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2018
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Efficacy of different vinegar solutions in removal of *Candida albicans* from denture acrylic resin

Tarana Garach

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree of Msc (Dent) (OMP)

Johannesburg,2018

DECLARATION:

I, Tarana Garach, declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Master of Science (Oral Medicine) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

_____

_____day of _____ in 20_____

ACKNOWLEDGMENTS:

I would like to thank both my mother and my father for their love, time, sacrifice and encouragement. A thank you to my brother Sachin and sister Akshita for the constant support.

I would like to acknowledge both my supervisors Dr J. Molepo and Prof. S. Shangase for their guidance and encouragement throughout this project.

I would also like to acknowledge Miss Mulalo Molaudzi for the many hours spent in the laboratory with me.

Thank you to Adedayo Tunde Ajidahun for his assistance with the statistical analysis for this project.

Thank you to the Faculty Research Committee, Wits University, for the FRC research grant that enabled this research to be performed.

DEDICATION

In loving memory of my father

Deenash Bhugwanjee Garach

1955 - 2017

PRESENTATIONS ARISING FROM THIS PROJECT

Poster –School of Oral Health Sciences research day, University of the Witwatersrand, August 2017

ABSTRACT

Background and Aims: Denture hygiene has become an important aspect in managing patients who often present with signs of denture stomatitis associated with *Candida* infection. There is a need for denture disinfectants which are of low cost and are easily accessible to denture wearers. The aim of this study was to investigate the efficacy of different vinegar solutions in removal of *C. albicans* from denture acrylic resin.

Materials and Methods: Hundred and ninety-two acrylic plates were used. White wine vinegar (6%), rice vinegar (5.5%), and apple cider vinegar (5%) were used as disinfectants. Distilled water and 0.2% Chlorhexidine were used as controls.

Cultures of *C. albicans* ATCC 90028 and a HIV strains were grown in Saboraud's dextrose agar. Sterile acrylic resin plates were immersed in test tubes and 200µl of *C. albicans* suspension was added to each tube. Contaminated acrylic plates were divided into 5 groups of 6 plates each. Plates were immersed in 20 ml of white wine vinegar (WWV), Rice vinegar (RV), Apple cider vinegar (ACV), sterile distilled H_2O, and chlorhexidine (CHX). These were incubated at room temperature for 30minutes, 1 hour and 8hours. Two non-exposed plates were included as controls.

Results: ACV, WWV and RV equally eliminated both *C. albicans* ATCC 90028 and HIV strains from acrylic plates at 8 hours (% Kill=100). All tested vinegars failed to completely eliminate *C. albicans* strains at 30 minutes and 1 hour, with no statistical significant difference for ATCC strain($p<0.05$) and with statistical difference for ACV ($p=0.03$) and RV ($p=0.01$) respectively for HIV strain. CHX completely eliminated ATCC strain at all tested times (%=100), but failed to completely eliminate HIV strain at 30 minutes and 1 hour. Sterile water, a negative control failed to completely eliminate both *C. albicans* strains at all tested times.

Conclusions: The results of the current study confirm that vinegar can be used to remove *C. albicans* from dentures, if used for 8 hours.

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LIST OF ABREVIATIONS:

C. albicans: *Candida albicans*

hrs: hours

^{0}C: degrees celcius

mm: millimetres

min: minutes

µl: microliters

ml: millilitres

WWV: white wine vinegar

RV: rice vinegar

ACV: Apple Cider Vinegar

CHX: Chlorhexidine

PBS: Phosphate Buffered Saline

CFU: colony forming unit

LP: phospholipases

GPI: glycosylphosphatidylinositol

SAPs: Secreted aspartyl proteinases

RPM: revolutions per minute

SDA: Sabourad Dextrose Agar

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

According to the World Health Organization, around 30% of people aged between 65-74 years are edentulous globally (World Health Organization 2012), with many of them replacing lost teeth with dentures.

In 1988, the South African National Oral Health Strategy (SANOHS) reported the prevalence of edentulism to be 10.36 % and 26.6% for population groups of ages 35 to 44 years and 60 to 64 years respectively (National Policy for Oral Health in South Africa, 1988).

Denture hygiene has therefore become an important aspect in managing these patients as they often present with signs of denture stomatitis, associated with *Candida* infection (Douglas 2003).

Denture stomatitis is an inflammatory reaction of the denture-bearing mucosa, characterized by varying degrees of erythema. It is one of the most common problems in elders wearing partial or complete dentures, and is more common in women (Arendorf, 1987). The disease affects between 15 and 70% of denture wearers (Gendreau & Loewy, 2011). Several factors contribute to the onset of the disease, including patient age, age of denture, oral hygiene, diet, smoking, denture trauma, continuous denture wearing, salivary flow, denture base material, cellular immunity, pH of the denture plaque and oral microorganisms (Shulman *et al*, 2005; Coco *et al.,* 2008; Gasparoto *et al.,* 2009; Gendreau & Loewy 2011; Skupien *et al.,* 2013). However, *Candida albicans* is reported as the primary causative agent of the disease (Ramage *et al.,* 2004; Jackson *et al.,* 2014).

Denture base acrylic resin is a significant contributing factor to the oral colonization of the denture by *C. albicans*. Dentures have rough fitting surfaces which are conducive for adherence of candidal species, amongst others, and formation of the biofilm (Wady *et al.,* 2012). In order to control this colonization and prevent *Candida* infections, the removal of these organisms from dentures is important. Antifungal agents are used to eradicate *C. albicans* and relieve denture stomatitis, but if the dentures are not

properly decontaminated and their cleanliness maintained, denture stomatitis recurs when treatment is discontinued (Glass *et al.,* 2004).

Several disinfectants have been used to remove *C. albicans* from dentures. Chlorhexidine gluconate (2%) antiseptic mouthwash has been used as a soaking solution to suppress adhesion of *Candida* to acrylic dentures (McCourtie *et al.,* 1986). However, the use of chlorhexidine leads to a brown discolouration of the denture, and has a bitter taste which may reduce compliance (Flotra *et al.,* 1971). Sodium hypochlorite can reduce the number of *Candida* on the denture surface effectively by immersing dentures in a solution of 0.02% sodium hypochlorite. However, sodium hypochlorite bleaches dentures if they are immersed in this solution for long periods (Abelson 1985).

Although previous studies have tested the removal of *C. albicans* from a denture base using acetic acid/ vinegar (Pinto *et al.,* 2008; Jafari *et al.,* 2012; Yadav *et al.,* 2013; Gouveia *et al.,* 2014; Mota *et al.,* 2014), none have studied and compared the effect of different types of vinegars nor have guidelines on optimal times required for these solutions to remove *C. albicans* from dentures.

1.2 *CANDIDA ALBICANS*

C. albicans is a unicellular, dimorphic yeast, which can grow as ovoid or spherical cells in standard laboratory media or as hyphal cells when conditions such as temperature, nutrients and pH are changed (Sudbery 2011; Si *et al.,* 2013).The yeast is commonly grown in Sabouraud's dextrose agar medium, with a pH of less than 6 to suppress commensal oral bacteria, where it appears as cream-colored colonies (Samaranayake 2002).

C. albicans is one of the most common causes of opportunistic infections in human immunodeficiency virus (HIV)-infected individuals. It is reported that up to 90% of HIV-positive individuals are affected by oral candidiasis at least once in the course of their disease (Hung *et al.,* 2005). In a South African study, Candida was found to be more prevalent in HIV-positive (75%) as compared to HIV-negative (68%) patients (Patel *et al.,* 2006). High quantities of *Candida* and variety of *Candida* species are found in HIV positive as compared to healthy individuals. *C. albicans* isolated from the oral cavities

of HIV positive patients has increased adherence ability and therefore considered to have increased virulence (Sweet *et al.,* 1995; Jain *et al.,* 2010; Mane *et al.,* 2011).

1.2.1 Adhesion of *Candida albicans* to Dentures

Candida albicans is a normal flora of the oral cavity in 45-65% of healthy individuals (Farah *et al.,* 2010). The organism becomes opportunistic in denture wearers, as dentures decrease the flow of oxygen and saliva to the underlying tissue, leading to production of acidic and anaerobic environment, favouring yeast overgrowth. This increases the prevalence of *C. albicans* in denture wearers to 60-100% (Salerno *et al.,* 2011; Kanaguchi *et al.,* 2012; Loster *et al.,* 2012; Sampaio-Maia *et al.,* 2012).

The use of denture base acrylic resins plays a significant role in contributing to the oral colonization by *C. albicans*. The initial attachment of *C. albicans* to the denture depends on physical properties of the denture surface such as porosity, surface free energy, hydrophobicity (Yildirim *et al.,* 2005) and roughness (Pereira-Cenc *et al.,* 2007). These factors contribute to *C. albicans* adhesion, which is a crucial step in biofilm formation (Hoshing *et al.,* 2011; Lazarin *et al.,* 2014). Salivary coating of acrylic has been reported to decrease the adherence of *C. albicans (*Devarhubli *et al.,* 2011). This was found to be due to saliva decreasing surface roughness, as well as surface free energy of the denture acrylic. In another study, the production of biofilm formation by *C. albicans* according to the surface roughness of a denture was described. The surface of acrylics was manually abraded to compare biofilm formation by mass, and the type of surface of the acrylic was shown to be vital in the attachment of fungal cells. These investigators found that more roughened surfaces retained more organisms, and that old dentures that have been abraded due to use of toothbrushes harboured more microorganisms than new dentures (Jackson *et al.,* 2014).

The type of denture base has been reported to affect *C. albicans* adherence. The most commonly used denture material is heat cured polymethyl methacrylate (PMMA) due to its cost-effectiveness, easy availability and manipulation (Phoenix *et al.,* 2004). PMMA denture bases with a surface roughness above 0.2 μm have been shown to be easily colonized by *C. albicans* (Quirynen *et al.,* 1990; Radford *et al.,* 1999).

Valplast dentures have been reported to have less *C. albicans* adherence than PMMA based dentures (Ahmad *et al.,* 2012), while metal based dentures were shown to have less adherence than acrylic based dentures (Devarhubli *et al.,* 2011).

1.2.2 *C. albicans* virulence factors

*C. albicans*is a notorious opportunistic pathogen, and the major factor contributing to its virulence is its ability to persist on mucosal epithelia of healthy individuals (Shepherd *et al.,* 1985). The virulence factors expressed by *C. albicans* vary depending on the type of infection (i.e., mucosal or systemic), the site and stage of infection, and the nature of the host response (P´erez *et al.,* 2011).

Various virulence factors involved in the pathogenicity of *C. albicans* include expression of adhesins and invasins on the cell surface, biofilm formation, phenotypic switching, secretion of hydrolytic enzymes and transition between hypha and yeast forms.

Initial colonization requires adherence of *Candida* to host cells, which include epithelial, endothelial and phagocytic cells. The adhesins expressed by *C. albicans*bind to host peptide ligands and assist adhesion with other microbial pathogens, causing polymicrobial infections (Lamont & Jenkinson 2000). *C. albicans*cell wall adhesins such as CaEap1 may be highly involved in the adhesion to denture acrylic (Li & Palecek, 2003).

C. albicans virulence factors allow this organism to adhere to and colonize surfaces, thereby forming biofilms (Ramage *et al.,* 2004). A biofilm is a thin layer of microorganisms adhering to the surface of a structure which may be organic or inorganic together with the polymers that they secrete (Redding *et al.,* 2009). The individual organisms that are found are embedded in a matrix of these extracellular polymers (Douglas 2003).*Candida* biofilms consist mainly of intricate networks of yeast cells and hyphae deeply embedded into cracks and imperfections of the biomaterials. These adhered organisms may be better protected from any washing action of saliva (Redding *et al.,* 2009).The structure of biofilm allows entry of nutrients and exit of wastes. It also makes the fungal cells inaccessible to host immune system cells and boosts cell communication (Costa *et al.,* 2013). These biofilms are multi-drug

resistant and can withstand antifungal concentrations 1000-fold higher than those inhibiting planktonic cells (Chandra *et al.,* 2001).

C. albicans have special sets of glycosylphosphatidylinositol (GPI)-linked cell surface glycoproteins, which allow the organism to adhere to the surface of the host cell or other surfaces such as dentures. *C. albicans* cell wall proteins are important in conferring cell surface properties; including hydrophobicity, immunogenicity, charge, and permeability (Yin *et al.,* 2008). These proteins are also involved in binding to salivary proteins on the denture acquired pellicle, adhesion to denture surfaces and biofilm formation (Klotz *et al.,* 2007).

Switching from yeast to hyphae form facilitates the invasion process on the biotic surfaces or host cells (Mayer *et al.,* 2013). *C. albicans* cell wall protein, hyphal wall protein 1 (HWP1), is mainly expressed in hyphal forms and plays a role in biofilms formation (Nobile *et al.,* 2006).

C. albicans produces enzymes such as phospholipases and proteases which are involved in its pathogenicity. Secreted aspartyl proteinases (SAPs) have been identified in *C. albicans* and their activity is enhanced in acidic conditions, where they are involved in digesting or destroying cell membranes and degrading host surface molecules (Schaller *et al.,* 2005). This is suggested to be a contributing factor in enhancing the ability of *C. albicans* to form denture plaque on the denture surface. These acidic proteases have also been shown to deactivate secretory immunoglobulin (IgA), allowing colonization of mucosal surfaces by *C. albicans* (Akiyama *et al.,* 1993).

C. albicans produces cut phospholipases (LPs), a group of heterogeneous enzymes (PLA, PLB, PLC and PLD), with the ability to hydrolyze glycerophospholipids, major components of the cell membrane (Guijas *et al.,* 2014). LPB has the major phospholipase activity as it disrupts host cell membrane (Francoise *et al.,* 2013; Deepa *et al.,* 2015).

The ability of *C. albicans* to produce SAPS and phospholipases enables it to adjust and respond to its environment, making it an excellent opportunist pathogen.

1.3 DENTURE STOMATITIS

Denture Stomatitis is an inflammatory lesion of the palatal mucosa bearing complete or partial removable dentures. Various factors influence the onset and severity of the disease, but the most common causative factors include poor denture fit, increasing age of the denture user, increased age of dentures, *Candida* infection, and poor denture hygiene (Gendreau &Loewy 2011; Skupien *et al.,* 2013). However, *Candida albicans* is reported as the primary causative agent of the disease (Ramage *et al.,* 2004; Jackson *et al.,* 2014).

The disease is common and recurring, and is observed in approximately 11% to 67% of otherwise healthy denture wearers (Arendorf & Walker 1987). Patients with denture stomatitis may complain of a burning sensation, discomfort, or bad taste, but in the majority of cases they are unaware of the problem. The disease is reported as the most common oral mucosal lesion associated with removable dentures (Cueto *et al.,* 2013), and affects one in every three complete denture wearers (Zissis *et al.,* 2006).

Denture stomatitis occurs in 3 stages based on the appearance, as classified by Newton (1962): **Type I: Punctiform hyperemia**, where hyperemia signs of the minor palatine salivary glands occur; with an erythematous punctiform appearance where small or diffuse areas of the palate may be affected. **Type II: Diffuse hyperemia**, smooth and atrophic mucosa, with erythematous appearance of the denture bearing area, It is considered the most common presentation of Denture Stomatitis. **Type III: Granular hyperemia**, more common in dentures with suction chambers. Affect the central region of the palate, with rough and nodular appearance of the mucosa (de Oliveira *et al.,* 2010).

1.3.1 Treatment of Denture Stomatitis

The treatment of denture stomatitis includes denture and oral cavity hygiene instructions, removal of the denture, use of antifungal agents, and making a new denture (Williams & Lewis 2000). A variety of chemical and mechanical means have been suggested, such as tooth brushing, mechanical brushing of dentures and the use of mouthwashes (Williams *et al.,* 2010).

Antifungal agents used include topical (first line) and systemic. Topical agents used include miconazole 2%, which is commercially available in form of gel, and can be applied directly on a previously cleaned denture surface. It is used 2 to 3 times a day for one or two weeks, guided by patient response (Newton 1962). Nystatin, available as liquid suspension, cream and pastille is used on the oral mucosa several times a day(Lalla *et al.,* 2013). If topical antifungal agents are not effective, other antifungal agents such as fluconazole, ketoconazole, amphotericin B, miconazole and clotrimazole are recommended (Montejo 2011; Spellberg *et al.,* 2006; Ramage *et al.,* 2002; Redding *et al.,* 2009).

1.4 DENTURE DISINFECTION

The main causative factor of denture stomatitis is the presence of biofilms formed by bacteria and yeasts (Pavarina *et al.,* 2003; Montagner *et al.,* 2009). Therefore, denture cleansing is very important in preventing or treating infections in patients with dentures (Shay 2000).

C. albicans adherence to the denture can be disrupted by using mechanical and/or chemical disinfection procedures on the dentures (da Silva *et al.,* 2011). Several mechanical methods are used including toothbrushes, nailbrushes, magnetic stirrers, agitators, sonic vibrators, and ultrasonic cleansers (Arita *et al.,* 2005).For handicapped denture wearers with limited motor capacity, chemical disinfection procedures are recommended (de Freitas *et al.,* 2011; Silva *et al.,* 2011).

Denture care products should have bactericidal and fungicidal properties, be able to remove inorganic/ organic deposits and stains, be easy to handle, be non-toxic, be compatible with the denture materials, and cheap (Jagger & Harrison 1995).

1.5 DISINFECTANTS

Several chemical disinfectants have been used to remove *C. albicans* from dentures.

1.5.1 Chlorhexidine

Chlorhexidine is considered the gold standard agent for its effectiveness in controlling dental plaque (Jones, 1997). It is bactericidal and also effective against some yeasts and viruses (Marsh 1992). Chlorhexidine is reported to accumulate on the cell surfaces of yeasts at low concentrations, causing cell membrane disruption and leakage of cytoplasmic component. Higher chlorhexidine concentrations produce coagulation of cytoplasmic constituents in microorganisms (da Silva *et al.,* 2011).

Chlorhexidine gluconate antiseptic mouthwash has been used to suppress adhesion of *Candida* by immersing acrylic dentures in this solution (McCourtie *et al.,* 1986). In a study by Lee *et al.,* (2011), denture acrylic resin samples were soaked in 0.2% chlorhexidine for removal of *C. albicans*, and at this concentration, chlorhexidine was found not to be effective. Higher concentrations (0.5% and 2%) of chlorhexidine were used in other studies and were found to be effective in removing *C. albicans* biofilms (Sena *et al.,* 2006; Theraud *et al.,* 2004). However, chlorhexidine has several disadvantages including its bitter taste, which may last as long as 4 hours (Flora *et al.,* 1971). Other side effects include increased calculus formation, irritation of the mucosa, and staining of teeth, tongue, and restorations (Sreenivasan & Gaffar 2002). Patient compliance in using this antiseptic is slow due to the inconvenience of obtaining the product by prescription and its cost.

1.5.2 Sodium hypochlorite

Sodium hypochlorite based denture cleansers are fungicidal, and are known to dissolve mucin and other organic substances easily (Harrison *et al.,* 2004). The antimicrobial activity of sodium hypochlorite is based on its higher pH, which is >11 (Estrela *et al.,* 2002). The high pH of sodium hypochlorite compromises cytoplasmic membrane integrity with irreversible enzymatic inhibition, biosynthetic alterations in cell metabolism and phospholipid destruction (Estrela *et al.,* 2003).

Several studies reported the effectiveness of sodium hypochlorite in removing biofilms from dentures (Chau *et al.,* 1995; Pavarina *et al.,* 2003; Barnabé *et al.,* 2004; Karakis *et al.,* 2016). However, sodium hypochlorite bleaches dentures if they are immersed in this solution for long periods (Abelson 1985).

1.5.3 Vinegar

1.5.3.1 White vinegar

Vinegar is a sour liquid comprised mainly of acetic acid, which is prepared by the fermentation of alcoholic beverages, mainly white and red wines. This solution is cheap and easily available in South African markets.

The mode of action of vinegar is said to be through necrosis or apoptosis. It enters the cell through the protein channel on the cell membrane using diffusion. It then accumulates in the cell and acidifies the internal atmosphere causing cell death by apoptosis or necrosis (Lastauskienė *et al.,* 2014).

Vinegar has been used in previous studies as a cleaning agent for treatment of wounds to inhibit growth of bacteria and fungi (Rund 1996).This solution has also been shown to be effective in some areas of dentistry. Different concentrations of vinegar have been reported to be effective against elimination of bacteria in toothbrushes (Peker *et al.,* 2014). In a different study, vinegar accounted for 40% reduction in microbial content of canal after being used as an irrigant and intracanal treatment (Estrela *et al.,* 2004).

At a cellular level, along with formic acid, had the most antifungal activity of all the solutions tested (Lastauskiene *et al.,* 2014). Diluted vinegar is less effective than alkaline peroxide in removing biofilms when dentures are soaked for 30minutes (Yadav *et al.,* 2013). Unfortunately, in the study researchers did not specify the strength of vinegar, nor the type of acrylic used (Yadav *et al.,* 2013). Gouveia *et al.* (2014) also explored different antimicrobial solutions and their effect on growth of *C. albicans*. They reported that a long contact time was required for an antimicrobial agent to remove *C. albicans* biofilm. The results of the study showed the importance of determining optimal time frame in investigating maximum effect of any agent being tested.

Pinto *et al.,* tested 4% red wine vinegar on multiple *Candida* species. They found that 10% vinegar used in the study could not completely remove *C. albicans* from the dentures (Pinto *et al.,* 2008). In contrast, Jafari *et al.,* tested 5% and 10% vinegar

solutions on adhered *C. albicans,* and found that the 10% solution removed 100% of the *C. albicans* attached to a resin plate (Jafari *et al.,* 2012). These contrasting findings show that more studies are required to investigate various concentrations of vinegar and time frames required to remove *C. albicans* from a resin plate.

1.5.3.2 Apple Cider Vinegar

Apple cider vinegar is a solution of acetic acid produced by fermentation of apples. It has antifungal and antibacterial activity due to its maleic acid content (Brown 2008), and like vinegar, it is widely available in South African markets.

In dentistry, apple cider vinegar has been used as an antimicrobial agent in preparation of root canals (Dornelles-Morgental *et al.,* 2011). Mota *et al.* (2014) tested the antifungal activity of apple cider vinegar on *Candida* species adhered to dentures, and found that it had a fungistatic effect from 0-180 minutes, and a fungicidal effect from 30-180 minutes. The authors also found that it does not discolour the acrylic surface, does not have any effects on surface roughness of the acrylic and prevented *C. albicans* adherence to the acrylic.

A study by Hashizume *et al.* (2015) aimed to find a cost effective alternative to conventional denture cleansers. Denture acrylics were immersed in disinfectant solutions for 10 minutes before testing its efficacy. Vinegar (4.5% acetic acid) was found to be more effective than 10 v hydrogen peroxide. These authors suggested that although the vinegar solution was not able to eliminate *Candida albicans* completely from the dentures, its antifungal effect could be greater if the period of immersion of the denture was longer and that further studies are necessary to validate this possibility (Hashizume *et al.,* 2015).

1.5.3.3 Rice Vinegar

Rice Vinegar is produced from rice wine and is milder, sweeter and more delicate than regular vinegar. Rice vinegar has been shown to have many benefits including reducing risk of cancer in rats (Shimoji *et al.,* 2004). A study by Choi *et al.,* showed that rice vinegar inhibited growth of bacterial species including *Staphylococcus Aureus, Escherichia coli; Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella typhirium, Yersinia entercolitica* and *Lodderomyces elongisporus*. The results of that study reported rice vinegar to be more effective than other antibiotics

such as tetracycline (Choi *et al.,* 2015). However, there have been no studies that tested antifungal effect of rice vinegar on *C. albicans.*

1.6 SIGNIFICANCE OF STUDY

The access to dental care is improving, and more people are retaining their natural teeth for longer periods of their lives; however the loss of teeth remains significant especially among the elderly, therefore the risk of being affected by denture stomatitis remains (Petersen & Yamamoto 2005).

Although several denture cleansers are readily available on the market, less than 60% of denture wearers use them due to their high cost. Therefore there is a need for denture disinfectants which are of low cost and are easily accessible to denture wearers.

Despite a number of studies having tested the removal of *C. albicans* from a denture base using acetic acid/ vinegar none have studied and compared the effect of different types of vinegars nor made recommendations on optimal times required for these solutions to remove *C. albicans* from denture surfaces. In addition, the use of apple cider vinegar to remove *C. albicans* from acrylic resin material has not been widely tested, and there are no studies on the use of rice vinegar as a denture cleaner. The knowledge gained from this study may have an impact in the way denture hygiene is managed, and could also have major public health effects due to the low cost and easy availability of vinegar.

1.7 AIM OF STUDY

The aim of the study was to investigate the efficacy of different vinegar solutions in removal of *C. albicans* from denture acrylic resin.

1.8 OBJECTIVES

- To investigate the effect of white, rice and apple cider vinegar in removing *C. albicans* from denture acrylic plates.
- To investigate the time required for white, rice and apple cider vinegar to remove *C. albicans* from acrylic plates.

- To compare the effect of white, rice and apple cider vinegar on removal of *C. albicans* from acrylic plates at different times

CHAPTER 2: MATERIALS AND METHODS

2.1 ACRYLIC PLATES AND DISINFECTANTS

A total of 192 acrylic plates (96 for each *C. albicans* strain) were used in the study, and the sample size was determined in reference to previous studies(Pinto *et al.,* 2008; Jafari *et al.,* 2012; Mota *et al.,* 2014), wherein a maximum sample size of 74 was used. Acrylic plates measuring 10×10×3mm were prepared according to the manufacturer's instructions (Confi-Dent agencies, Johannesburg, South Africa), and kept in a flask containing normal saline and sterilized in an autoclave at 121ºC/15 min according to a method described in a previous study (Jafari *et al.,* 2012). White wine vinegar (6%), rice vinegar (5.5%), and apple cider vinegar (5%) were purchased in the local supermarkets for use as disinfectants. Sterile distilled water and Chlorhexidine were included as negative and positive control respectively.

2.2 CULTURES AND INOCULA

Cultures of *C. albicans* strain ATCC 90028 and a clinical *C. albicans* isolate were obtained from the Department of Oral Biological Sciences laboratory. *C. albicans* strain ATCC 90028 was a purchased strain, while a clinical isolate was obtained from an HIV positive patient from a previous study (Ethical approval No: M120423). A waiver to use the isolate was obtained from Human Research Ethics Committee (waiver certificate number is W-CJ-150205-3) (Appendix A). These strains were grown on Sabouraud Dextrose Agar plates (SDA) at 37^0C for 48hrs. Culture inocula were prepared by suspending colonies in 20 ml of sterile normal saline and turbidity adjusted to 0.5 McFarland standard.

2.3 TEST PROCEDURE

The test procedure as described by Jafari *et al.* (2012) was followed, with slight modifications. Sterile acrylic resin plates were immersed in 3 ml sterile Sabouraud's Broth in test tubes and 200µl of *C. albicans* suspension was added to each tube. The tubes were then closed and incubated at 37°C in a shaker (100 rpm) for 48hrs. For each *C. albicans* strain, 96 acrylic plates were used.

2.3.1 Disinfection of acrylic plates

For disinfection procedure, contaminated acrylic plates were randomly divided into 5 groups of 6 plates each. Group 1 plates were immersed using a sterile forceps in 20 ml of 6% white wine vinegar (WWV), group 2 in 20ml of 5.5% rice vinegar (RV), group 3 in 20ml of 5% apple cider vinegar (ACV), group 4 in 20ml of distilled water (H_2O), and group 5 in 20ml of Chlorhexidine (CHX). These were incubated at room temperature for 30minutes, 1 hour and 8hours, and 2 plates were removed from each group using a sterile forceps at each time frame and processed to determine colony forming units (CFUs) (Figures 2.1 and 2.2). This procedure was repeated 3 times for reproducibility of results. CHX and H_2O were included as positive and negative control respectively. Two plates not exposed to any disinfectant were included as controls.

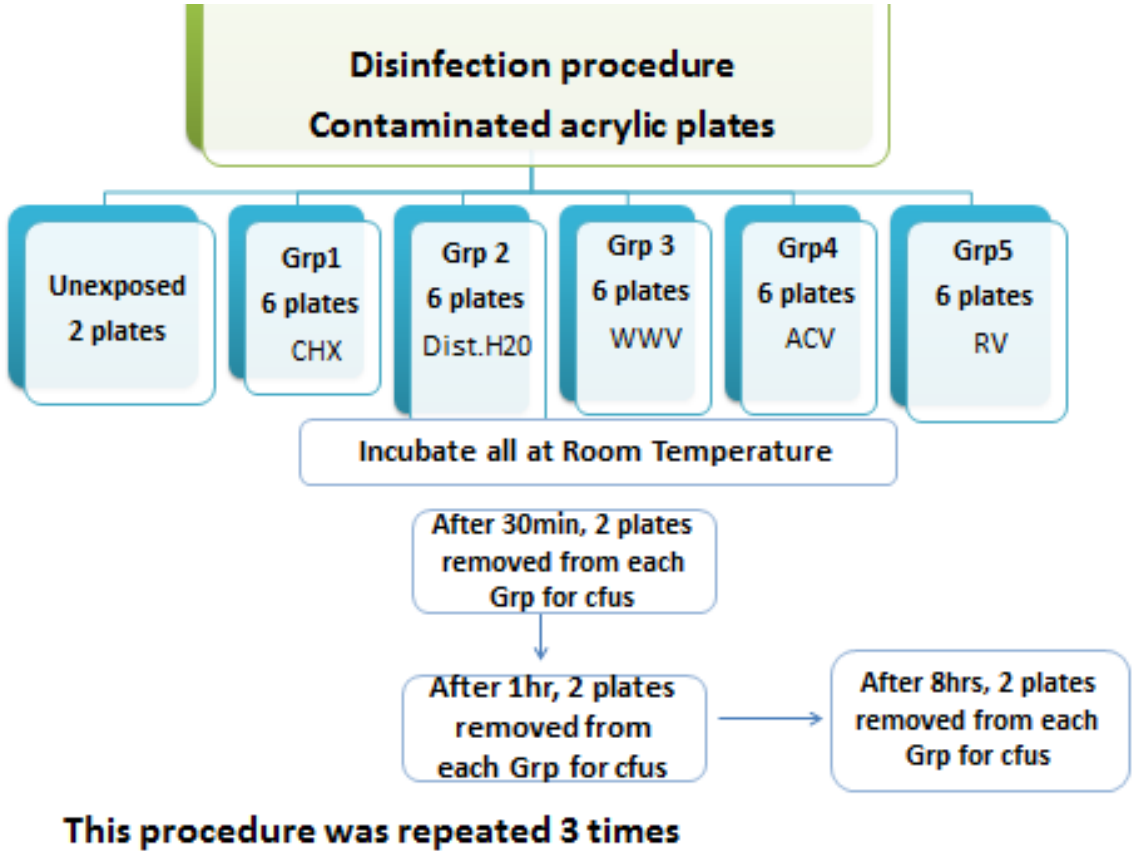


Figure 2.1: Disinfection procedure for acrylic plates contaminated with *C. albicans* clinical strain

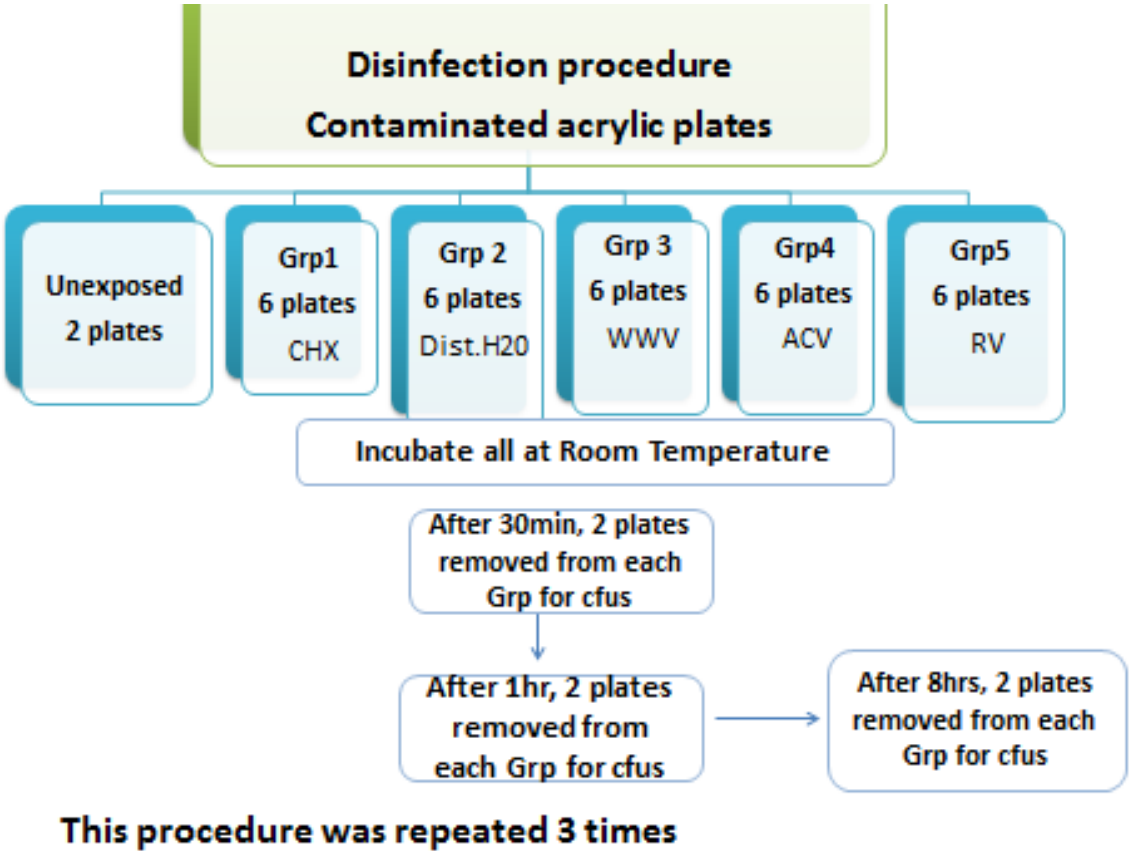


Figure 2.2: Disinfection procedure for acrylics contaminated with *C. albicans*ATCC 9008 strain

2.3.2 Colony forming units count

To determine CFUs, the disinfected acrylic plates were transferred to tubes containing 20ml of sterile saline and vortexed to remove the adhered cells. Serial dilutions were prepared from this suspension by mixing 0.1ml of each suspension with 0.9ml of sterile phosphate buffered saline (1:10), which was further diluted by adding 0.1ml of this suspension into another 0.9ml of PBS (1:100). One hundred microliters of these dilutions were spread in duplicate on Sabouraud's dextrose agar plates, and incubated for 48 hours at 37°C.

These tests were repeated three times for each of the 2 *C. albicans* strains for reproducibility of results (Figure 2.3)

After 48 hours of incubation, colonies were counted and CFUs calculated by multiplying the colony counts by dilution factors and dividing by volume of inoculum put on plate. The average count of the dilution plates at 30minutes, 1hour and 8hours were used for further calculations. The counts were expressed as CFUper acrylic plate. The control count represented the original level of contamination before disinfection and the test count represented the live organisms obtained due to failure of disinfection by each disinfectant.

The percentage of fungi killed was calculated using the formula: control count-test count/control count X100. The log reduction was calculated by subtracting the log of surviving organisms from the log of challenged organisms.

Percentage kill of different disinfectants were compared. Log reduction shows the actual reduction in the microbial counts, where 1 log reduction means 90% reduction in the number of viable organisms.

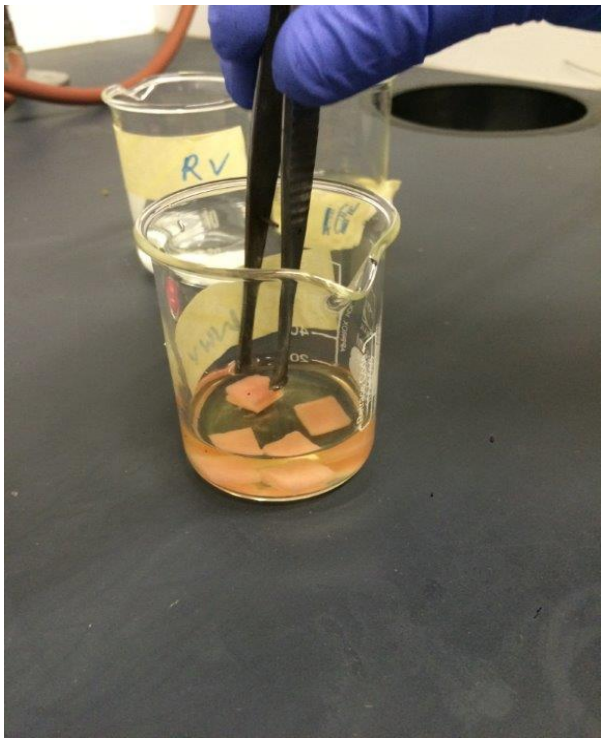


Figure 2.3: Acrylic resin plates

2.4 STATISTICAL ANALYSIS

Descriptive analysis was used to express the results. Percentage kill of disinfectants were obtained and compared using all three repeat results. Paired T-test was used to determine significant difference of the percentage kill means of disinfectants between the HIV and ATCC strains at various times. A significant level of less than 0.05 ($P < 0.05$) was used.

2.5 VALIDITY AND REPEATABILITY

All procedures were repeated 3 times for reproducibility of results. Two plates not exposed to any disinfectant were included as controls (number of challenged organisms)

CHAPTER 3: RESULTS

The mean count of CFUs on acrylic plates not exposed to disinfectants was 48666 for ATCC strain and 26000 for HIV strain (controls/challenged organisms).

3.1 DISINFECTION OF ACRYLIC PLATES CONTAMINATED WITH *C. ALBICANS*ATCC 90028 STRAIN

CHX showed a 100% kill and Log reduction of 4.53 of *C. albicans* at 30 minutes, 1 hour and 8 hours, whilst ACV, WWV and RV showed similar results only at 8 hours. RV showed the least %Kill and Log reduction at 83.36 and 0.82 respectively. Water as the negative control performed the worst in %Kill and log reduction (Tables 3.1 and 3.2; Figure 3.1).

When comparing the percentage kill of the three vinegars at 30 minutes and 1hour, WWV had the highest percentage kill.

Table 3.1: Disinfection of Acrylic plates artificially contaminated with *C. albicans* ATCC 90028 strain(%Kill)

Disinfecting solution	Time	Repeats	No. of challenged organismscfu/acrylic plate (a)	No. of surviving organismscfu/acrylic plate (b)	No. of killed organisms a-b	% Killeda-b/a X 100
Apple Cider Vinegar	30 mins	1	56000	2200	53800	96
		2	8750	1900	6850	78
		3	81250	6350	74900	92
Mean±SD						**88.85±9.35**
	1hr	1	56000	300	55700	99
		2	8750	1150	7600	87
		3	81250	2350	78900	97
Mean±SD						**94.48±6.70**
	8hr	1	56000	0	56000	100
		2	8750	0	8750	100
		3	81250	0	81250	100
Mean±SD						**100±0.0**
Chlorhexidine	30 mins	1	56000	0	56000	100
		2	8750	0	8750	100
		3	81250	0	81250	100
Mean±SD						**100±0**
	1hr	1	56000	0	56000	100
		2	8750	0	8750	100
		3	81250	0	81250	100
Mean±SD						**100±0**
	8hr	1	56000	0	56000	100
		2	8750	0	8750	100
		3	81250	0	81250	100
Mean±SD						**100±0.0**
White Vinegar	30 mins	1	56000	3150	52850	94
		2	8750	1250	7500	86
		3	81250	3200	78050	96
Mean±SD						**92.05±5.7**
	1hr	1	56000	150	55850	100
		2	8750	450	8300	95
		3	81250	2050	79200	97
Mean±SD						**97.36±2.5**
	8hr	1	56000	0	56000	100
		2	8750	0	8750	100
		3	81250	0	81250	100
Mean±SD						**100±0.0**
Rice Vinegar	30 mins	1	56000	7150	48850	87
		2	8750	850	7900	90
		3	81250	22300	58950	73
Mean±SD						**83.36±9.1**
	1hr	1	56000	3850	52150	93
		2	8750	600	8150	93
		3	81250	9900	71350	88
Mean±SD						**91.36±2.8**
	8hr	1	56000	0	56000	100
		2	8750	0	8750	100
		3	81250	0	81250	100
Mean±SD						**100±0.0**
Water	30 mins	1	56000	7250	48750	87
		2	8750	2050	6700	77
		3	81250	4450	76800	95
Mean±SD						**86.05±9.0**
	1hr	1	56000	6150	49850	89
		2	8750	5050	3700	42
		3	81250	5350	75900	93
Mean±SD						**74.91±28.3**
	8hr	1	56000	20700	35300	63
		2	8750	2450	6300	72
		3	81250	11100	70150	86
Mean±SD						**73.79±11.5**

Challenged Org=organisms on acrylic plates not exposed to disinfectants

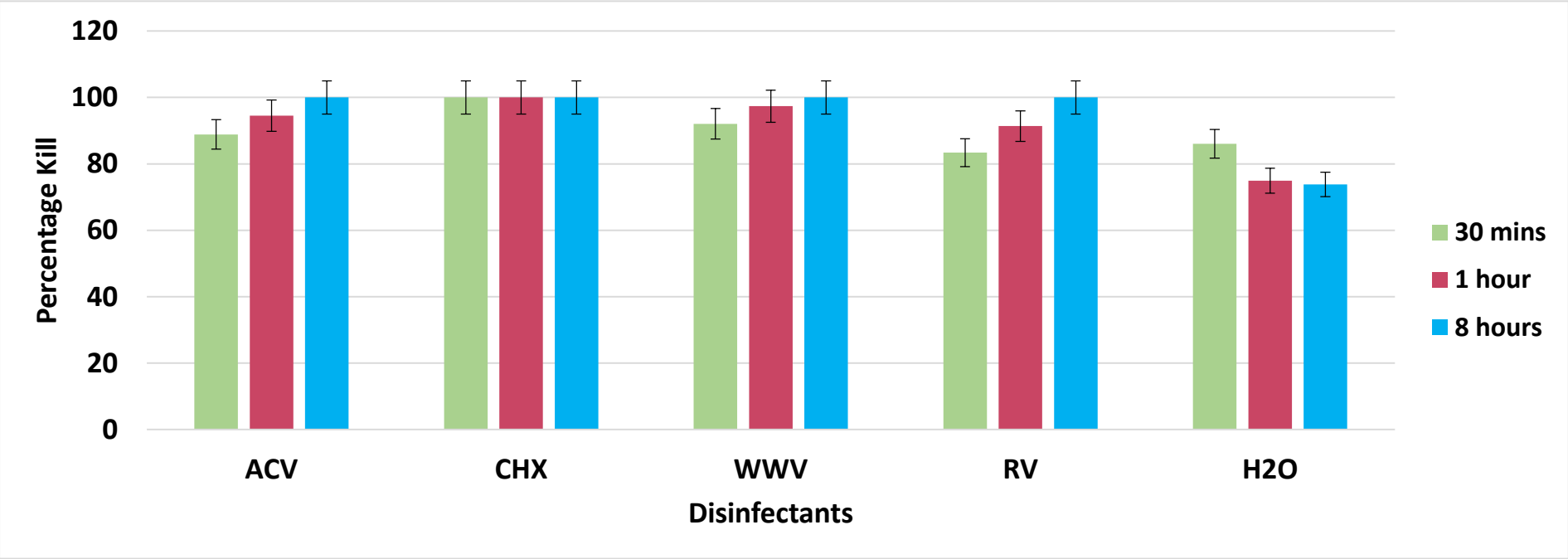


Figure 3.1: Comparison of percentage kill of different disinfectants at different times (ATCC 90028 strain)

ACV=Apple cider vinegar**; CHX**= Chlorhexidine**; WWV**=White wine vinegar**; RV**= Rice vinegar**;** H_2O= **water**

Table 3.2: Disinfection of Acrylic plates artificially contaminated with *C. albicans* ATCC 90028 strain (Log reduction)

Disinfecting solution	Time	Repeats	No. of challenged org (log cfu/acrylic plate (a)	No. of surviving org (log cfu/acrylic plate (b)	log reduction a-b
Apple Cider Vinegar	30 mins	1	4.75	3.34	1.41
		2	3.94	3.28	0.66
		3	4.9	3.8	1.1
Mean					**1.06**
	1hr	1	4.75	2.48	2.27
		2	3.94	3.06	0.88
		3	4.9	3.37	1.53
Mean					**1.56**
	8hr	1	4.75	0	4.75
		2	3.94	0	3.94
		3	4.9	0	4.9
Mean					**4.53**
Chlorhexidine	30 mins	1	4.75	0	4.75
		2	3.94	0	3.94
		3	4.9	0	4.9
Mean					**4.53**
	1hr	1	4.75	0	4.75
		2	3.94	0	3.94
		3	4.9	0	4.9
Mean					**4.53**
	8hr	1	4.75	0	4.75
		2	3.94	0	3.94
		3	4.9	0	4.9
Mean					**4.53**
White Vinegar	30 mins	1	4.75	3.5	1.25
		2	3.94	3.09	0.85
		3	4.9	3.51	1.39
Mean					**1.16**
	1hr	1	4.75	2.18	2.57
		2	3.94	2.65	1.29
		3	4.9	3.31	1.59
Mean					**1.44**
	8hr	1	4.75	0	4.75
		2	3.94	0	3.94
		3	4.9	0	4.9
Mean					**4.53**
Rice Vinegar	30 mins	1	4.75	3.85	0.9
		2	3.94	2.92	1.02
		3	4.9	4.35	0.55
Mean					**0.82**
	1hr	1	4.75	3.58	1.17
		2	3.94	2.78	0.96
		3	4.9	3.99	0.91
Mean					**1.01**
	8hr	1	4.75	0	4.75
		2	3.94	0	3.94
		3	4.9	0	4.9
Mean					**4.53**
Water	30 mins	1	4.75	3.86	0.89
		2	3.94	3.31	0.63
		3	4.9	3.65	1.25
Mean					**0.92**
	1hr	1	4.75	3.79	0.96
		2	3.94	3.7	0.24
		3	4.9	3.73	1.17
Mean					**0.79**
	8hr	1	4.75	4.32	0.43
		2	3.94	3.39	0.55
		3	4.9	4.04	0.86
Mean					**0.61**

3.2 DISINFECTION OF ACRYLIC PLATES ARTIFICIALLY CONTAMINATED WITH HIV STRAIN

All disinfectants (ACV, CHX, WWV and RV) showed similar results at 8hours;a 100% Kill; and a log reduction of 4.41). At 30 minutes and one hour CHX recorded a 99.58% Kill= 3.02Log reduction) and a 99.17% Kill= 2.91Log reduction respectively.

Of the remaining three disinfectants, WWV recorded the highest results in % Kill and Log reduction at 82.59 and 1.92; and 72.64 and 1.72 respectively. RV recorded the least values after one hour at 65.45 and 0.46 for % Kill and Log reduction respectively. Water, a negative control, showed the least results overall at 30 minutes, 1 hour and 8 hours (% Kill=76.77; 71.09; 78.10; Log reduction=0.94, 0.81 and 0.66 respectively) (Tables 3.3 and 3.4; Figure 3.3).

Table 3.3: Disinfection of Acrylic plates artificially contaminated with *C. albicans* HIV strain (%Kill)

Disinfecting solution	Time	Repeats	No. of challenged org cfu/acrylic plate (a)	No. of surviving org cfu/acrylic plate (b)	No. of killed org a-b	% Killed a-b/a X 100
Apple Cider Vinegar	30 mins	1	23500	9350	14150	60
		2	26050	13700	12350	47
		3	28450	8000	20450	72
Mean±SD						**59.83±12.5**
	1hr	1	23500	7550	15950	68
		2	26050	14450	11600	45
		3	28450	3900	24550	86
Mean±SD						**66.23±20.1**
	8hr	1	23500	0	23500	100
		2	26050	0	26050	100
		3	28450	0	28450	100
Mean±SD						**100±00**
Chlorhexidine	30 mins	1	23500	0	23500	100
		2	26050	50	26000	100
		3	28450	300	28150	99
Mean±SD						**99.58±0.57**
	1hr	1	23500	0	23500	100
		2	26050	50	26000	100
		3	28450	650	27800	98
Mean±SD						**99.17±1.2**
	8hr	1	23500	0	23500	100
		2	26050	0	26050	100
		3	28450	0	28450	100
Mean±SD						**100±00**
White Vinegar	30 mins	1	23500	0	23500	100
		2	26050	11000	15050	58
		3	28450	2850	25600	90
Mean±SD						**82.59±21.9**
	1hr	1	23500	0	23500	100
		2	26050	12500	13550	52
		3	28450	9700	18750	66
Mean±SD						**72.64±24.6**
	8hr	1	23500	0	23500	100
		2	26050	0	26050	100
		3	28450	0	28450	100
Mean±SD						**100±00**
Rice Vinegar	30 mins	1	23500	5850	17650	75
		2	26050	2800	23250	89
		3	28450	7150	21300	75
Mean±SD						**79.74±8.1**
	1hr	1	23500	9500	14000	60
		2	26050	8500	17550	67
		3	28450	8700	19750	69
Mean±SD						**65.45±4.7**
	8hr	1	23500	0	23500	100
		2	26050	0	26050	100
		3	28450	0	28450	100
Mean±SD						**100±00**
Water	30 mins	1	23500	1900	21600	92
		2	26050	12200	13850	53
		3	28450	4200	24250	85
Mean±SD						**76.77±20.7**
	1hr	1	23500	1100	22400	95
		2	26050	18400	7650	29
		3	28450	3250	25200	89
Mean±SD						**71.09±36.4**
	8hr	1	23500	5800	17700	75
		2	26050	5650	20400	78
		3	28450	5500	22950	81
Mean±SD						**78.10±3.0**

Challenged Org=organisms on acrylic plates not exposed to disinfectants (control)

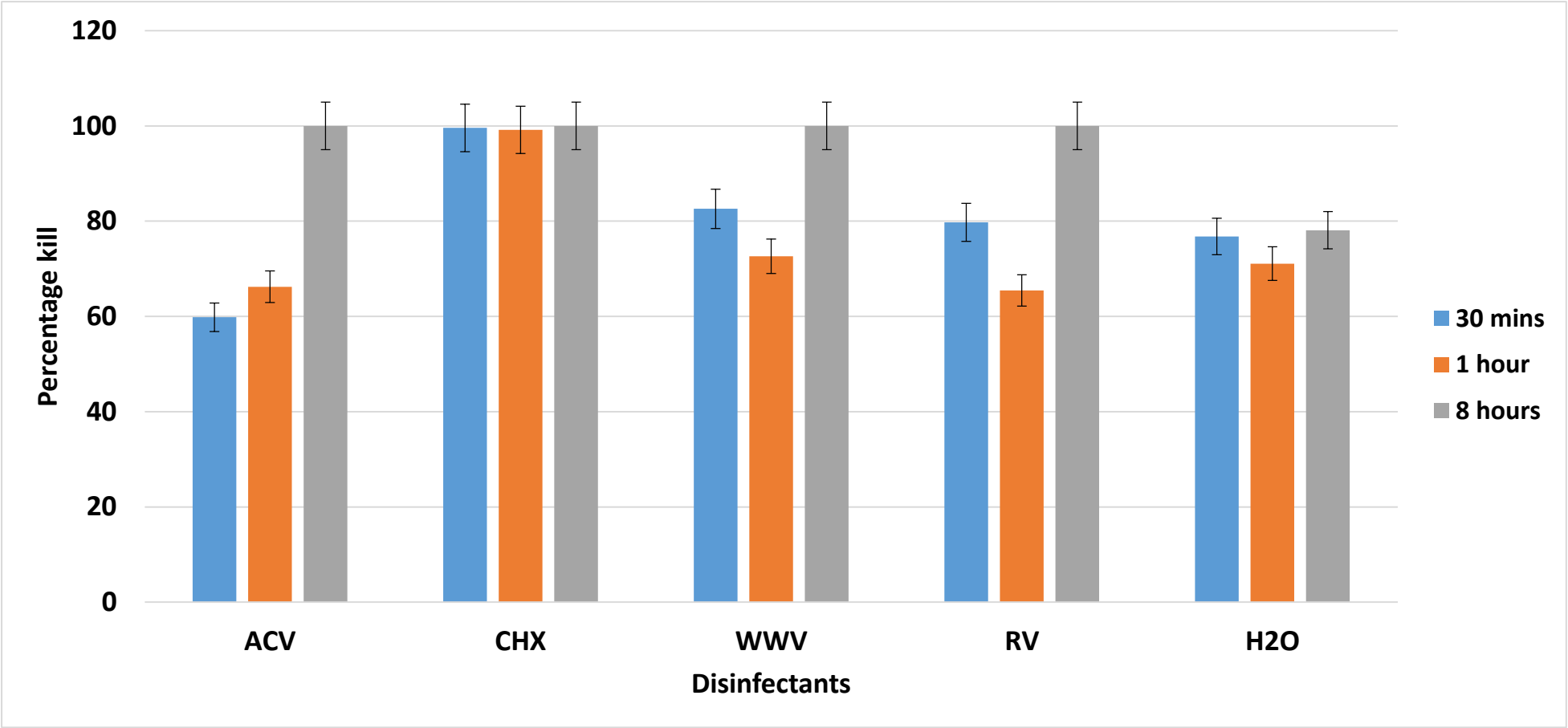


Figure 3.2: Comparison of percentage kill of different disinfectants at different times (HIV strain)

ACV=Apple cider vinegar; **CHX=** Chlorhexidine**; WWV=**White wine vinegar**; RV=** Rice vinegar**; H_2O=** Water

Table 3.4 Disinfection of Acrylic plates artificially contaminated with *C. albicans* HIV strain (Log

Disinfecting solution	Time	Repeats	No. of challenged org (log cfu/acrylic plate (a)	No. of surviving org (log cfu/acrylic plate (b)	log reduction a-b
Apple Cider Vinegar	30 mins	1	4.37	3.97	0.4
		2	4.42	4.14	0.28
		3	4.45	3.9	0.55
Mean					**0.41**
	1hr	1	4.37	3.88	0.49
		2	4.42	4.16	0.26
		3	4.45	3.59	0.86
Mean					**0.54**
	8hr	1	4.37	0	4.37
		2	4.42	0	4.42
		3	4.45	0	4.45
Mean					**4.41**
Chlorhexidine	30 mins	1	4.37	0	4.37
		2	4.42	1.7	2.72
		3	4.45	2.48	1.97
Mean					**3.02**
	1hr	1	4.37	0	4.37
		2	4.42	1.7	2.72
		3	4.45	2.81	1.64
Mean					**2.91**
	8hr	1	4.37	0	4.37
		2	4.42	0	4.42
		3	4.45	0	4.45
Mean					**4.41**
White Vinegar	30 mins	1	4.37	0	4.37
		2	4.42	4.04	0.38
		3	4.45	3.45	1
Mean					**1.92**
	1hr	1	4.37	0	4.37
		2	4.42	4.09	0.33
		3	4.45	3.98	0.47
Mean					**1.72**
	8hr	1	4.37	0	4.37
		2	4.42	0	4.42
		3	4.45	0	4.45
Mean					**4.41**
Rice Vinegar	30 mins	1	4.37	3.76	0.61
		2	4.42	3.45	0.97
		3	4.45	3.85	0.6
Mean					**0.73**
	1hr	1	4.37	3.98	0.39
		2	4.42	3.93	0.49
		3	4.45	3.94	0.51
Mean					**0.46**
	8hr	1	4.37	0	4.37
		2	4.42	0	4.42
		3	4.45	0	4.45
Mean					**4.41**
Water	30 mins	1	4.37	3.29	1.08
		2	4.42	4.09	0.33
		3	4.45	3.62	1.41
Mean					**0.94**
	1hr	1	4.37	3.04	1.33
		2	4.42	4.26	0.16
		3	4.45	3.51	0.94
Mean					**0.81**
	8hr	1	4.37	3.76	0.61
		2	4.42	3.75	0.67
		3	4.45	3.74	0.71
Mean					**0.66**

reduction)Challenged Org=organisms on acrylic plates not exposed to disinfectants

3.3 SUMMARY RESULTS OF DISINFECTION OF ACRYLIC PLATES CONTAMINATED WITH ATCC 90028 AND HIV STRAINS

Table 3.5 and Figure 3.4 show summary of results obtained in the study. When the results of the two strains were compared, ACV, WWV, RV and CHX were effective in removing both strains at 8 hours (%Kill=100). CHX was the most effective disinfectant in removing both strains, however, it was more effective in removing ATCC 90028 strain at all times (% Kill= 100), as compared to HIV strain, where it could not completely remove the strain at 30 minutes (% Kill=99.58) and 1 hour (% Kill=99.17). H_2O was ineffective in completely removing both strains from acrylic plates at all times. ACV, WWV and RV failed to completely remove both strains at 30 minutes and 1 hour; however, all these vinegars removed the ATCC 90028 strain better as compared to the HIV strain.

Table 3.5: Summary of % Kill results of different disinfectants at different times between HIV and ATCC 90028 strains

Strains	Time	No. of Challenged Orgs Mean CFU /acrylic plate	Mean ± SD % Killed				
			ACV	WWV	RV	CHX	H_2O
ATCC 90028 STRAIN	30min	48666	88.85±9.35	92.05±5.7	83.36±9.	100±0.0	86.05±9.0
	1hr	48666	94.48±6.70	97.36±2.5	91.36±2.8	100±0.0	74.91±28.3
	8hrs	48666	100±0.0	100±0.0	100±0.0	100±0.0	73.79±11.5
HIV STRAIN	30min	26000	59.83±12.5	82.59±21.9	79.74±8.1	99.58±0.57	76.77±20.7
	1hr	26000	66.23±20.1	72.64±24.6	65.45±4.7	99.17±1.2	71.09±36.4
	8hrs	26000	100±0.0	100±0.0	100±0.0	100±0.0	78.10±3.0

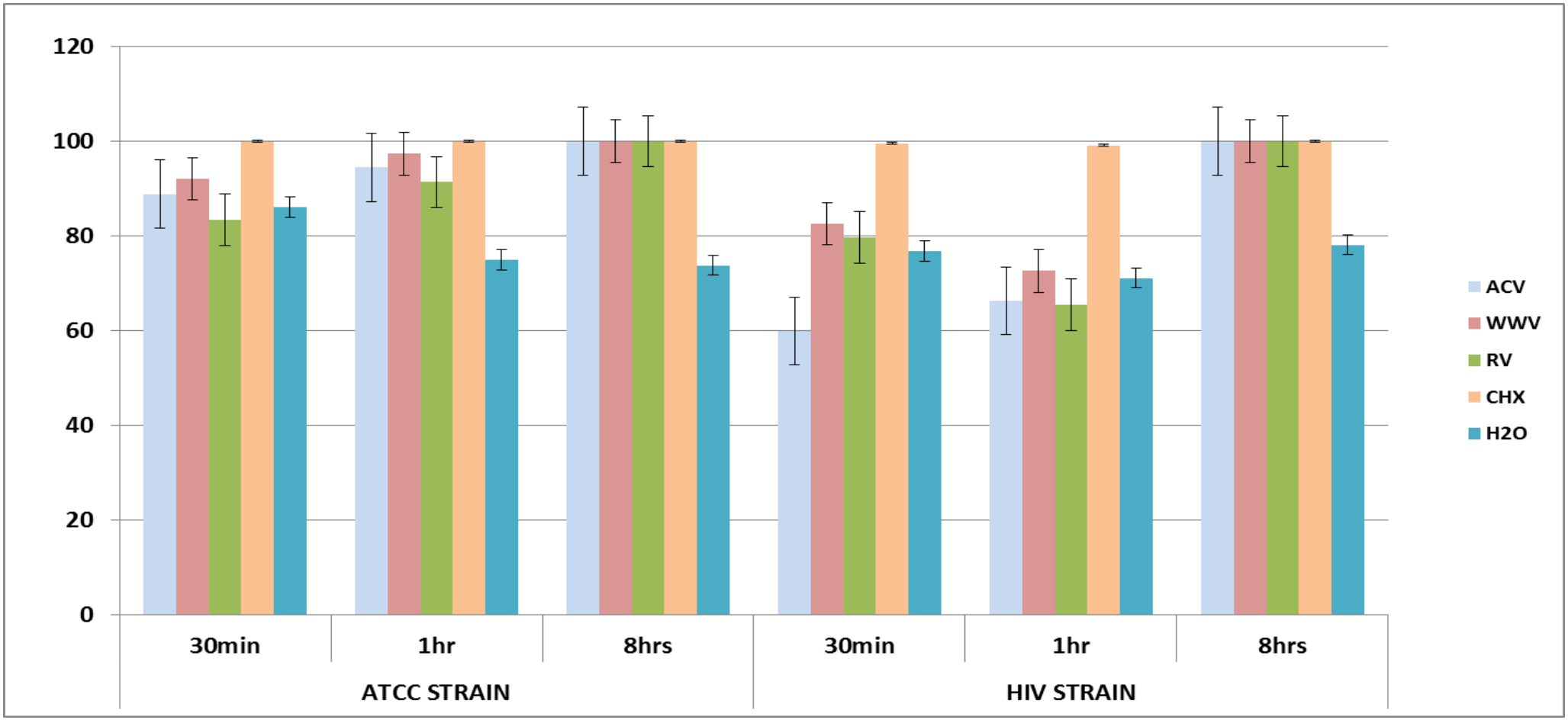


Figure 3.3: Comparison of percentage kill of different disinfectants at different times between HIV and ATCC 90028 strains

ACV=Apple cider vinegar**; CHX=** Chlorhexidine**; WWV=**White wine vinegar**; RV=** Rice vinegar**; H2O=** Water

3.4 COMPARISON AND STATISTICAL ANALYSIS OF DISINFECTION OF ACRYLIC PLATES CONTAMINATED WITH ATCC 90028 AND HIV STRAINS

For ATCC strain, there was no statistical difference in the percentage removed between ACV, RV and WWV at 30 minutes and 1 hour ($p<0.05$). There was statistical significant difference in the percentage removed for water at 30 minutes ($p=0.01$) and 1 hour ($p=0.04$). Chlorhexidine was able to completely eliminate the ATCC 90028 strain at every time interval. However it could not eliminate the HIV strain.

For HIV strain, there was statistical significant difference in the percentage removed for ACV at 30 minutes ($p=0.03$), RV at 1 hour ($p=0.01$) and H_2O at 8 hours ($p=0.00$). There was no statistical significant difference in the percentage removed for chlorhexidine at 30 minutes and 1 hour.

These results are shown in Figure 3.5.

Table 3.6: Paired T-Test for significant difference between ATCC 90028 and HIV strains at various times

	HIV strain		ATCC 90028 strain	
Apple cider vinegar	**t**	**p-value**	**t**	**p-value**
Time 0-30	6.02	**0.03***	2.43	0.14
Time 30-1	-1.23	0.35	-2.33	0.15
Time 0-1	2.79	0.11	2.13	0.17
White wine vinegar				
Time 0-30	1.4	0.3	3.95	0.06
Time 30-1	1.34	0.31	-2.42	0.14
Time 0-1	1.95	0.19	1.5	0.27
Rice vinegar				
Time 0-30	4.09	0.06	1.59	0.25
Time 30-1	3.03	0.09	-1.46	0.28
Time 0-1	29.13	**0.001***	1.76	0.22
Water				
Time 0-30	1.95	0.19	3.05	**0.01***
Time 0-1	1.39	0.3	16.8	**0.004***
Time 30-1	0.63	0.59	0.79	0.51
Time 1 -8	65.24	**0***	2.17	0.16
Chlorhexidine				
Time 0-30	1.26	0.34	-	-
Time 30-1	1	0.42		
Time 0-1	1.12	0.38	-	-

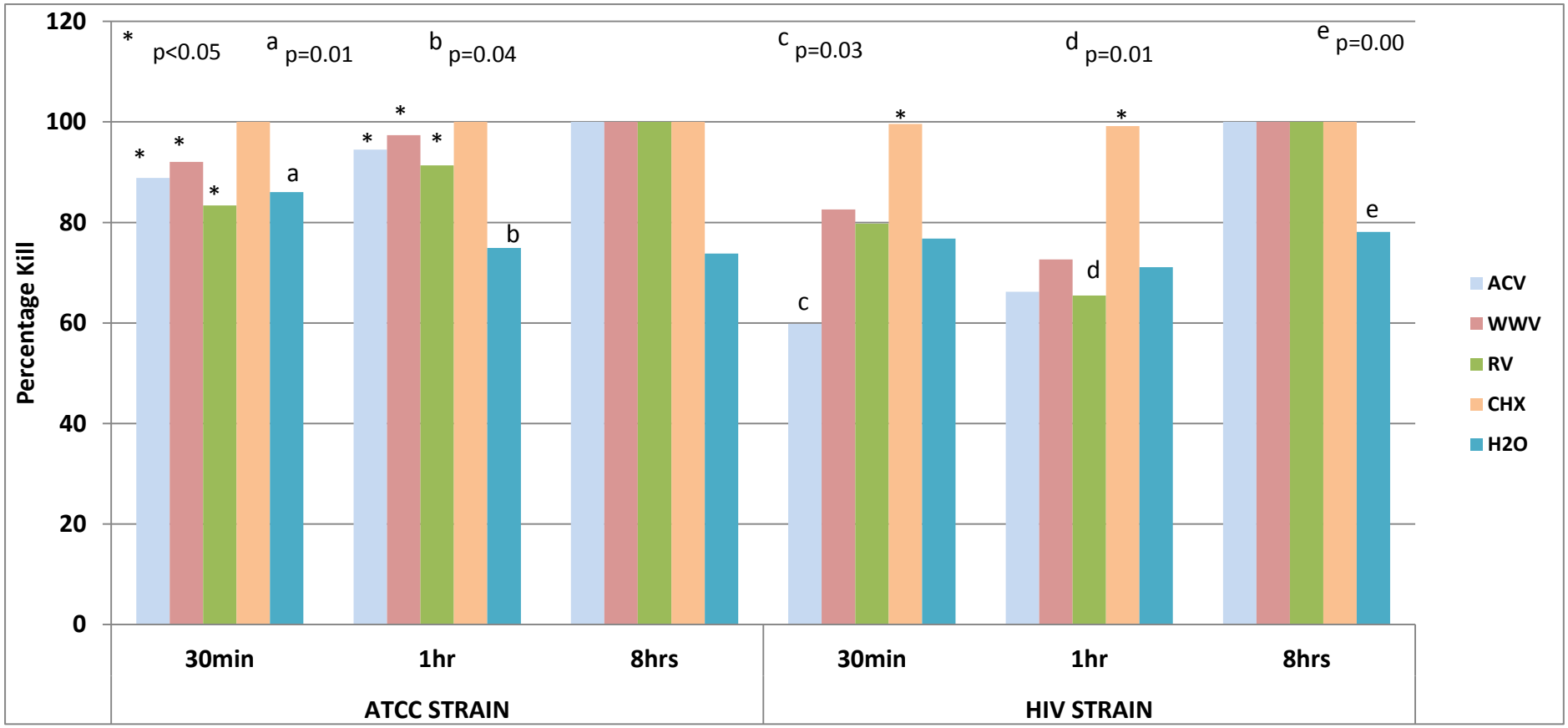


Figure 3.4: Comparison of different disinfectants at different times between HIV and ATCC 90028 strains

ACV=Apple cider vinegar**; CHX=** Chlorhexidine**; WWV=**White wine vinegar**; RV=** Rice vinegar**; H2O=** Water**.** Superscripts *, a, b, c, d, e represent statistical significance differences

CHAPTER 4: DISCUSSION

Around 30% of people aged 65-74 are edentulous globally (WHO 2012), with many of them replacing lost teeth with dentures. Denture hygiene has therefore become an important aspect in managing these patients as they often present with signs of denture stomatitis (Douglas 2003). Denture stomatitis is caused mainly by the adherence and colonization of *C. albicans* on the surface of the denture. Removal of *Candida* plaque on dentures is important in controlling the colonization of this yeast and preventing infections caused by *C. albicans*.

Although several denture cleansers are readily available on the market, less than 60% of denture wearers use them due to the high cost (Pinto *et al.,* 2008). Therefore, there is a need for denture disinfectants which are of low cost and that are easily accessible to denture wearers. Vinegar is cheap and easily available in South African markets.

Previous studies have tested the removal of *C. albicans* from a denture base using acetic acid/ vinegar (Pinto *et al.,* 2008; da Silva *et al.,* 2008; Jafari *et al.,* 2012; Mota *et al.,* 2014; Lavanya & Kumar 2015), but none have studied and compared the effect of different types of vinegars in removing *C. albicans* from dentures. In addition, the use of apple cider vinegar to remove *C. albicans* from acrylic resin material has not been widely tested, and there are no studies on the use of rice vinegar as a denture cleanser. This study aimed at investigating the efficacy of different vinegar solutions in removal of *C. albicans* from denture acrylic resin.

4.1 DISINFECTION OF ACRYLIC PLATES CONTAMINATED WITH *C. ALBICANS* ATCC 90028 AND HIV STRAINS

Apple cider vinegar has been reported to have antifungal and antibacterial activity due to its maleic acid content (Brown 2008). In the current study apple cider vinegar eliminated *C. albicans* ATCC 90028 and HIV strains from acrylic plates at 8 hours (% Kill=100), but failed to completely eliminate both strains at 30 minutes and 1 hour. There are very few documented studies on the effect of apple cider vinegar against *C. albicans* adhered to denture acrylic. The results of the current study contradict another where the *in vitro* antifungal activity of apple cider vinegar on *Candida* species (including *C. albicans*) adhered to acrylic dentures was tested. Apple cider vinegar was reported to be fungicidal between 30 - 180 minutes, which is less time compared

to the current study, although a lower concentration (4%) was used in their study as compared to the current study (5%) (Mota *et al.,* 2014).

Other studies reported the effectiveness of apple cider vinegar against *C. albicans,* although the organism was not attached to denture acrylic. A recent study reported that 5% apple cider vinegar was effective against *C. albicans* after 24 hours exposure using a gel diffusion method (Yagnik *et al.,* (2018). In another study, antifungal activity against *C. albicans* was tested by using gel-diffusion method and 60% and 80% concentrations of apple cider vinegar were found to be effective against *C. albicans* (Al-Sahili & Jumaah 2017). Another study also used gel diffusion method and reported 5% apple cider vinegar to be effective against fluconazole resistant *C. albicans* from patients with otomycosis (Hayder *et al.,* 2011).

Apple cider vinegar has easy application, low cost and low toxicity and the results of the current study suggest that it can be used by denture wearers to clean their dentures overnight.

In the current study 6% white wine vinegar completely eliminated *C. albicans* ATCC 90028 and HIV strains from acrylic plates at 8 hours (% Kill=100), but could not completely eliminate these strains at 30 minutes and 1 hour. These results are comparable with those of a study by Jafari *et al* where 10% vinegar removed 100% of the *C. albicans* attached to a resin plate after 8 hours, although different concentrations of white vinegar were used (Jafari et. al 2012).

The results of the current study are in agreement with other studies in which a high concentration of vinegar was used to remove *C. albicans* from dentures. Another study evaluated the effectiveness of 100% vinegar in the disinfection of acrylic resin specimens contaminated *in vitro* with *C. albicans*. In that study, vinegar was found to be as effective as 1% hypochlorite and 2% glutaraldehyde after 10 minutes of exposure (da Silva *et al.,* 2008). A study was done where 100% white wine vinegar was tested in removing *C. albicans* from acrylic resin specimens. The results showed that the ability of *C. albicans* to form biofilms and survive on acrylic specimens immersed in 100% vinegar for 10 minutes was negligible (Lavanya & Kumar 2015). Another author evaluated the effect of 100% vinegar in removing *C. albicans* from acrylic resin specimens after 10 minutes exposure (Hashizume *et al.,* 2015). Vinegar

was found to be more effective than 10 v hydrogen peroxide (Hashizume *et al.*, 2015). In another such study heat cured acrylic resin dentures were contaminated with *C. albicans* and treated with 100% vinegar for 10 minutes. Vinegar was found to be most effective in removing *C. albicans* from dentures (Yildirim *et al.,* 2014).

A previous study exposed heat cured resin specimens contaminated with *C. albicans* to 50 ml/200 ml of vinegar and found that the number of *C. albicans* colonies was reduced when acrylic resin plates were immersed for 8 hours rather than 1 hour ($P < 0.05$). However, *C. albicans* was not completely eliminated at 8 hours, which differs with the current study (Ali *et al.,* 2015).

In contrast to the results of the current study, Pinto *et al.* (2008) reported that 10% vinegar could not eliminate *C. albicans* from dentures and saliva of denture wearers after 45 days of overnight immersion of dentures. Similar contrasting results were reported by another author, who tested the efficacy of household vinegar in reducing *C. albicans* colonization on soft denture relining material after 10 minutes of exposure and household vinegar showed no antifungal potential compared to the control (Buergers *et al.,* 2009). Another author investigated the effectiveness of vinegar in the removal of plaque from the tissue surface of the denture. The dentures were soaked in vinegar diluted in equal amount of water for 30 minutes and vinegar was found to be less effective than alkaline peroxide in removing biofilms, which is in contrast to results of the current study (Yadav *et al.,* 2013) Similar contrasting results were reported in another study, where acrylic resin plates contaminated with *C. albicans* were exposed to white vinegar for 10 minutes, and white vinegar was found to be inefficient in reducing the number of *C. albicans* colonies that adhered to plates (Sousa *et al.,* 2009). A study evaluated the efficacy of two commercial solutions, 4% vinegar and 50% vinegar diluted in water against *C. albicans* adhered to acrylic denture base resin. These vinegar solutions were less effective in removing *C. albicans* after 8 hours exposure as compared to commercial solutions, in contrast to the current study (Kumar *et al.,* 2012).

In the current study 5.5% rice vinegar completely eliminated *C. albicans* ATCC 90028 and HIV strains from acrylic plates at 8 hours (% Kill=100), but could not completely eliminate these strains at 30 minutes and 1 hour. There are no documented studies

that tested antifungal effect of rice vinegar against *C. albicans or C. albicans* attached to acrylic resin. However, a recent study by reported that rice vinegar inhibited growth of bacterial species including *Staphylococcus aureus, Escherichia coli; Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella typhirium, Yersinia entercolitica* and *Lodderomyces elongisporus*. The results of that study showed that rice vinegar was more effective than other antibiotics such as tetracycline (Choi *et al.,* 2015). The results of the current study show that denture wearers can use rice vinegar to disinfect their dentures overnight. This is the first documented study where rice vinegar was tested for denture disinfection and was found to be effective as a denture cleanser.

The results of the current study show that 5.5% rice vinegar, 6% white wine vinegar and 5% apple cider vinegar can be used as denture cleansers, which can allow denture wearers to soak their dentures at night to be ready for use the next day.

In searching the literature, no South African studies on removal of *C. albicans* from dentures using vinegars could be found; therefore the results of the current study could not be compared to those of other South African studies.

Chlorhexidine is considered the gold standard agent for its effectiveness in controlling dental plaque (Jones, 1997). In the current study, 0.12% chlorhexidine completely eliminated *C. albicans* ATCC 90028 strain at 30 minutes, 1 hour and 8 hours from acrylic resin, and was the most effective disinfectant (% Kill=100). These results are in concordance with other studies where various concentrations of chlorhexidine were used, at various times. Another such study reported 100% removal of *C. albicans* by 2% Chlorhexidine gluconate after 10 minutes immersion of dentures (Lavanya & Kumar 2015). Similarly, another author evaluated the antifungal activity of denture cleansers used against *Candida albicans* after 0, 30 and 60 minutes exposure. Chlorhexidine (0.12%) was found to be effective in eliminating *C. albicans* at all tested times (Gouveia *et al.,* 2014).

The ability of chlorhexidine to eliminate *C. albicans* can be explained by its potent antifungal effect which interferes with cellular permeability and plasma membrane integrity (Balagopal & Arjunkumar 2013).The elimination of *C. albicans* from denture

acrylic by chlorhexidine supports the role of chlorhexidine as a potential therapeutic agent in the prevention and treatment of denture stomatitis. However chlorhexidine has disadvantages that have led to this disinfectant not being recommended for daily use, including bitter taste and discoloration of dentures (Budtz-Jorgensen 1979). In addition, denture wearers in poor settings may not use it as a denture cleanser due to the inconvenience of obtaining the product by prescription and its cost (Eden 2008).

In the current study, 0.12% chlorhexidine failed to completely eliminate HIV strain at 30 minutes and 1 hour. These results are in agreement with other studies where chlorhexidine reduced the counts of *C. albicans* without completely eliminating the organism. A previous author reported that 0.12% chlorhexidine decreased the number of *C. albicans* colonies adhered to heat-activated acrylic resin after 10 minute exposure, but could not completely eliminate the organism (Sousa *et al.,* 2009). Another study evaluated the effectiveness of 2% chlorhexidine digluconate against *C. albicans* on acrylic resin specimens after 10 minutes of exposure. Chlorhexidine was found to be effective in reducing the counts of *C. albicans* without completely eliminating the organisms (da Silva *et al.,* 2008). In a similar study denture acrylics were immersed in 2% chlorhexidine for 10 minutes and this disinfectant was the most effective in reducing *C. albicans* adhered to acrylic resin (Hashizume *et al.,* 2015). In another study acrylic resin specimens were contaminated *in vitro* with *C. albicans* and immersed in 2% chlorhexidine digluconate. This disinfectant was found to be less effective than 1% sodium hypochlorite in reducing the number of colonies after 10 minutes of exposure (Pawashe *et al.,* 2017).

Other studies reported contrasting results where chlorhexidine was found to be inefficient or less efficient against *C. albicans*. A study reported that sodium hypochlorite-based substances and hydrogen peroxide were more efficient disinfectants against *C. albicans* than 2% chlorhexidine after 10 minute exposure of denture-base acrylic resins (Montagner *et al.,* 2009). In another study by denture acrylic resin samples were soaked in 0.2% chlorhexidine for 15 minutes for removal of *C. albicans*, and chlorhexidine was found to be ineffective in reducing the counts of *C. albicans* (Lee *et al.,* 2011).

The failure of apple cider vinegar, white wine vinegar, rice vinegar and 0.12% chlorhexidine to completely remove the HIV strain in the current study supports previous reports that *C. albicans* strains from HIV infected individuals are more virulent than those from non-HIV infected individuals (Jain *et al.,* 2010; Mane *et al.,* 2011; de Paula Menezes *et al.,* 2016; Freire *et al.,* 2017). Although not statistically significant, the % kill for the HIV strain was higher at 30 minutes than at 1hour with WWV and RV as compared to ATCC 90028 strain. This could be due to fungal cells aggregation during growth, thus yielding a false low number of colonies. In addition some fungal cells may adhere to the walls of the test tubes or pipettes and not be delivered onto the plates, resulting in low number of colonies (Bhatt *et al.,* 2002) .

Although water in the current study could reduce *C. albicans* from denture acrylic, it failed to completely eliminate *C. albicans* at 30 minutes, 1 hour and 8 hours (% Kill=86.3; 74.7; 73.6 respectively). These results are in agreement with a previous study where contaminated denture acrylic resin samples were immersed in 250 ml of sterile distilled water for 15 minutes, and water was found to be ineffective in completely removing *C albicans* from the denture samples (Lee *et al.,* 2011).

The ability of water to reduce *C. albicans* from acrylic resin can be explained by that water is hypotonic as compared to the cellular contents of microorganisms, and this lead to osmotic pressure, causing the flow of water into the cells and disrupting the microorganisms (Pelczar *et al.,* 1993).

When the effectiveness of the vinegars were compared at different times, apple cider, white wine and rice vinegars equally eliminated both *C. albicans* ATCC 90028 and HIV strains from acrylic plates at 8 hours (% Kill=100). All these tested vinegars failed to completely eliminate *C. albicans* strains at 30 minutes and 1 hour, with no statistical significant difference for ATCC strain($p<0.05$) and with statistical difference for ACV ($p=0.03$) and RV ($p=0.01$) respectively for HIV strain. These results suggest that denture wearers can soak their dentures in these vinegars overnight for use on the next day, which will be convenient for them. Chlorhexidine was the most effective disinfectant for eliminating ATCC strain at all tested times. All tested vinegars, water (negative control) and chlorhexidine (positive control) failed to completely eliminate HIV strain at 30 minutes and 1 hour. Sterile distilled water was the least effective as it failed to completely eliminate both ATCC and HIV strains at all tested times.

CHAPTER 5: CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

Dentures contaminated with *C. albicans* are associated with development of denture stomatitis; therefore cleaning and disinfection of dentures is of utmost importance. There is a need for denture disinfectants which are effective, of low cost and toxicity, and which are easily accessible to denture wearers.

The results of this study show that apple cider, rice and white wine vinegars completely eliminated both *C. albicans* strains from acrylic plates at 8 hours. These results shows that these vinegars can be used as denture cleansers, and that denture wearers can soak their dentures in these vinegars overnight for use the next day. Although apple cider, rice and white wine vinegars could reduce both *C. albicans* strains at 30 minutes and 1 hour, they failed to completely eliminate both these strains at these times. These results also confirm previous reports that the disinfection of acrylic dentures is time-dependent. The current study is the first where rice vinegar was tested for denture disinfection and was found to be effective as a denture cleanser.

Chlorhexidine was the most effective disinfectant as it completely eliminated *C. albicans* ATCC 90028 strain at 30 minutes, 1 hour and 8 hours. However this disinfectant failed to eliminate HIV strain at 30 minutes and 1 hour. This is a cause for concern as disinfectant is used as a gold standard in the removal of dental plaque. The failure of all vinegars and chlorhexidine to completely remove the HIV strain from acrylic plates at 30 minutes and 1 hour confirms previous reports that *C. albicans* strains from HIV positive individuals are more virulent that those from HIV negative individuals. This means that they are more adherent to acrylic plates and require more time for them to be completely eliminated. Sterile distilled water failed to completely eliminate both *C. albicans* strains at all tested times and is therefore not recommended to be used for disinfecting dentures.

The results of the current study confirm that vinegar can be used to remove *C. albicans* from dentures, and that it might be a promising therapeutic and preventive measure

against denture stomatitis, especially because of its low toxicity, cost and availability (da Silva *et al.,* 2008).

5.2 LIMITATIONS

The current study was conducted *in vitro*. *In vitro* studies are conducted in artificial settings and have limitations as they do not reflect the complex oral conditions of a patient in a clinical setting. In a clinical setting, each patient has a clinical isolate which is different from those isolates used in *in vitro* studies, such as the ATTC strains. The antifungal susceptibility of the HIV strain used in the current study was not known, and clinical isolates from some patients may be resistant to the commonly used antifungal agents. It would be interesting to note the performance of the vinegar solutions with regards to these antifungal resistant isolates. The long term effects of vinegar on the porosity of denture acrylic is not known and requires further study to assess its effect on mechanical strength of the denture acrylic over time.

5.3 RECOMMENDATIONS

In vivo studies are required to confirm the use of vinegar in the challenges of the oral environment. *In vitro* studies could be conducted where isolates that are known to be resistant to antifungals are used to determine the effect of vinegar on these isolates. The current study used undiluted vinegar and further studies using diluted vinegar could determine how long one bottle of vinegar could last. Further studies could be conducted to determine how many days the same vinegar solution could be used before it loses its effectiveness. This would further confirm the cost-effectiveness of vinegar.

APPENDIX A

ETHICS WAIVER APPROVALCERTIFICATE

Human Research Ethics Committee (Medical)

Research Office Secretariat: Senate House Room SH10005, 10th floor. Tel +27 (0)11-717-1252
Medical School Secretariat: Tobias Health Sciences Building, 2rd floor Tel +27 (0)11-717-2700
Private Bag 3, Wits 2050, www.wits.ac.za. Fax +27 (0)11-717-1265

Ref: W-CJ-150205-3 (title change) 13/07/2015

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigator: **Dr T Garach. (Student no 0701148Y)**

Project title: **Efficacy of different vinegar solutions in removal of *Candida albicans* from denture acrylic resin.**

Reason: This is a laboratory study using a commercial culture of Candida albicans. There are no human participants

UNIVERSITY OF THE WITWATERSRAND
PROF PE CLEATON-JONES
HREC (MEDICAL)
2015 -07- 13
JOHANNESBURG

Professor Peter Cleaton-Jones

Chair: Human Research Ethics Committee (Medical)

Copy – HREC (Medical) Secretariat: Zanele Ndlovu, Langutani Masingi.

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