have studied SOW as disinfectant for instruments. Middleton et al. (2000) studied on the disinfection of bronchoscopes by using SOW and revealed that SOW may be an effective alternative disinfectant for bronchoscopes. Some studies also described the use of SOW on humans for various indications like ulcers, peritoneal lavage and hand washing (Inoue et al. 1997; Sakashita et al. 2002). SOW was able to destroy microorganisms including fungi, viruses and bacteria with electrical energy (Thomson 2000). A study found that patients admitted to the intensive care unit with nosocomial wound infection showed high incidence of

gram-negative bacilli (91.4%) including *Klebsiella pneumoniae*, *Proteus mirabilis* and *Acinetobacter baumannii* (Baviskar et al. 2019).

Wound healing depends on maintaining the viability of the healing tissues and controlling bacterial balance. In this study, we evaluated the antimicrobial effect as well as the cytotoxic effect of the product. Our findings correlated with many studies and clinical trial. A study found that a super oxidised solution with neutral pH was effective in *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella* Typhi *and Candida albicans* with up to reduction of 99.999% after 30 seconds exposure (Landa-Solis et al. 2005). Another study showed that HClO had a broad-spectrum antibacterial activity that can eradicate even antibiotic-resistant strains of bacteria including Methicillin-resistant Staphylococcus aureus (MRSA) and Vancomycin-Resistant Enterococci (VRE). It was also shown to have fungicidal activity against *Candida* species and *Aspergillus species* (Lineaweaver et al. 1985). A previous study reported that artificially contaminated eggs with *Salmonella Typhi* or *Escherichia coli* had reduced CFUs of >1.45 $\log_{10}$ CFU/egg and >6.39 $\log_{10}$ CFU/egg, respectively, after 30 seconds treatment of Neutral Electrolysed Water (Medina-Gudiño et al. 2020).

The treatment of systemic infections using topical antimicrobial agents in wound management should be avoided because it can promote the development of resistance. The World Health Organisation (WHO) also does not recommend the use of topical antimicrobial agents for rinsing and dressing wound (WHO 2013). The study conducted in West Yorkshire revealed that 50% of the fusidic acid-resistant *Staphylococcus aureus* strains originated from dermatology patients who had been exposed to topical fucidin within the six months preceding the study. This highlights the issue of inappropriate topical antibiotic usage in dermatology patients, resulting in the development of fusidic acid resistance (Shah & Mohanraj 2003). For this reason, Dermacyn with SOW might be a choice for antiseptic in wound management.

A study conducted by Gutierrez in 2006 explored applications of SOW and found that it has moistening effects and minimum toxicity. They recommended the use of SOW for wound care management. We found Dermacyn to have very minimum cytotoxicity in human fibroblast cells. Duc et al. (2007) tested the cytotoxic effect of 12 commonly used antiseptics and Dermacyn was not cytotoxic when tested on two human skin substitutes and a full-thickness autograft by in vitro assay. Currently, a study reported pH-neutral SOW infused transurethral in the bladder of rats has no adverse effects on the uroepithelium by histopathological evidence (Pérez-Salas et al. 2021).

Technically, we found some variations of antimicrobial efficacy between tested products with different expiry dates. The tested product close to expiry date showed lower antimicrobial efficacy compare to newly manufacture tested product. It is

possible because the component HClO that responsible for the bactericidal effect of wound care solution has self-decomposed and decreased significantly during storage. A study showed that electrolysed water needs to be used immediately or stored in closed containers after preparation to prevent the loss of available chlorine which is one of the main factors for contributing the antimicrobial activity (Cui et al. 2009). In addition, we found an increased in cytotoxicity with the newer product. The amount of HClO component in newly manufacture product is maintain in optimum concentration. The increased cytotoxicity was possibly due to high HOCl concentration. Previous study also showed that cytotoxic and antimicrobial activity increased with rising HClO solution concentrations and extended exposure times (Severing et al. 2019). These results are in good agreement with our study.

The main limitation of this study is that the test was conducted in vitro, so it cannot be assumed that the same results of cytotoxicity effect and antimicrobial efficacy could be transferable to the wound care and translated into clinical effectiveness.

CONCLUSION

In conclusion, the present study suggests that Dermacyn™ Wound Care has great bactericidal activity at concentrations that are safe for human cells. It can be broadly applied within a comprehensive wound management.

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