



RESEARCH ARTICLE

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COMPARISON OF DISINFECTANT AGENTS AND POSSIBLE APPLICATION AGAINST COVID-19. ARE DISINFECTANTS REALLY EFFECTIVE?

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ABSTRACT

The current pandemic caused by the Sars-Cov-2 virus directs the use of disinfectant agents more frequently, especially the recommendations and recommendations of World Health Organization (WHO) agencies. Therefore, we aimed this study to evaluate the efficacy of germination and disinfection of disposal sites – location with a high concentration of contaminants – from the dental clinic of a Brazilian university, under the microbicidal action of three different chemical agents: 70% Alcohol (v.v⁻¹), 1% Peracetic Acid (v.v⁻¹) and a common commercial product with the following formula: 0.42% o-benzyl p-chlorophenol. Through the collection and analysis of samples, it was observed that 70% alcohol was effective for disinfecting surfaces contaminated by general microorganisms, proving its possible effectiveness and indications for use against SARS-CoV-2. However, our evidence has shown that although peracetic acid has activity against viruses, further studies are needed for its applicability against COVID-19 at the concentrations analyzed in this study. On the other hand, o-benzyl p-chlorophenol in this study did not demonstrate effectiveness against microorganisms and, therefore, should not be used in coping with COVID-19.

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INTRODUCTION

Healthcare-associated infections are common in hospitals and clinics (JAKUBOVICS; GREENWOOD; MEECHAN, 2014). In addition, there is cross-infection, which consists of the transfer of an infectious agent from one individual to another in a clinical setting (KHAN *et al.*, 2010). With the emergence of the new coronavirus (SARS-CoV-2)(ZHENG, 2020), the existence of already known and recurrent viruses such as human immunodeficiency virus and hepatitis B virus, and the resurgence of ancient pathogens such as Mycobacterium tuberculosis, interest in biosafety for employees of laboratories and health units is back on the agenda (AHMAD *et al.*, 2020; SEWELL, 1995). In the UK, in 2011, approximately 6.4% of hospital patients acquired infections as a result of procedures, devices or interventions (JAKUBOVICS; GREENWOOD; MEECHAN, 2014). In this way, hospital and clinical environments become a susceptible medium of exposure of professionals and patients to biological risks involving a large

number of microorganisms that may be present in biological matrices, such as gingival fluids, spittle, blood and contaminated, in addition to surfaces not sanitized. Therefore, cross-infection control involves taking standard precautions to minimize the risk of transmission, regardless of the health status of the patient or healthcare professional (JAKUBOVICS; GREENWOOD; MEECHAN, 2014). Whereas, human coronaviruses, such as Severe Acute Respiratory Syndrome (SARS) coronavirus, Middle East Respiratory Syndrome coronavirus (MERS) or endemic human coronavirus (HCoV) can persist on inanimate surfaces such as metal, glass or plastic for up to 9 days (KAMPF *et al.*, 2020). For this, there are several devices that allow the disinfection or coating of surfaces touched by the patient, or even by the professional himself; or even various forms of sterilization, such as "cold" or disinfection of thermosensitive materials and "heat" sterilization of non-thermosensitive instruments (DUTRA *et al.*, 2008; EKLUND, 2003; LEDINGHAM *et al.*, 2014). Thus, as new methods are being studied, as shown by studies of Queiroz *et al.*, 2020 (QUEIROZ *et al.*, 2020), that demonstrate

the use of photodynamic therapy as a possible form of action against COVID-19 and microorganisms. Based on this precept, it is possible to reduce the number of potentially pathogenic microorganisms through aseptic care, as they are no longer a source of infection (KAMPF *et al.*, 2020). However, the lack of knowledge about biosafety or even neglect in relation to it is still alarming in different health environments. The incorrect use of sterilization methods, the bacteriological resistance of several types of viruses and the lack of care of professionals at risk have contributed to the increase in the number of cases of infections (RUTALA; WEBER, 2016). With the appearance of the new coronavirus (COVID-19), it was necessary to return with greater attention to this care (WU *et al.*, 2020). Therefore, the forms of disinfection and degermination are extremely important for health professionals, as well as for the good functioning not only of clinics, but of health as a general welfare. The objective of this study was to verify the antimicrobial capacity and to carefully analyze the efficiency of different chemical agents in microbial control in healthcare environments, verifying methods to reduce the spread of microorganisms in clinical environments.

METHODOLOGY

The experiments were carried out at the Laboratory of Microbiology of the Paraná Northern State University - UENP, Jacarezinho, Brazil - PR.

General lines: For the development of this work, an observational study was carried out at the disposal sites of a dental clinic at a Brazilian public university. The research was carried out during the period of activity of the place evaluating the conditions of cleaning, degermination and disinfection of filters separating debris from dental chairs, under microbicidal action of three different chemical agents: 70% Alcohol ($v.v^{-1}$), 1% Peracetic Acid ($v.v^{-1}$) and 0.42% o-benzyl p-chlorophenol (commercial product). The collection of materials was carried out in two stages: before and after the use of the evaluated disinfectants, with the aid of sterile swabs soaked in sterile phosphate-saline buffer (PBS). For better analysis, dental chairs were disinfected with 70% alcohol, collections were made before and after cleaning with the aid of sterile swabs soaked in phosphate-saline buffer (PBS).

Preparation of petri dishes and buffer solution: The cultivation of microorganisms was based on the work of Silva *et al.* (SILVA *et al.*, 2019), for this purpose, petri dishes (42.5 mm in diameter) were made with 20mL of the “Brain Heart Infusion Agar” (BHI) culture medium. Afterwards, the microorganisms were inoculated from the test sites, sample collection was based on the Machado *et al.* (MACHADO *et al.*, 2020) modified, then preparing a phosphate buffered saline solution (PBS) with pH 7.2 (10x) using 8.25g NaCl (Sodium Chloride), 1.05g $Na_2 HPO_4$ (Sodium Phosphate - Sodium Phosphate) and 0.35g of $NaH_2PO_4 + H_2O$ (Monobasic Sodium Phosphate) in 1000mL of deionized water. The pH was checked in a pHmeter (Tecnal TEC-5), and the sample was sterilized in a vertical autoclave (Primatec CS).

Proof Board: This study was carried out through the collection of samples before (negative control) and after the disinfection procedure, based on the technique of Jett *et al.* (JETT *et al.*, 1997) using sterile swab. The collection was carried out in the sinks (in the sterilization room and clinics),

in addition to samples of the debris separating filters of the dental chairs at the University Dental Clinic. For this purpose, these sites were subjected to individual disinfection with 70% alcohol, 1% peracetic acid and 0.42% o-benzyl p-chlorophenol. Then, the samples were placed in autoclave sterilized petri dishes and soaked in saline solutions. To monitor bacterial growth, the plates were placed in greenhouses at 36.5°C, for a period of 0, 12 to 24 hours. For the fungal evaluation, the plates were submitted to a temperature of 25°C for a period of 120 to 168 hours. To monitor the negative control, plates without inoculation were used. In a second step, an individual analysis of samples from 4 chairs was carried out using 70% alcohol. The collection was performed with swabs soaked in phosphate-saline buffer (PBS), before (positive control) and after disinfection; 12 samples of each were collected. In sequence, they were inoculated into petri dishes containing “Brain Heart Infusion Agar” (BHI), placed in an oven at 37°C, monitored and photographed for a period of 12 hours. The determination of the growth area and its macroscopic aspects, after the stage of inoculation in the greenhouse, was through images photographed at a distance of 5 cm and analyzed by the ImageJ® software (version 1.52a) by prior calibration of the software.

Determination of antimicrobial activity: In order to determine the microbicidal effect of chemical agents (70% alcohol, 1% peracetic acid and 0.42% o-benzyl p-chlorophenol) on the possible microorganisms (bacteria and yeast), inoculation of the sites was carried out disposal and chairs after disinfection. For this, a sterile swab was used for each collection, moistened in the sterile PBS solution that were rubbed in the established place (before and after cleaning), with subsequent peaking in the culture media (JETT *et al.*, 1997). Then, the inoculated plates were placed in an oven (DeLeo) at 36.5°C for growth verification. The colonies formed were classified as positive (showed growth) or negative (showed no growth). To verify fungal growth, the temperature of the greenhouse was reduced to 28°C, and qualified as positive or negative according to the growth after 120h and 168h. As a negative control, plates without inoculation were used, and all samples were treated in a biological chapel (Shimadzu AUY220) and free from contamination. All materials involved were previously sterilized in a vertical autoclave (Primatec CS). A second analysis was made using 70% alcohol using samples from 4 dental chairs at Dental clinic, before and after cleaning, with the help of swabs soaked in phosphate-saline buffer (PBS); 12 samples were taken from each chair before and after the disinfection process. Microbial growth was determined after processing the ImageJ software (version 1.52a) (BARAPATRE; AADIL; JHA, 2016).

Statistical Analysis: Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) - version 25. The normality of the data was tested with the Shapiro-Wilk test. Since the variable area of growth (cm^2) presented a normal distribution for o-benzyl p-chlorophenol 0.42% (Shapiro-Wilk test: $p > 0.05$). The t-test - independent samples or the Mann-Whitney test was used to compare the means between the growth area of the treaty (after the disinfection procedure) with the negative control. To analyze the results of the differences between the three groups after the disinfection treatment, the Kruskal-Wallis non-parametric test was used. In the statistical test, a significance level of 5% was

considered. Thus, the differences were considered statistically significant if the significance was less than 0.05 ($p < 0.05$). The graph was made in OriginPro (version 9.1).

RESULTS

Table 1 shows the statistical results obtained for the area of growth of microorganisms for the different disinfectant products used in the disposal areas, after 168 hours in the microbial growth oven. The results demonstrate that peracetic acid (1%) and alcohol (70%) were statistically efficient in disinfecting the samples (49.33 ± 0.62 and 48.60 ± 2.21 , respectively), after the cleaning procedure, because for all samples the result was 0 (zero) ($p < 0.05$). In addition, no statistical difference was demonstrated between the treatments of peracetic acid (1%) and alcohol (70%) ($p < 0.05$) (Figure 1).

However, the use of o-benzyl p-chlorophenol (0.42% - commercial product) did not prove to be effective in eliminating microorganisms after the disinfection procedure (49.23 ± 0.68 - positive control and 48.11 ± 1.03 - after disinfection procedure). Therefore, the results demonstrate that there was no statistical difference between before and after cleaning ($p > 0.05$). In the second analysis, performed on 4 dental chairs, they were disinfected using only 70% alcohol, for which statistical differences were demonstrated between disinfection and negative control ($p < 0.05$). After the application of this chemical compound, no microbial growth was observed (Graph 1). The samples without treatment, for all chairs, showed an intense microbial growth (Figure 2) as can be seen in "dental chair 4", which presented 27.15 ± 1.11 of microbial growth area to 0 (zero), after disinfection. Thus, it is suggested that 70% alcohol is an efficient method of disinfection against microorganisms.

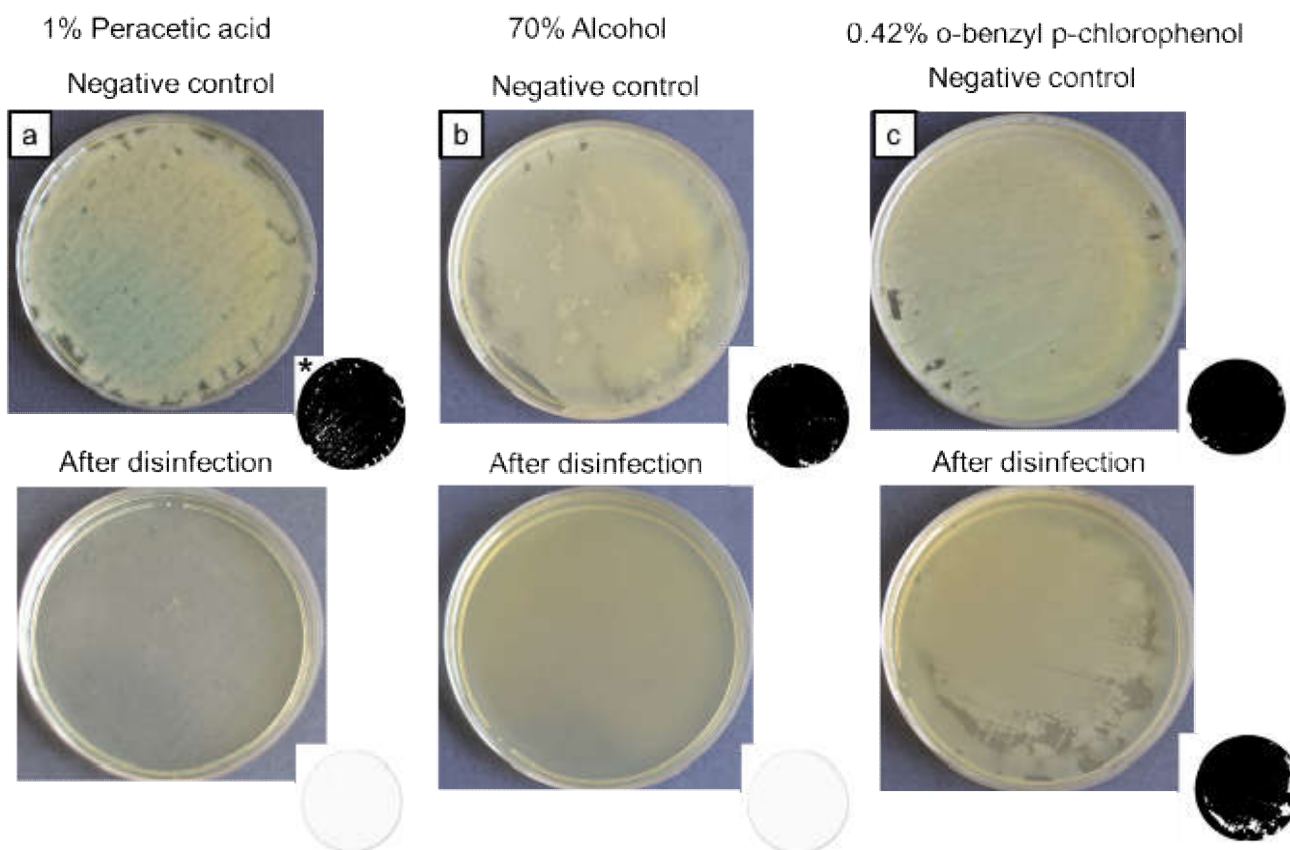


Figure 1. Samples from the disposal areas of the dental clinic showing the activity of the disinfectant products used before and after processing: a) 1% Peracetic acid; b) 70% Alcohol; c) 0.42% o-benzyl p-chlorophenol. *figures after processing in ImageJ® software (version 1.52a).

Table 1. Characterization and comparison of growth area values (cm^2) for antimicrobial activity for the tested chemical agents

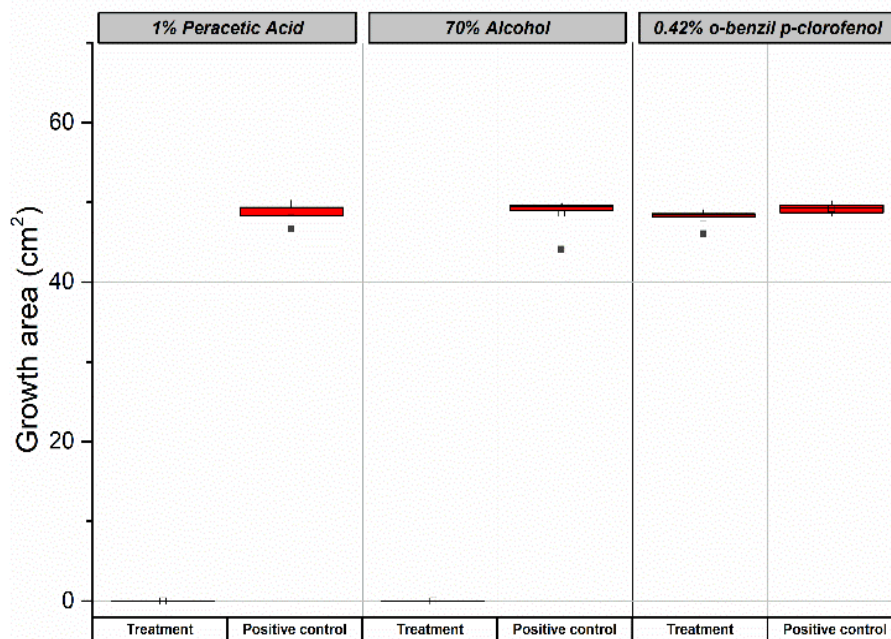
Group	Negative control	After the disinfection procedure	Test Mann-Whitney ⁽²⁾	Teste T - Independence ⁽³⁾
1% Peracetic acid	Mean = 49.33 SD = 0.62 (n = 6)	All values equal to 0 (n = 6)	$p < 0.05^{(2)}$	-
70% Alcohol	Mean = 48.60 SD = 2.21 (n = 6)	All values equal to 0 (n = 6)	$p < 0.05^{(2)}$	-
0.42% o-benzyl p-chlorophenol	Mean = 49.23 SD = 0.68 (n = 6) Kruskal-Wallis test ⁽¹⁾	Mean = 48.11 SD = 1.03 (n = 6)	-	$p > 0.05^{(3)}$
			$p > 0.05$	

⁽¹⁾Significance level of the Kruskal-Wallis test for the comparison between the 3 groups; ⁽²⁾Significance level of the Mann-Whitney Test for comparison with the negative control; ⁽³⁾Significance level of the Independent t-test for comparison with the negative control.

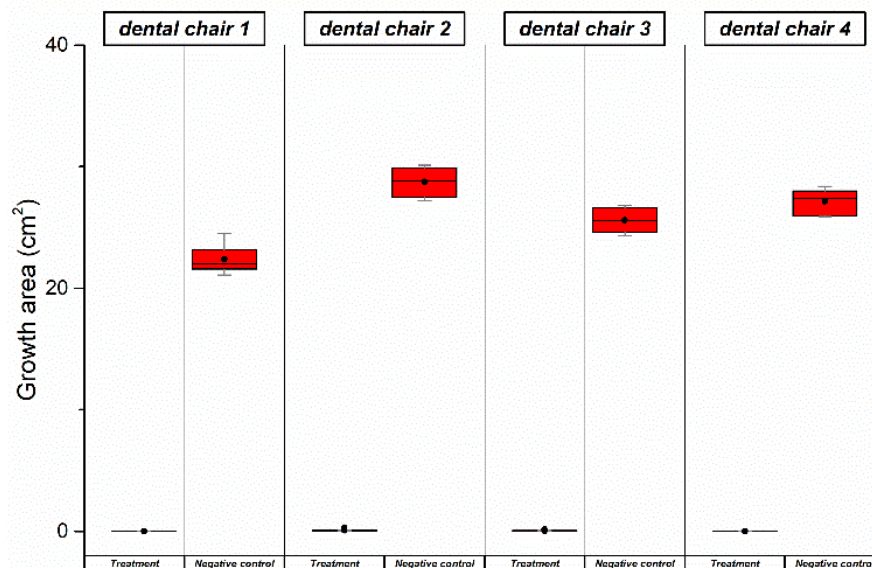
Table 2. Characterization and comparison of growth area values (cm²) for the action of 70% alcohol as a cleaning and disinfection procedure

Group	Negative Control	After the disinfection procedure	Test Mann-Whitney ⁽¹⁾
Dental chair1	Mean = 28.39 SD = 1.24 (n = 12)	All values equal to 0 (n = 12)	p < 0.05
Dental chair2	Mean = 28.73 SD = 1.21 (n = 12)	Mean = 0.06 SD = 0.12 (n = 12)	p < 0.05
Dental chair3	Mean = 25.58 SD = 1.08 (n = 12)	Mean = 0.02 SD = 0.06 (n = 12)	p < 0.05
Dental chair4	Mean = 27.15 SD = 1.11 (n = 12)	All values equal to 0 (n = 12)	p < 0.05

(1) Significance level of the Mann-Whitney Test for comparison with the control negative.



Graph 1. Boxplot with growth area values in each group of the dental clinic disposal areas, before and after the degermination procedure, using 1% peracetic acid, 70% alcohol and 0.42% o-benzil p-chlorophenol, respectively.



Graph 2. Boxplot with values of the growth area of the groups of microorganisms collected in the dental clinic chairs before and after the disinfection procedure using 70% alcohol



Figure 2: Samples before and after processing. On the left the samples collected before cleaning with 70% alcohol. On the right, there are the collection samples after cleaning with 70% alcohol

DISCUSSION

The results obtained in this study demonstrated that 1% peracetic acid and 70% alcohol were effective for disinfecting surfaces contaminated by general microorganisms, as shown in the literature (YANG; WANG, 2020). However, o-benzyl p-chlorophenol did not demonstrate significant efficacy in disinfection, in addition, Butaye (BUTAYE, 2014) demonstrated that this type of disinfectant is not effective against viruses and bacterial spores. This study particularly evaluated bacteria and fungi in disposal areas and chairs at a dental clinic at a Brazilian state university. As expected, 70% alcohol was effective in decontamination by reducing the number of bacterial cells and live yeasts on the analyzed surfaces. Its antimicrobial action is due to its chemical composition, with two carbon atoms, and its high solubility in water, which allow to break the hydrogen bonds that exist between the amino acid residues, which results in a structural collapse of the affected proteins and, consequently, the loss of their activities and inactivation of microorganisms (LIMA *et al.*, 2020). As for the applicability in relation to viruses, and its action against SARS-CoV-2, 70% alcohol causes structural breakdown of viral proteins, decomposes the biomolecules of the phospholipid bilayer and, thus, inactivates the virus from the breakdown of its envelope lipid and thus destruction of its nucleocapsid (LAMAS *et al.*, 2020). This fact made 70% alcohol a member of the disinfection protocol against COVID-19 (LIMA *et al.*, 2020; MOORER, 2003; RAHMAN; BAHAR, 2020).

The World Health Organization (WHO) (OMS, 2020) among the protocols for coping with the COVID-19 pandemic, the use of 70% alcohol as a disinfectant to be used after washing hands and cleaning surfaces and places that may act as a source of infection. Lamas *et al.* (LAMAS *et al.*, 2020) demonstrated that the cleaning of possible sources of infection such as door handles, doors and cytological samples, through the use of 70% alcohol, proved to be effective in inactivating SARS-CoV-2 in laboratories. Peracetic acid has disinfectant activity based on the oxidation of cellular constituents of microorganisms, disruption of osmotic function, transport by membrane lipoproteins and protein denaturation (NASCIMENTO, 2015). However, Nascimento *et al.* (NASCIMENTO *et al.*, 2015) states that this chemical

compound has an action on the bases of the DNA molecule, inactivating catalase - an enzyme that neutralizes the action of hydroxyl free radicals. These properties, as affirm HE *et al.* (HE *et al.*, 2020), make peracetic acid be able to completely inactivating a virus, and can be used to fight different types of viruses, such as SARS-CoV-2. Studies of Pradhan *et al.* (PRADHAN *et al.*, 2020) confirmed that peracetic acid in concentrations of 0.2% is effective in destroying COVID-19 viral particles after the exposure time of 30s. At the present time, the world is experiencing a pandemic caused by the SARS-CoV-2 virus (SINGHAL, 2020), where there are no vaccines or effective methods of treatment against the signs and symptoms of COVID-19 disease (PRADHAN *et al.*, 2020). An analysis of 22 studies revealed that human coronaviruses can persist on inanimate surfaces such as metal, glass or plastic for up to 9 days (KAMPF *et al.*, 2020); but it can be effectively inactivated by surface disinfection procedures. Queiroz *et al.* (QUEIROZ *et al.*, 2020) suggests the use of photodynamic therapy as a possible action to face and decontaminate surfaces. However, chemicals that are easily accessible to the population are more effective and faster tools, such as ethanol (62-71%), which has an excellent action against SARS-CoV-2 (KAMPF *et al.*, 2020). Therefore, knowing the properties of 70% alcohol is of utmost importance to make it applicable against this new virus.

In the current scenario, preventive measures such as hand washing and hygiene with 70% alcohol have been suggested by WHO (OMS, 2020), shows extreme efficiency in combating COVID-19 (MAHMOOD *et al.*, 2020). Several Brazilian health agencies indicate 70% ethanol as a sanitizer against countless microorganisms, and in the most current one against COVID-19 (ANVISA, 2020). Peracetic acid has an effective activity against viruses, as stated by HE *et al.* (HE *et al.*, 2020), however, there are no studies that prove its real effectiveness against SARS-CoV-2 in the concentrations analyzed in this study. The o-benzyl p-chlorophenol in this study did not demonstrate effectiveness against microorganisms and may be related to the literature (BUTAYE, 2014), that this compound is ineffective against viruses and, therefore, should not be used in coping with COVID-19. Therefore, knowing the disinfectant agents and their antimicrobial properties allows their application as methods of prevention and disinfection. The evidence presented in this study shows the effectiveness of 70% alcohol against bacteria, fungi and viruses, which is a really effective and safe product for defense against SARS-CoV-2.

Conclusion

The results obtained in this study demonstrated that the 70% alcohol was effective for the disinfection of surfaces contaminated by general microorganisms, thus proving the effectiveness of this agent against general microorganisms and, consequently, its recommendation made by the health agencies against the SARS-CoV-2 virus. Peracetic acid was effective against the microorganisms analyzed in the samples, having present activity against viruses, as shown in the literature. However, further studies are needed to assess its applicability against COVID-19 at the concentrations analyzed in this study. O-benzyl p-chlorophenol 0.42%, in this study, did not demonstrate effectiveness against microorganisms and, therefore, should not be used as a disinfectant to face the current pandemic caused by COVID-19.

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