

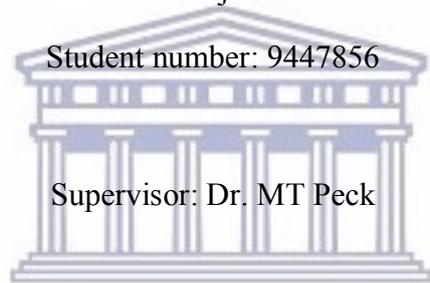
The antimicrobial efficacy of a carbohydrate derived fulvic acid as a pre-periodontal procedure mouth rinse

A mini-thesis submitted as partial fulfillment for the requirements of the M. Ch. D in Oral
Medicine and Periodontics at the Faculty of Dentistry University of the Western Cape

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Key words

Mouthwash

Chlorhexidine digluconate (CHX)

Carbohydrate derived fulvic acid (CHD-FA)

Antimicrobial

Efficacy

Pre-procedural

Oral rinse

Colony forming units (CFU)

Infection

Bacteraemia



Abstract

The aim of this study was to assess whether a mouthwash containing carbohydrate derived fulvic acid, is effective in reducing the salivary microbial count pre-operatively. Endeavours have been made to reduce the risk of infection, bacteraemia and cross-contamination during dental procedures by the application of topical antimicrobial agents. To date chlorhexidine is the most widely evaluated and efficacious agent against oral biofilms but there have been reports of adverse effects ranging from contact dermatitis to severe anaphylactic shock. A new mouth rinse containing carbohydrate derived fulvic acid are reported to have broad spectrum antimicrobial activity against specific oral microbes and *Candida albicans* with no side effects.

Methods:

Saliva samples were collected at baseline than after patients rinsed with fifteen (15) ml sterile saline for sixty (60) seconds. The last saliva sampling was collected at end of the oral procedure. These saliva samples where than cultured in the laboratory. Colony forming units (CFU) were counted at baseline, after fifteen (15) minutes and at the end of the oral procedure. This was repeated for both test and control samples and the three readings were compared.

Results:

The mean microbial log counts at fifteen (15) minutes were statically significantly smaller than the baseline microbial count mean (P-value = 0.0012). The end microbial mean was also significantly smaller than the baseline mean count (P-value= 0.0035). This means that at 15 minutes and end of the treatment, the readings were not significantly different from each other (P-value= 0.627). These values were similar for both carbohydrate derived fulvic acid and chlorohexidine. However. the mean microbial count for the chlorohexidine was significantly smaller than CHD-FA.

Conclusion:

CHD-FA reduced oral microflora significantly fifteen minutes after rinsing. A second objective of this study was to determine whether CHD-FA was still active at the end of the surgery. The colony forming units in CHD-FA group increased at the end of the surgical procedure, but never reached the baseline count.

Declaration

I hereby declare that “The antimicrobial efficacy of a carbohydrate derived fulvic acid as a pre-periodontal procedure mouth rinse” is my own work and that it has never been submitted before for any examination or degree in any university. All the sources that I have used and quoted have been acknowledged by complete references.

Gadija Abrahams

Signed *G. Abrahams*

Date: 17 July 2017



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I wish to acknowledge my gratitude to the following people for their assistance given to me in this research project:

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Dr Basson, for his guidance on all the technical aspects of this project.



Dedication

I dedicate this work to my mother and family, if not for all their support and patience, this study would not have been possible.



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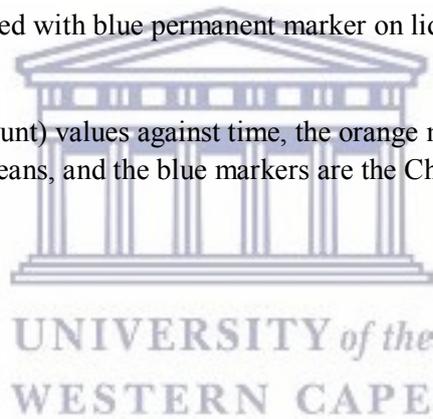
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List of abbreviations

CHX	Chlorhexidine
CHD-FA	Carbohydrate derived fulvic acid
ml	Millilitre
CFU	Colony forming units
MIC	Minimum Inhibitory Concentration
DAAC	Danish Anaesthesia Allergy Centre
PI	Povidone iodine
CPC	Cetylpyridinium chloride
EO	Essential oils
DNA	Deoxyribonucleic acid
ADA	American Dental association
<i>S.mutans</i>	Streptococcus mutans
<i>E. faecalis</i>	Enterococcus faecalis
<i>P. gingivalis</i>	Porphyromonas gingivalis
<i>C albicans.</i>	Candida Albicans
µm	Micrometres
AHA	American Heart Association
BSAC	British Society of Antimicrobial Chemotherapy

Chapter 1

Introduction

Mouth rinses also known as mouth wash are simply a means for delivery of active substances in the oral cavity where, after 20 to 30 seconds of rinsing, all surfaces of the dentition have come into contact with the mouth rinse (Van der Weijden, *et al.*, 2015). There are various uses for mouth rinses which would depend on the clinical situations (Lang & Lindhe, 2015).

The normal oral microbiota is complex, consisting of both anaerobic and aerobic bacteria. The average concentration is about 10^7 - 10^8 colonies per 1 ml (millilitre) (Kosutic, *et al.*, 2009). Oral surgical wounds can become infected by various modes of microbial transmission (Hennessy & Joyce, 2004). However qualitative microbial analysis has shown that local contamination of wounds by intra-oral bacterial flora is the primary source of infections and temporarily reducing these bacterial counts can reduce the risk of post-operative infections (Kosutic, *et al.*, 2009). These bacteria can also transiently enter the bloodstream following invasive and non-invasive dental procedures (Borgnakke, 2015; Bölükbaşı, *et al.*, 2012). The main objective of antiseptic pre-operative mouth rinses is to reduce the bacterial load in the oral cavity at the time of dental treatment with the aim of reducing the risk of developing bacteraemia (Ugwumba, *et al.*, 2014).

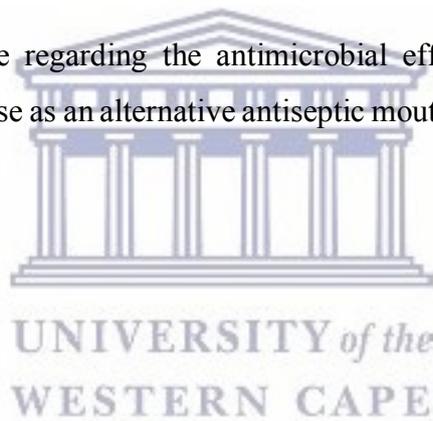
It has been found that during routine dental procedures using sonic or ultrasonic devices, splatter and aerosols are produced and may carry infectious micro-organisms, raising concerns of contamination. Pre-procedural rinsing with an antimicrobial mouth rinse can reduce the bacteria in these aerosols (Trenter & Walmslet, 2003; Lang & Lindhe, 2015; Feres, *et al.*, 2010).

There is no standard protocol for the use of pre-operative antimicrobial mouth rinses but chlorhexidine (CHX) in different concentrations is still the most widely used pre-operative antimicrobial for various surgical procedures (Veksler, *et al.*, 1991; Garvey, *et al.*, 2001; Young, *et al.*, 2002; Gürkan, *et al.*, 2006; Todkar, *et al.*, 2012; Bonez, *et al.*, 2013; Opstrup, *et al.*, 2014; Ugwumba, *et al.*, 2014; Kosutic, *et al.*, 2009).

The combination of CHX's bactericidal, bacteriostatic effects as well as its ability to be released slowly from surfaces hours after initial use, makes it an advantageous product for pre-operative use, and is therefore considered to be the gold standard (Marchetti, *et al.*, 2011). However there have been reports of adverse effects to CHX, ranging from contact dermatitis to severe anaphylactic shock (Todkar, *et al.*, 2012; Gürgan, *et al.*, 2006; Ebo, *et al.*, 1988; Garvey, *et al.*, 2001). Additionally, alcohol added to some forms of CHX, is contraindicated in patients undergoing radiation therapy for head and neck cancers, mucositis as well as those who are sensitive to alcohol (Gürgan, *et al.*, 2006; Todkar, *et al.*, 2012).

A mouthwash containing carbohydrate derived fulvic acid (CHD-FA), has recently been introduced and available without prescription. The current literature reports that it has broad spectrum antibacterial activity against oral bacteria and oral candida. It is also non-toxic to humans and may even have anti-inflammatory and wound healing capabilities (Sherry, *et al.* 2012; Sherry, *et al.*, 2013).

There is insufficient literature regarding the antimicrobial efficacy of CHD-FA as a pre-operative mouth rinse and its use as an alternative antiseptic mouth rinse to patients with known allergy to CHX.



Chapter 2

Literature review

1. Mouth rinses

Mouth rinsing has been practised by humans for more than 2000 years ago. In the 1880's Willoughby D. Miller, a dentist trained in microbiology, was the first to suggest the use of an antimicrobial mouthwash containing phenolic compounds to combat gingival inflammation (cited in Van der Weijden, *et al.*, 2015). Commercially available mouth rinses were introduced in the 1970's. These were formulated specifically for supra gingival plaque control and gingivitis and may be used in the clinic or at home as an adjunct to daily oral hygiene practices (Moran, 2008; Lindhe, *et al.*, 2006).

Mouth rinses provide a convenient vehicle for the delivery of a range of therapeutic agents to the oral cavity. These therapeutic agents are varied, but may include agents such as, bis-biquanides, phenolic compounds, povidone iodine, hydrogen peroxide, sanguinarine and fluoride (Walker, 1988; Young, *et al.*, 2002; Veksler, *et al.*, 1991; Hermes, *et al.*, 1998; Kosutic, *et al.*, 2009; Hennessy & Joyce, 2004; Ugwumba, *et al.*, 2014; Gupta, *et al.*, 2012). Antimicrobial mouth rinses have shown to augment home care and provide an effective means to reduce or even remove bacterial plaque, thereby limiting gingivitis and the possible progression to periodontitis (Haffejee *et al.*, 2008). One of the most commonly used antiseptic currently incorporated into mouth rinses is chlorhexidine (Veksler, *et al.*, 1991; Garvey, *et al.*, 2001; Young, *et al.*, 2002; Gürkan, *et al.*, 2006; Todkar, *et al.*, 2012; Bonez, *et al.*, 2013; Opstrup, *et al.*, 2014; Ugwumba, *et al.*, 2014; Kosutic, *et al.*, 2009).

Recently an increasing number of naturally occurring compounds derived from plants, microorganisms and marine organisms have been incorporated in mouth rinses (Chen, *et al.*, 2014). Several clinical trials indicate that these agents yield positive therapeutic results for a number of oral pathological conditions and many of them have also been shown to possess anti-inflammatory, anti-oxidant and antimicrobial properties (Sherry, *et al.*, 2012; Sherry, *et al.*, 2013; Duss, *et al.*, 2010).

2. Compounds contained in antimicrobial mouth rinses

2.1 Chemical compound containing mouth rinses

2.1.1 Chlorohexidine digluconate (CHX)

Chlorhexidine (CHX) was developed in the 1940's and used as an antiseptic cleaner for wounds, surgical scrub as well as a hand wash in the United Kingdom hospitals (Gupta, *et al.*, 2012). It is a cationic bis-biquanide and has wide range antimicrobial effects. CHX has different antibacterial activities at different concentrations being bacteriostatic at low concentrations and bactericidal at higher concentrations. These include inhibiting microbial growth and killing microbes directly (Walker, 1988). It also has anti-fungal properties and has proven to be effective in treating denture stomatitis (Gupta, *et al.*, 2012).

Bacteriostatic activity of CHX involves increasing the permeability of the bacterial cell wall thereby allowing certain low molecular weight components and ions leak out of the cell, altering their intracellular concentrations. Bactericidal activity leads to chemical precipitation of the cytoplasm and leads to cell death (Jones, 1997). This activity is effective against both Gram-positive and Gram-negative organisms but with different susceptibilities. This susceptibility is expressed as Low Minimum Inhibitory Concentration (MIC) (Gupta, *et al.*, 2012).

CHX is widely used in different concentrations, as a mouthwash, for sterilisation of surgical instrument and disinfection of surgical surfaces (Bonez, *et al.*, 2013; Veksler, *et al.*, 1991). It is also routinely used pre-operatively for various dental procedures including periodontal surgery and inter-alveolar tooth extractions (Hermesch, *et al.*, 1998; Gupta, *et al.*, 2012). In studies conducted by Ugwenda *et al.* (2014) and Veksler *et al.* (1991), chlorhexidine at concentrations of 0.2% and 0.12% respectively, were used pre-operatively for various dental procedures. These studies concluded that chlorhexidine added value in reducing microbial levels prior to dental procedures and thus would reduce the risk of post-operative infections as well as bacteraemia (Ugwenda, *et al.*, 2014; Veksler, *et al.*, 1991; Bonez, *et al.*, 2013).

The side effects of CHX are dependent on the duration of its use. With long-term use, it could result in staining of teeth as well as other surfaces of the oral cavity principally the dorsum of the tongue, alterations in taste perception, mucosal erosions and unilateral or bilateral parotid

swelling (Lindhe, *et al.*, 2006). There is significant amount of alcohol contained within CHX. The use of alcohol containing mouth wash is contraindicated in patients with a mucositis or undergoing head and neck radiations therapy as it could cause xerostomia and lead to ulcerations. CHX without alcohol has proven to be just as effective as those with alcohol, however the long-term side effects remained. This included staining, altering the taste sensation as well as mild burning sensation (Todkar, *et al.*, 2012; Gürgan, *et al.*, 2006). Allergy to CHX can present in the form of a contact dermatitis to severe anaphylactic shock (Ebo, *et al.*, 1988). The incidence of allergy and anaphylactic shock is unknown.

Ebo *et al* (1988) described two clinical cases, a 43-year-old male who developed anaphylactic shock in one surgery and a rash at the surgical incision areas a few weeks later, 2% CHX was used preoperative to disinfect the surgical site. The second clinical case was a boy who developed anaphylactic shock to CHX during surgery. A skin prick allergy test specific for CHX was positive in both these patients.

Garvey *et al* (2001) described four cases of CHX allergy. These patients developed anaphylactic reactions while under general anaesthesia. CHX was used in different concentration to clean the surgical areas, as well as in a gel form in procedures to place the urethral catheter. These patients were subsequently tested at the Danish Allergy Centre. The results were conclusive that the reactions were caused because of CHX allergy. Interestingly these patients also had had previous exposure to CHX with reactions ranging from itching to rashes or faints. It also seemed likely that the risk to allergy was higher when CHX was used repeatedly on broken skin or the mucosal surface as would occur during invasive or surgical procedures (Garvey, *et al.*, 2001).

Garvey *et al* (2007) conducted a retrospective study analysing serum samples of patients who had an allergic reaction during surgery and anaesthesia. Serum samples were obtained from the Danish Anaesthesia Allergy Centre (DAAC). This centre has been routinely testing all patients with a suspected anaphylaxis during anaesthesia since 2009 in Denmark. Of the 174 patients that had been in the database from 1999 to 2005, 12 patients tested positive for allergy to CHX using the skin test (Garvey, *et al.*, 2007). Opstrup *et al* (2014) did a histamine release test; skin prick test and intradermal test to test for allergy to CHX. The patients were obtained via the DAAC, which had evaluated patients for suspected perioperative allergy to CHX from 2004 to 2014. Of the total 228 patients used for this study 32 patients had one or more positive test for CHX allergy (Opstrup, *et al.*, 2014). There have also been concerns raised regarding the

emergence of resistant bacteria because of long term CHX usage in the oral cavity (Walker, 1988; Bonez *et al.*, 2013).

2.1.2 Povidone-Iodine (PI)

Iodine is recognised as an effective germicide being active against a wide variety of microorganisms, such as viruses, bacteria, protozoa, yeast, and fungi. However, its use often is contraindicated because of its insolubility, instability, staining and irritating properties. When combined with povidone, an organic polymer which is water-soluble, a complex is formed in which iodine's toxic properties are lost without its bactericidal activity being affected (Zinner, *et al.*, 1961)

Povidone-iodine displays an affinity for the cell membrane thereby delivering free iodine directly to the bacterial cell surface. It has a broad spectrum of activity against bacteria, fungi, protozoa and viruses (Farah, 2009). It has been evaluated at various concentrations ranging from 0.5% -10% and its antibacterial properties are considered to be short term when compared to CHX. PI has little anti-plaque activities and does not provide any adjunct to chemical treatment of chronic periodontitis (Addy, *et al.*, 1977; Zanatta, *et al.*, 2006). Absorption of excess iodine has been postulated to adversely affect thyroid function (Farah, 2009; Lindhe, *et al.*, 2006).

2.1.3 Cetylpyridinium chloride (CPC)

Cetylpyridinium chloride (CPC) was initially described in 1940 using a range of bacteria and has been formulated in mouth rinses at concentrations that commonly range from 0.01% to 0.1%. (Hu, *et al.*, 2009). CPC is a cationic quaternary ammonium compound with surface-active properties. It has a broad antimicrobial spectrum, with rapid killing of gram-positive pathogens and yeast (Van der Weijden, *et al.*, 2015). Its mechanism of action relies on the hydrophilic part of the CPC molecule interacting with the bacterial cell membrane leading to loss of cell components, disruption of cell metabolism, inhibition of cell growth, and finally cell death (Feres, *et al.*, 2010; Van der Weijden, *et al.*, 2015). CPC may cause brown staining of teeth (Van der Weijden, *et al.*, 2015). Others side effects related to CPC use are mild changes in taste and a burning feeling (Feres, *et al.*, 2010).

2.2 Natural compounds containing mouth rinses

Natural products have been used for folk medicine purposes throughout the world for thousands of years. Many of them have pharmacological properties, such as antimicrobial, anti-inflammatory and cytostatic effects. The ancient Romans included teeth cleaning as part of their religious ceremonies and human urine was a secret ingredient included into their mouthwash. Until the 18th century, urine continued to be an active ingredient in toothpaste and mouthwash because of the ammonia's cleansing abilities (Kureja & Dodwad, 2012). Numerous natural compound containing mouth rinses have been extensively tested in in vitro and in vivo studies, only a single systematic review (on an essential oils (EO) mouth rinse (Listerine®)) is currently available. Systematic evidence for the efficacy of other natural compound containing mouth rinses is still lacking (Chen, *et al.*, 2014).

2.2.1 Sanguinarine

Sanguinarine is a (toxic) quaternary ammonium salt from the group of benzyloquinoline alkaloids. It is extracted from some plants, including bloodroot (*Sanguinaria canadensis*) and it is also found in the root, stem, and leaves of the opium poppy (Van der Weijden, *et al.*, 2015). Sanguinarine has been found to inhibit the multiplication of bacteria, fungi and viruses by intercalating DNA, inhibiting DNA synthesis, reversing transcriptase and affecting membrane permeability. Sanguinarine was associated with oral leukoplakia, a potentially malignant lesion (Vlachojannis, *et al.*, 2012; Moran, 2008). In 2001, bloodroot was removed from the Viadent® product formula and recently the brand has disappeared altogether from the worldwide market (Vlachojannis, *et al.*, 2012).

2.2.2 Essential oils (EO)

Essential oils (EO) are a natural compound extracted from plants. They are used in an over-the-counter mouthwash containing a fixed formula of 2 phenol-related EOs, thymol 0.064% and eucalyptol 0.092%, mixed with menthol 0.042% and methyl salicylate 0.060% in a 22% alcohol vehicle (Corelli, *et al.*, 2012; Van der Weijden, *et al.*, 2015). The first official approval of essential oil mouth rinses dates back to 1987 and was based on clinical studies that fulfilled the American Dental Association (ADA) criteria (Van Leeuwen, *et al.*, 2011). The antimicrobial mechanisms of action of EO against bacteria are complex. At high concentrations, there is disruption of the cell wall and precipitation of cell proteins, whereas at lower concentrations, there is inactivation of essential enzymes. The anti-inflammatory action has been proposed based on antioxidant activity (Van der Weijden, *et al.*, 2015). In the

randomised parallel clinical trial by Cortelli *et al* (2012) concluded that EO were clinically superior in reducing plaque and well as gingivitis parameter after 6 months of use when compared with 0.05% CPC (Cortelli, *et al.*, 2012). In a similar randomised controlled clinical trial Sharma *et al* (2010) also compared the efficacy EO to 0.05% CPC in reducing plaque and gingivitis. Their study also demonstrated the superior efficacy of a mouth rinse containing a fixed combination of EO's (Listerine® Antiseptic) compared to a commercially available mouth rinse containing 0.05% CPC (Colgate Plax) over the course of six months (Sharma, *et al.*, 2010). A systematic review by Van Leeuwen *et al* (2011) also compared the antiplaque and anti-gingivitis efficacy of EO to other mouth rinses. Their review concluded that compared the CHX, EO were no different to control long term gingival inflammation but that CHX was more effective in reducing plaque. Side effects with long term use include staining but not as severe as with long term CHX use (Van Leeuwen, *et al.*, 2011).

2.2.3 Carbohydrate derived fulvic acid (CHD-FA)

Fulvic acid is the main compound of humic acids and had been used in the past as a natural remedy. Humic substances occur whenever organic matter is decomposed and are found in compost, sewage, soils, lignites, carbonaceous shales and brown coals (Sherry, *et al.*, 2013). Carbohydrate derived fulvic acid (CHD-FA) is a heat stable low molecular weight, water soluble, colloidal, cationic material with proposed antifungal and antibacterial properties. CHD-FA is a pure form of fulvic acid produced by a patented process, rendering it free from heavy metals and environmental pollutants which are normally found in fulvic acid from environmental sources (Sherry, *et al.*, 2012).

CHD-FA was evaluated against chlorhexidine. Tests were conducted to evaluate the broad spectrum antimicrobial activity of CHD-FA against pathogens found in the oral cavity, pathogens involved in biofilm formation, as well as any immunomodulatory properties. CHD-FA had fast broad-spectrum antimicrobial activity against, *S.mutans*, *E. faecalis*. and *P. gingivalis*. It was also capable of disrupting the biofilm architecture. It killed periodontal pathogens that were tested as polymicrobial biofilms, and after 30 min reduced the biofilm by 90%. This effect was also observed against *C albicans*. CHD-FA also showed a reduction of some pro-inflammatory mediators. This was however an in vitro study and patient factors could not be considered. Moreover, biofilm models where replicated that does not fully represent all the mixed biofilms found in the oral cavity (Sherry, *et al.*, 2012; Sherry, *et al.*, 2013).

Sabi *et al* (2012) evaluated the safety and anti-inflammatory as well as wound healing characteristics of a CHD-FA. In the tested mice, it did not produce any hypersensitivity when applied topically and was non-toxic to liver and kidney. Oral dosages were provided to the rats and this also showed no liver and kidney toxicity (Sabi, *et al.*, 2012).

Gandy *et al* (2012) wanted to determine the acute and subacute safety and proof-of-concept efficacy of CHD-FA. They tested CHD-FA in 30 male volunteers. The volunteers in the study were administered 40 ml oral dosages of 3.8% CHD-FA. CHD-FA proved to be safe with no liver or kidney toxicity. There was also no hypersensitivity reaction to CHD-FA. They advised that 15 ml could be used safely used daily for systemic use (Gandy, *et al.*, 2012).

Table 1: Literature on efficacy and on the safety of CHD-FA

Author	Aim and outcome of the study
Sherry, <i>et al.</i>, 2012	An <i>in vitro</i> investigation of CHD-FA against fungal species <i>Candida albicans</i> . CHD-FA shown to have fungicidal activity and disrupts cell membrane activity.
Sherry, <i>et al.</i>, 2013	An <i>in vitro</i> investigation on the biological properties of CHD-FA as a potential novel therapy for the management of oral biofilm infections. CHD-FA was highly active against all of the oral bacteria tested.
Sabi, <i>et al.</i>, 2012	A clinic study to evaluate the safety and anti-inflammatory and wound healing characteristics of CHD-FA in rats. CHD-FA was a safe compound with anti-inflammatory and wound-healing properties.
Gandy, <i>et al.</i>, 2012	To determine the acute and subacute safety and proof-of-concept efficacy of CHD-FA. No adverse events occurred, establishing CHD-FA to be safe at doses up to 40 ml twice daily for a week.

3. Indication for single use of antimicrobial mouth rinses

There are various uses for mouth rinses for different clinical situations as well as the duration and the objective of the intervention. The mouth rinses can be for single use, short-term or long-term use (Lang & Lindhe, 2015). Mouth rinses for single pre-operatively use may be considered with the aim to decrease the bacterial load; to decrease the risk of bacteraemia and to decrease the risk of infection of the surgical area (Lang & Lindhe, 2015; Feres, *et al.*, 2010; Ugwenda, *et al.*, 2014; Kosutic, *et al.*, 2009).

Mouth rinses are used for short term where patients are unable to mechanically clean following oral procedures that result in pain and discomfort, to prevent or control biofilm formation. Short term use is also indicated for certain infective conditions e.g. necrotising gingivitis therapy, candidiasis, peri-implantitis, peri-implant mucositis and during basic periodontal therapy.

Long term use of various anti-microbial mouth rinses is indicated to prevent dental biofilm formation and as part of the supportive periodontal therapy programme as well as for the prevention of certain oral conditions, seen in patients who are immunosuppressed, caries prevention and candidiasis prevention. (Lang & Lindhe, 2015).

3.1 Decreasing the oral bacterial load

The use of pre-procedural mouth rinses containing antiseptic agents is an effective and feasible way to reduce viable bacteria in the oral cavity and various studies have demonstrated this efficacy (Purohit, *et al.*, 2009, Rani, *et al.*, 2014, Feres, *et al.*, 2010). The oral cavity being part of the oro-nasal pharynx, harbours bacteria and viruses from the nose, throat and respiratory tract. Dental plaque, saliva and oral fluids are major sources of these organisms. Dental procedures that have a potential to aerosolise saliva, will cause airborne contamination with organisms from these sources. These airborne particles are the result of the combined action of water sprays, compressed air, organic particles, such as tissue and tooth dust, and organic fluids, such as blood and saliva from the site where the instrument is used (Purohit, *et al.*, 2009).

Splatter during routine dental procedure as well as aerosols produced with debridement using sonic or ultrasonic, which may carry infectious micro-organisms, raise concerns of cross contamination. This places both the dental team and patients at risk of infectious agents (Trenter & Walmslet, 2003; Lang & Lindhe, 2015; Feres, *et al.*, 2010). Splatter being defined as airborne particles larger than 50 µm (micrometres) in diameter. These particles are too large to become suspended in the air and are airborne only briefly. Aerosols are defined as particles with a diameter of 5µm (micrometres) and because of their diameter being so small they can remain airborne for extended periods before they settle on environmental surface or enter the respiratory tract. Aerosols can be inhaled into the lungs to reach alveoli and carry the potential risk of transmitting infections (Purohit, *et al.*, 2009).

Various studies have compared the efficacy of pre-procedural herbal mouth rinses to CHX. They have found that pre-procedural mouth rinses have been effective in reducing bacterial

splatter as well as aerosol contamination (Rani, *et al.*, 2014; Gupta, *et al.*, 2014). Gupta *et al* (2014) suggested that a routine pre-procedural mouth rinse could eliminate most bacterial aerosols generated using an ultrasonic unit (Gupta, *et al.*, 2014).

Ferez *et al* (2010) in their clinical trial concluded that mouth rinses containing 0.05 percent CPC and 0.12 percent CHX are equally effective during ultrasonic scaling and their use could help decrease the level of microbial contamination in the dental office. CPC has fewer side effects than CHX and may be a good alternative to 0.12 CHX as a pre-procedural mouth rinse (Feres, *et al.*, 2010).

Table 2: Literature of mouth rinses evaluated as an effective primary measure in reducing aerosol cross-contamination

Author	Active ingredient	Results
Litsky, <i>et al.</i> , 1970	CPC 1:2000 (Cepacol®) rinsed with 10-20 seconds	CPC significantly reduced bacterial counts
Purohit, <i>et al.</i> , 2009	15 cc of 0.12 % CHX mouth-rinse for 30 seconds	CHX significantly reduced CFU
Reddy. <i>et al.</i> , 2012	0.2% CHX, compared 0.2% of tempered CHX rinse for 60 seconds	Both CHX and tempered CHX significantly reduced CFU
Shetty, <i>et al.</i> , 2013	0.2% CHX (Rexidine®) compared with Tea tree oil (Emoform®) rinse for 2 minutes	Both CHX and Tea tree oil reduced CFU, CHX was superior
Gupta, <i>et al.</i> , 2014	0.2% CHX compared with herbal mouthwash (The herbal mouth wash is made from natural herb extracts bibhitaki, nagavalli, peelu, peppermint satva and yavani satva; and oils gandhapura taila and ela . 10ml rinses used for 1 minute	Both CHX and herbal mouthwash significantly reduced CFU and CHX was superior
Feres, <i>et al.</i> , 2010	0.05 % CPC compared with 0.12% CHX	CPC and CHX were equally effective in lowering the levels of splatter bacteria
Rani, <i>et al.</i> , 2014	20 ml of 0.2% CHX compared with 18 ml of an herbal mouthwash (Herbal mouthwash prepared from Pilu 5 mg, Bibhitaka 10 mg, Nagavalli 10 mg, Peppermint satva 1.6 mg, Yavanisatva 0.4 mg was used) rinse for 30 seconds	Both CHX and Herbal mouthwash reduced CFU

3.2 Decreasing the risk of bacteraemia

Bacteria can transiently enter the bloodstream, and in a healthy patient is it eliminated by the normal host defence systems however patients with heart disorders are at risk of developing infective endocarditis (Bölükbaşı, *et al.*, 2012; Tuna, *et al.*, 2012). Bacteraemia can occur following invasive dental procedure such as extractions and periodontal surgery as well as non-invasive procedures such as dental probing and oral hygiene procedures (Borgnakke, 2015; Bölükbaşı, *et al.*, 2012).

The American Heart Association (AHA) and the British Society of Antimicrobial Chemotherapy (BSAC) both advised that antibiotics only be used on patient at high risk of developing infective endocarditis (Tuna, *et al.*, 2012; Bölükbaşı, *et al.*, 2012). There are many adverse effects to using antibiotics prophylactically for dental treatment. This includes allergy and the development of antibiotic resistance. The AHA announced that bacteraemia is short lived after a dental procedure no more than 15 minutes (Tuna, *et al.*, 2012).

In the pilot study conducted by Tuna *et al* (2012), blood cultures before third molar extraction were collected and compared with blood collected at 1 min and 15 min into surgery. These samples all presented with bacteraemia. Samples of the control group were compared with two other groups. One had rinsed with 0.2% CHX and the other with 7.5% PI and the control group had rinsed with nothing. The CHX group had significantly less bacteraemia and the PI group had none after 15 minutes, showing a reduction in oral microbiota with pre-procedural antimicrobial rinses (Tuna, *et al.*, 2012).

Ugwumba *et al* (2014) in a similar clinical study randomly assigned 101 patients to either rinse with 0.2% CHX or sterile water 1 minute before dental manipulation. Blood samples were also collected at baseline, 1 minute and 15 minutes after the dental extraction. Their clinical study revealed that most of the bacteraemia occurred 1 minute after extractions and that the presence of bacteraemia in the 0.2% CHX was significantly lower than the control group. A single rinse with 0.2% CHX reduced the prevalence of post extraction bacteraemia in their study (Ugwumba, *et al.*, 2014).

The evidence is contradictory with regard to the effect of pre-operative mouth rinse with antiseptics on the prevalence of bacteraemia associated with dental procedures. The American Heart Association concluded in their report stated that topical antiseptic rinses do not penetrate beyond 3 mm into the periodontal pocket and therefore do not reach areas of ulcerated tissue

where bacteria most often gain entrance into circulation (Lang & Lindhe, 2015; Ugwumba, *et al.*, 2014). The main objective of antiseptic prophylaxis is to reduce the bacterial load in the oral cavity at the time of dental manipulation with the aim of reducing the risk of developing bacteraemia (Ugwumba, *et al.*, 2014).

3.3 Decreasing the risk of infection of the surgical area

The risk of post-operative infections is increased in intra-oral surgical procedures; as it is almost impossible attain an aseptic condition due to the extensive quantity of oral microbes in the normal mouth (Kosutic, *et al.*, 2009). Post-operative infections can have a detrimental outcome on surgery (Kosutic, *et al.*, 2009; Hermes, *et al.*, 1998; Caso, *et al.*, 2005). Attempts have been made to reduce the risk of this complication in intra-oral surgical procedures by reducing the bacterial load pre-operatively with the use of antimicrobial mouth rinses (Caso, *et al.*, 2005; Young, *et al.*, 2002; Veksler, *et al.*, 1991; Hermes, *et al.*, 1998; Kosutic, *et al.*, 2009; Hennessy & Joyce, 2004; Ugwumba, *et al.*, 2014; Gupta, *et al.*, 2012).

CHX in various concentrations and PI solution are the most frequently used pre-operative antiseptic solutions in intra-oral surgical procedures and their efficacy have been extensively evaluated (Kosutic, *et al.*, 2009; Hermes, *et al.*, 1998).

In the study conducted by Kosutic *et al* (2009) they compared the effectiveness 0.1 % CHX, PI and cetrimide as pre-operative antiseptic mouth rinses in reducing oral bacterial counts with that of a saline rinse. The results showed that all three antiseptic mouth rinses effectively reduced both aerobic and anaerobic bacteria whereas the saline was shown to be ineffective in reducing oral bacteria (Kosutic, *et al.*, 2009).

In another clinical study Young *et al* (2002) evaluated the effect that pre-operative rinsing with 0.1 % CHX would have on the bacterial contaminants present in bone debris collected during osteotomy site preparation for implant placement. Bone collected from the patients who had rinsed pre-operatively with CHX was compared with those that had rinsed with sterile water. The samples were tested for microbes, and 39 species were identified these included pathogenic species *Actinomyces odontolyticus*, *Clostridium bifermentans*, *Propionibacterium propionicum* and *Prevotella intermedia*, which have all been associated with diseases. The samples evaluated from the test group who had rinsed with chlorhexidine pre-operatively

yielded significantly fewer organisms. This outcome supports the pre-operative use of CHX to lower microbial count (Young, *et al.*, 2002).

Table 3: Literature of pre-procedural mouth rinses evaluated for reducing oral microbiota to prevent post-operative infection

Study	Active ingredient and purpose of study
Veksler, <i>et al.</i>, 1991	The purpose of this study was to assess the effect of pre-procedural rinsing (2 consecutive rinses) with 0.12% CHX on salivary bacteria during scaling and root planing procedures
Hermesch, <i>et al.</i>, 1998	The purposes of this study were to evaluate the use of 0.12% CHX as a prophylactic therapy for the prevention of alveolar osteitis and to further examine subject-based risk factors associated with alveolar osteitis.
Young, <i>et al.</i>, 2002	This study examined the effect of pre -operative rinsing with a 0.1% CHX mouth rinse on the bacterial contaminants present in collected bone debris bone.
Caso, <i>et al.</i>, 2005	The objective of this study was to assess if CHX rinse decreases the occurrence of alveolar osteitis following third molar removal.
Kosutic, <i>et al.</i>, 2009	The purpose of this study was to compare preoperative oral cavity decontamination using 3 different antiseptic solutions (1% PI, CHX and cetrimide) and a sterilized physiological solution (control group) to reduce intraoral bacterial counts during and at the end of clean/contaminated surgical procedures within the oral cavity and to determine the most efficient one.
Ugwumba, <i>et al.</i>, 2014	The aim of the study was to investigate the effect of preoperative 0.2% CHX mouthwash on the risk of bacteraemia following routine intra-alveolar tooth extraction

Chapter 3

Aims and Objectives

Aim

To compare the antimicrobial efficacy of carbohydrate fulvic acid as a pre- operative mouth rinse to chlorhexidine digluconate.

Objectives

To determine whether carbohydrate fulvic acid was able to reduce oral microflora at 15 minutes after initial rinse.

To determine whether carbohydrate fulvic was still active at the end of the surgery.



Chapter 4

Methodology

Study design

A randomized control trial included 20 patients who underwent periodontal procedure or minor oral surgery under local anaesthesia.

Study population

A total of 20 patients who, met the inclusion criteria, were recruited at the Oral Health Centre of University of the Western Cape, between January 2016 and September 2016. Prior to participation, each patient was given a detailed description of the study and all patients signed informed consent forms. All participants remained anonymous. The study was in accordance to the Helsinki declaration and the study protocol was approved by the ethics committee of the University of the Western Cape, South Africa. Project registration number: 15/7/33

Patient Selection

Patients that met the following inclusion criteria were enrolled in this study:

Inclusion criteria:

1. Patients indicated for periodontal surgery or minor oral surgery procedure
2. No medical conditions that affect periodontal status
3. Persons 18 years or over.
4. Cigarette smoking < 10 cigarettes per day)

Exclusion criteria:

1. Patient below the age of 18
2. Individuals that have used medications, that might affect the periodontium, during the past 30 days prior to sample collection
3. Patients with known allergy to any substance investigated in this study.
4. Smoking > 10 cigarettes per day

Conflict of interest

The author declares no conflict of interest.

Specimen sampling

Sample procedure

Patients scheduled for periodontal surgery were randomised on the day of the procedure into one of the two study groups by flip of a coin.

1. They were presented pre-operatively with a universal specimen jar, containing fifteen (15) ml sterile saline, and under supervision instructed to rinse for sixty (60) seconds and then expectorate directly back into the specimen jar. This allowed for the first saliva sample to be collected at baseline (figure 1)
2. Patients were then immediately instructed to rinse (supervised), with fifteen (15) ml of mouthwash (either 0.2% CHX or 20% CHD-FA) (see figure 2), for thirty (30) seconds and expectorate into spittoon. This was repeated.
3. Fifteen (15) minutes after the mouthwash rinse, the patients were then instructed to rinse with a further fifteen (15) ml sterile saline for sixty (60) seconds and expectorate into a universal jar and a second saliva sample was collected (Figure 1)
4. Thereafter the oral periodontal surgical procedure was undertaken and at the end of the procedure the patient was again instructed to rinse with fifteen (15) ml of sterile saline for sixty (60) seconds and to expectorate into a universal specimen jar. A final saliva sample was then collected. (see figure 3)

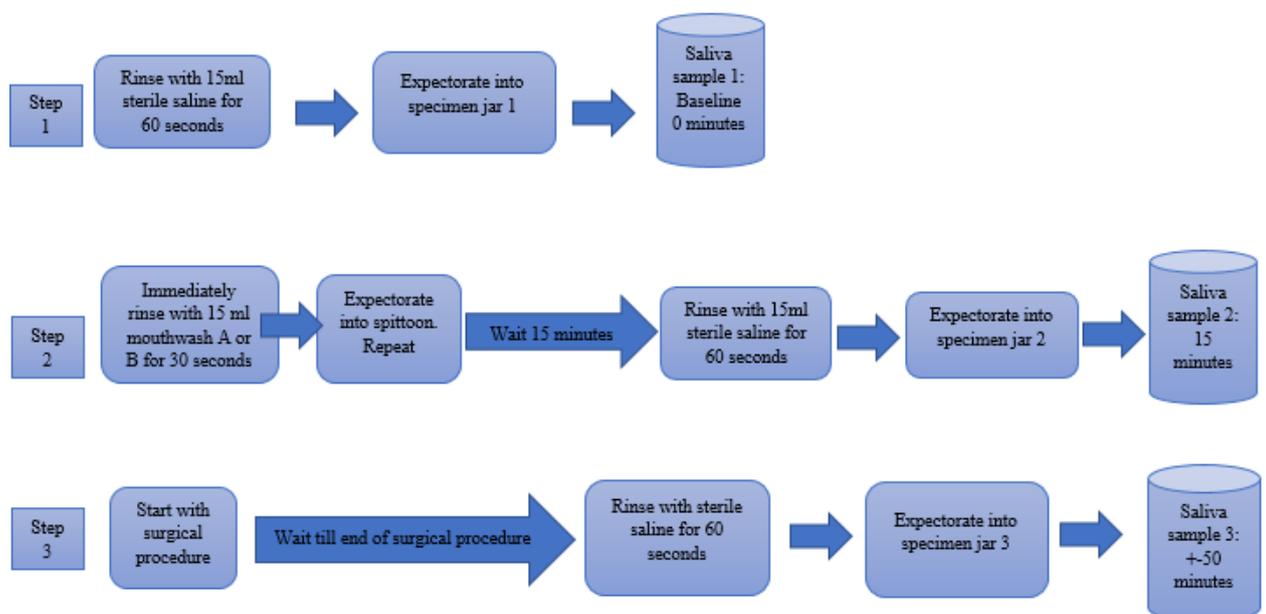


Figure 1: Methodology



Figure 2: Mouthwashes tested Carbohydrate derived fluvic acid (Biobalance™) and 0.2% Chlorhexidine



Figure 3: Universal specimen jar

Laboratory preparation of samples

Saliva samples collected at baseline were immediately taken to the lab and 0.1ml saliva samples were serially diluted with 0.9ml sterile buffered saline up to 10^9 (see figure 4). The same procedure of serial dilution was performed for saliva samples obtained at 15 minutes as well as at the end of surgery. 100 μ l of the saliva sample was inoculated onto previously prepared brain heart infusion agar plates and spread over the agar surface with a sterile glass rod. All agar plates were then placed in the incubator at 37 °C and left for 24 to 48 hours. After 24-48 hours, the agar plates were placed on a hand-counter with transmission light and magnifier and colony forming bacterial units were counted manually. All counted colonies

were marked with a pen on the glass to prevent repeated counting of the same colony and all values were recorded (see figure 5). This colony forming bacterial units (CFU) were counted thrice and the mean CFU counts was noted (see appendix 3)

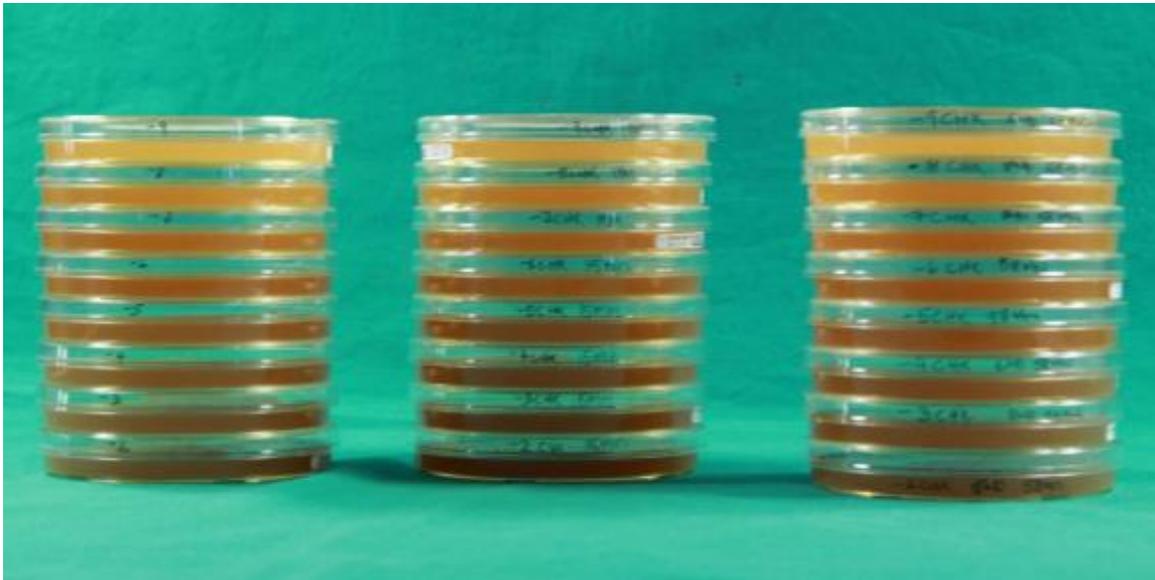


Figure 4: Brain heart infusions agar plates

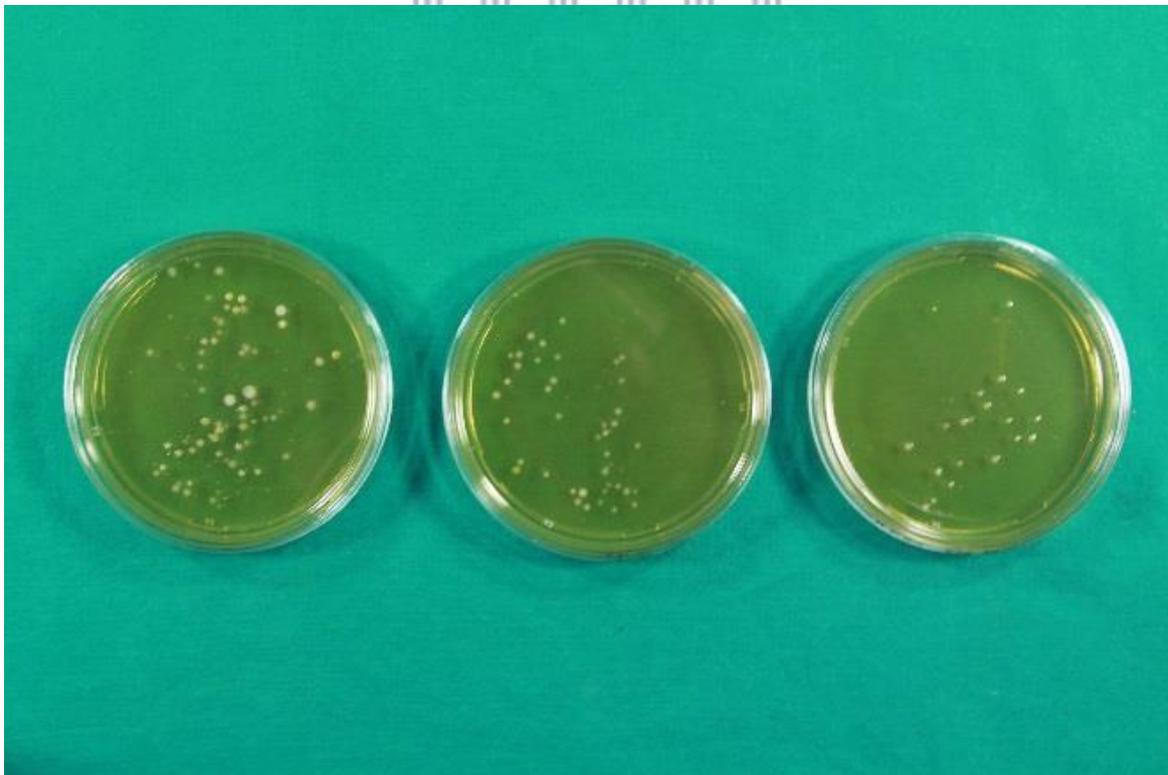


Figure 5: Brain heart infusion agar plates with CFU at baseline; 15 minutes and at end of oral surgical procedure

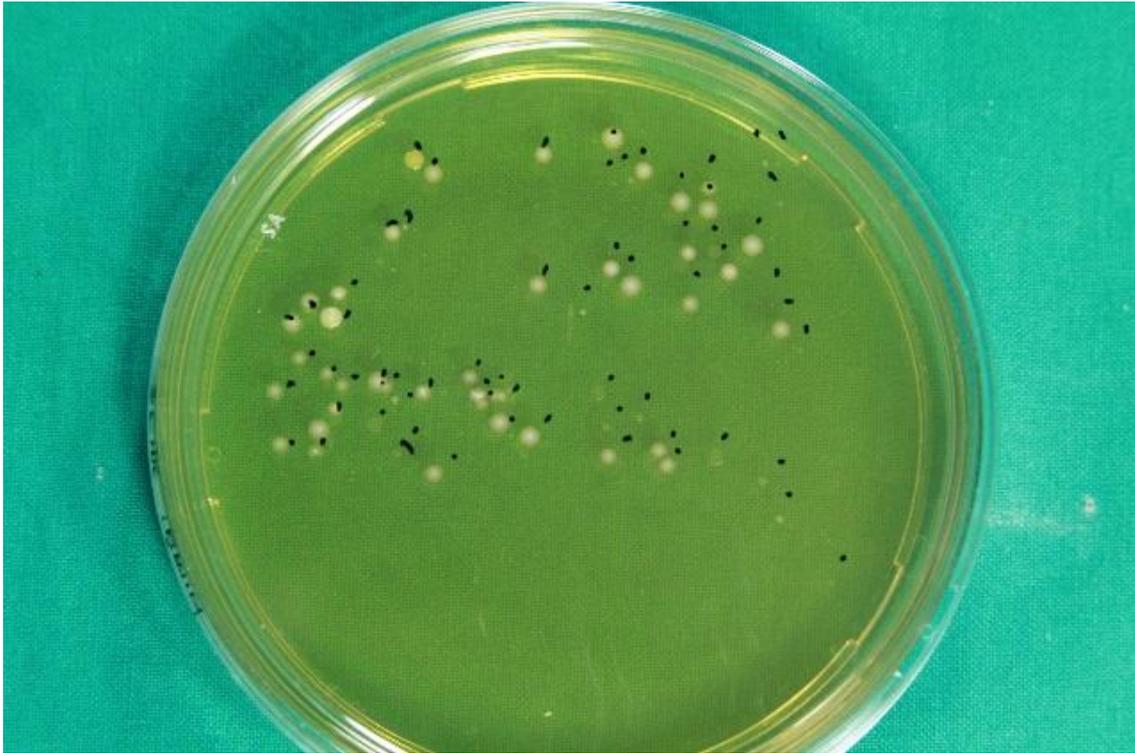


Figure 6: CFU counted and marked with blue permanent marker on lid of plates

Data capture and analysis

After 24-48 hours, the agar plated were placed on a hand-counter with transmission light and magnifier and colony forming bacterial units were counted manually. All counted colonies were marked with a permanent marker on the lid of agar plates, to prevent repeated counting of the same colony and all values were recorded. This colony forming bacterial units were counted thrice and the mean value was noted (see appendix 3)

All data was captured and recorded into a data capture sheet using Microsoft Excel© (Microsoft Corporation, Redmond, WA, USA). A statistician was consulted and the mean values and standard deviation of the microbial log (counts) at baseline, 15 minutes after antimicrobial rinse and at the end of surgery were calculated and compared. Analysis of the data was performed utilising the 'R software package' (R Core Team 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). Other factors including patient age, gender, length and type of periodontal surgery were also captured.

Chapter 5

Results

The proportion of males to females in the CHD-FA and the CHX group were similar and not significant. The mean age of the CHD-FA group was 34.0 years and the CHX group was 40.5 years. According to a Fisher exact test the distribution of surgery types was not significantly different in the rinse type groups.

Table 4: Means of the microbial counts (log) for CHD-FA

Baseline	15 minutes	End
18.118	15.604	15.922

Table 4 gives means of microbial log (counts) at baseline, 15 minutes and end of surgery for the CHD-FA. The mean time at end of surgery was 67.5 minutes for the CHD-FA group. By fitting a repeated measure linear model, it was found that the mean at 15 minutes was statistically significantly smaller than the baseline microbial mean (P-value=0.0012). The end microbial mean was also significantly smaller than the baseline microbial mean (P-value=0.0035), the means at 15 minutes and end are not significantly different from each other (P-value=0.627).

Table 5: Mean microbial log (counts) for chlorohexidine

Baseline	15 minutes	End
16.747	12.375	11.469

Table 5 gives means of microbial log (counts) at baseline, 15 minutes and end of surgery for the CHX group. The mean time at the end of surgery was 73.4 minutes for the CHX group. By fitting a repeated linear model, it was found that the mean at 15 minutes is statistically significantly smaller than the baseline mean (P-value=0.0016), the end mean is also significantly smaller than the baseline mean (P-value=0.0003), the means at 15 minutes and end are not significantly different from each other (P-value=0.435).

Table 6: Comparison of microbial counts of CHD-FA and CHX

	numDF	denDF	F-value	p-value
(Intercept)	1	36	531.8811	<.0001
Time	2	36	19.1499	<.0001
Type	1	18	5.3656	0.0325
Time*Type	2	36	2.6817	0.0821

Table 7: Mean microbial log count comparing CHD-FA to CHX

	Baseline	15 minutes	End
CHD-FA	18.118	15.604	15.922
CHX	16.747	12.375	11.460

Table 6 gives an analysis of the variance summary. When comparing CHD-F and CHX, a repeated measures linear model was fitted with factors Type (CHD-FA, CHX), Time (Baseline, 15 minutes, and end of the periodontal procedure) The type of periodontal procedure was found to be not significant (P-value=0.922).

The significant time effect (P-value<.0001) confirms that the results above, the significant type effect (P-value =0.0325) indicates the overall CHX mean were significantly smaller than the CHD-FA mean. The small P value (0.0821) for the interaction effect is the result of the means at Time=Baseline, where they are close to each other (See table 7).

Figure 7 is a plot of mean log (count) values against time; the orange markers are CHD-FA means and the blue ones CHX means. The arrows are confidence limits, mean $\pm 1.4 X$ (standard error), designed so that if CHD-FA and corresponding CHX arrows just touch significance of difference of the two means is indicated at level approximately 0.05. If they overlap the difference in means is not significant, if they are separated the means differ at significance level smaller than 0.05. At time=0 the arrows overlap (as they should), at 15 minutes they touch, and at the end they are clearly separated.

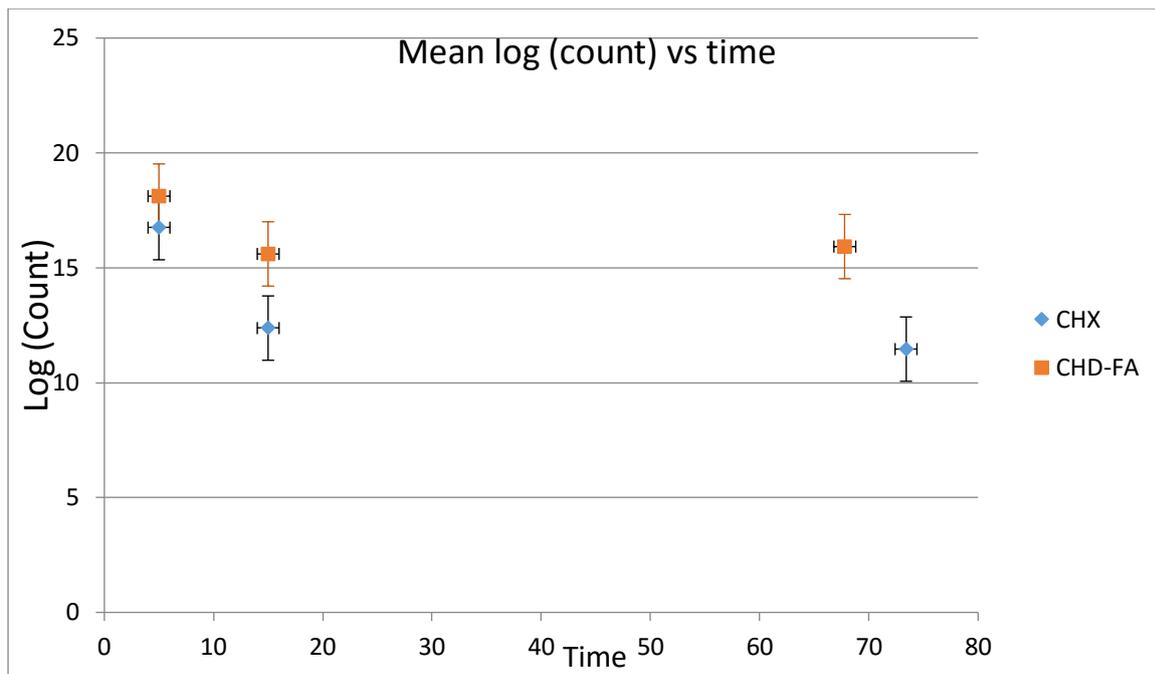
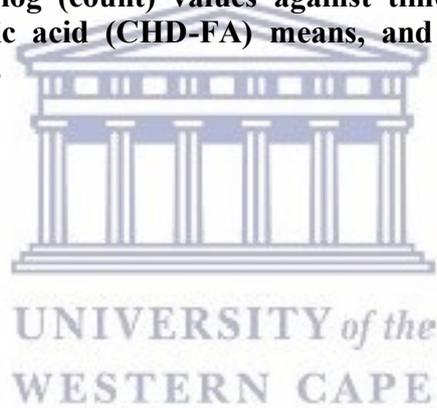


Figure 7: Mean microbial log (count) values against time, the orange markers are Carbohydrate derived fulvic acid (CHD-FA) means, and the blue markers are the Chlorhexidine (CHX) means



Chapter 6

Discussion

Several pre-procedural oral rinses have incorporated antiseptic agents as a means of reducing oral bacteria for various reasons. These include; to reduce cross contamination due to splatter, reduce the incidence of post-operative infection, and reduce the risk of bacteraemia (Feres, *et al.*, 2010; Shetty, *et al.*, 2013; Reddy, *et al.*, 2012; Tuna, *et al.*, 2012; Ugwumba, *et al.*, 2014; Kosutic, *et al.*, 2009). Recently there has been an increase in the use of mouth rinses that contain natural compounds. These natural compounds, are often one, or several herbal extracts that are incorporated into the mouth rinse as an alternative to traditional chemical compounds (Chen, *et al.*, 2014). Clinical research suggests that they may have a reduced incidence of adverse effects as compared to traditional antiseptic mouth rinses that often contain CHX (Duss, *et al.*, 2010).

The aim of this study was to determine whether a newly introduced commercially available mouth-rinse that contained 20% CHD-FA (a naturally derived compound), was able to reduce oral microflora to a significant degree when compared to a 0.2% CHX containing mouth rinse. The results of the present study indicate that the CHD-FA mouth rinse could reduce the total microbial count to a statistically significant degree within 15 minutes of the initial oral rinse (p-value=0.0012). The reduced microbial count was sustained for the duration of all surgical procedures (± 50 min), with a non-significant decrease between the mean microbial count at 15 minutes and the end of the surgery (P-value=0.627). This was similar to the results obtained from the control, CHX. However, when comparing the total bacterial reduction between the two products, the CHX containing mouth rinse showed a significantly greater reduction. This implies that although both mouth rinses could reduce the number of bacteria, CHX was able to do this to a greater degree (P-value <.0001).

The mechanism of antimicrobial action of CHX is well documented (Jones, 1997; Jenkins, *et al.*, 1988;). It binds strongly to the bacterial cell membrane and has different actions depending on its concentration. At a low concentration, it increases cell membrane permeability causing leakage of the intracellular components, and at higher concentrations, it causes precipitation of the of bacterial cytoplasm and cell death (Jones, 1997). CHX show an affinity for the different

oral surfaces and binds reversibly to teeth, mucosa and to the pellicle and saliva. It is then slowly released from these surfaces and allows for sustained antimicrobial effects i.e. its substantivity. Salivary levels of CHX can be detected for many hours after rinsing (Jenkins, *et al.*, 1988; Jones, 1997). This could explain why in our study the total microbial count in the CHX group continued to decrease for the duration of surgical procedure. (see Figure 7). The same effect was not seen in the CHD-FA group as the microbial count reached a plateau, after the initial fifteen (15) minute decrease, and steadily increased towards the end of the surgical procedure. CHX achieves plaque inhibition because of immediate bactericidal action at the time of application and a prolonged bacteriostatic action because of adsorption to the pellicle coated enamel surface (Jenkins, *et al.*, 1988).

The exact antimicrobial mechanism of CHD-FA is unknown. However, the effect on fungal species has been studied in more detail (Sherry, *et al.*, 2012). Sherry *et al.* (2012) showed that CHD-FA displayed antifungal activity against *Candida albicans*, both in planktonic and sessile states. It was shown to act non-specifically on the cell membrane, causing disruption of the membrane thereby leading to lysis of the cytoplasmic content. No known antifungal internal mechanism was demonstrated (Sherry, *et al.*, 2012). In another study by the same author, the antibacterial activity of CHD-FA was evaluated on orally derived biofilms (Sherry, *et al.*, 2013). CHD-FA at a concentration of 0.8% was found to be highly effective at inhibiting and killing all oral isolates tested. The mechanism of action in this study indicated a nonspecific action against the bacterial cell membrane (Sherry, *et al.*, 2013). We speculate that this mechanism may be similar to that previously demonstrated for *Candida albicans*.

Another interesting fact is that the pH of the two (2) mouth rinses tested were not similar. CHD-FA had a pH of 2.27 and CHX had a pH of 5.5 (tested with BECKMAN® ϕ^{TM} 32 pH meter, BECKMAN INSTRUMENTS, INC, USA). The lowered pH could possibly explain a bacteriocidal mechanism of CHD-FA since the exact antimicrobial mechanism is unknown. However, it is known that certain oral bacteria implicated in caries are able to survive the acidic environment by utilising certain tolerance mechanisms (Svensäter, *et al.*, 1997; Fozo, *et al.*, 2004). More research is required to elucidate the exact antimicrobial mechanism of action of CHD-FA.

Chapter 7

Limitations

Although the current study showed statistically significant results, several limitations exist. These can be summarised as follows;

- The study had a small sample group only twenty (20) patient, ten (10) for the test group and ten (10) for the control group.
- Manual counting of colony forming units are error prone.
- The exact time that the microbial reduction peaked could not be evaluated unless samples are taken immediately after the rinse and possibly every minute.
- This study used human oral microbial rinses whereas the other studies used a selection of fungal and bacterial strains cultured in a laboratory. These bacterial and fungal species were standardised and adjusted to specific final working concentrations for both planktonic and sessile testing, whereas in this study there was no standardised or adjusted working concentrations.
- A significant finding in the mentioned studies was that the concentrations of CHD-FA used were lower than that used in our study. The mouth-rinse evaluated in this study had a concentration of 20 % CHD-FA and it was combined with other ingredients which included Peppermint; Menthol; Apple flavour; Sorbitol; Sucralose and a colourant (unknown). Consequently, our results cannot be directly compared to previous CHD-FA studies due to the above-mentioned differences.
- The CHX mouth rinse used in our study was 0.2% and is not commercially available and only available with prescription from government institution.

Chapter 8

Conclusions

CHD-FA could possibly be used as a pre-operative mouth-rinse for short dental procedures in patient who have either adverse reaction to CHX or have difficulties with taste or burning sensations with the use of CHX. The mechanism of action on the microbial reduction of CHD-FA was not evaluated in this study. To our knowledge no other studies have been undertaken to evaluate the substantively and the exact mode of action of CHD-FA on all oral microbes. More research is required to elucidate the mechanism of action of CHD-FA.



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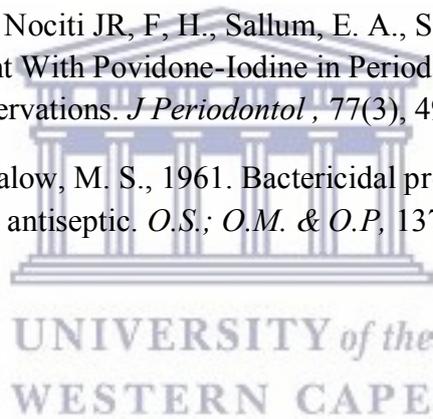
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Appendix 1

Information sheet

You are invited to participate in this study. This document will provide you with all the relevant information regarding this proposed study. Please read through all the information provided as to what is required from you as a participant and if you require clarity on any of the information provided please feel free to ask.

Title: The antimicrobial efficacy of a carbohydrate derived fulvic acid (CHD-FA) as a pre-operative mouth rinse before periodontal surgery

Principle researcher: Dr Gadija Abrahams

Position: Postgraduate student within the Department of Oral Medicine and Periodontology

Contact details:

Office number: (021) 937-3167

Email address: 9447856@myuwc.ac.za

What is the purpose of this research?

To determine if a new antimicrobial mouth rinse is able to reduce the bacteria in your saliva before surgery is performed and whether it is still active at the end of your procedure.

What would be required from you?

Saliva samples will be collected initially before any treatment is done. You will be asked to rinse with an antibacterial mouth rinse and your saliva samples will be collected two more times, 15 minutes after you have rinsed and again at the end of your surgery. All the bacteria will then be counted in all three samples and compared to see if there is any change than from the start. All samples will be destroyed at the end of the study

Why have you been invited?

You are invited to participate in this study because are 18 years or older, you have some or all of your teeth still in your mouth. You have no medical conditions or allergies to the product being tested. You are about to have some form of periodontal surgery.

Who cannot participate in this study?

If you have an underlying medical condition, are allergic to the product being tested, you are a smoker, have used antibiotics in the last 2 months or using immunosuppressive or cytotoxic medications, then you **MAY NOT** participate in this study.

Your decision to participate:

Should you decide to participate, you will be asked to sign a consent form. This indicates that you are willingly participating in the study. You can withdraw from the study time at no consequence to you and your treatment will be unaffected.

Are there any disadvantages to your participation?

There are no disadvantages to you taking part.

Are there any benefits to you taking part?

There are no benefits to you partaking in the study. It will provide us with information about the effectiveness of this new antibacterial mouth rinse.

Confidentiality

All your records will be kept confidential and all samples taken and data collected will have no information pertaining to your identity. However, your personal information may be given out if required by law.

What will happen on completion of this research study?

The results of this research will be submitted as a thesis for a specialist degree in Oral medicine and Periodontology. If approved by the university senate, the research will then be submitted for publication within a medical/dental scientific journal. The outcome of the study can be made available to you at your request.

Appendix 2

Informed consent

I..... (Full name of participant) has been informed about the research project entitled the antimicrobial efficacy of carbohydrate derived fulvic acid as a pre-operative mouth rinse before periodontal surgery.

I understand the content of this document, the purpose and procedures of this research project.

I have been given an opportunity to ask questions about the research and have had answers to my satisfaction and I am at liberty to withdraw from the project at any time, should I want to.

I declare that my participation in this study is entirely voluntary and that all samples will be destroyed appropriately at the end of the study.

If I have any further questions/concerns or queries related to the study I understand that I may contact the researcher at cell phone number 0823119582 or via e-mail 9447856@myuwc.ac.za

If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact:

DENTISTRY RESEARCH ETHICS COMMITTEE
UNIVERSITY of the
WESTERN CAPE

Francie van Zyl Drive

Private Bag X1

Tygerberg 7505

Cape Town, SOUTH AFRICA

Signature of Participant

Date

Appendix 3

Data capture Sheet

Sample bottle number:.....

Patient file number:

Time start of surgery:.....

Time end of surgery:.....

Patient details:.....

Age:.....

Gender:.....

Type of periodontal surgery:.....



Colony forming unit count at baseline

Count 1	Count 2	Count 3	Mean	multiply dilution factor
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Colony forming unit count at 15 minutes

Count 1	Count 2	Count 3	Mean	multiply dilution factor
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Colony forming unit count at end of surgery

Count 1	Count 2	Count 3	Mean	multiply dilution factor
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