IN VITRO ANTIMICROBIAL PROPERTIES OF A MOUTHRINSE CONTAINING GLYCERINE, POTASSIUM NITRATE AND SODIUM FLUORIDE

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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 Master of Science in dentistry

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DECLARATION

I, Nozizwe Ndlovu, declare that this research report is my own original work. It is being submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Dentistry. This work has not been submitted before in part or in full for any degree or examination at this or any other university.

…….
Nozizwe Ndlovu

…….day of …….2007

DEDICATION

I dedicate this to my Dad, my Mom for her loving support and encouragement. My brother, who always manages to bring lightness to even the most daunting situations. I also want to dedicate this work to my husband and my baby girl.

ABSTRACT

Introduction: Patients who have received radiation therapy due to oral cancers often present with complications such as salivary dysfunction, mucositis, soft tissue necrosis, infections and dental caries. The aim of this study was to investigate the antimicrobial properties of an experimental mouthrinse which also contains analgesic and anticaries compounds and can be used in the management of patients with oral cancers after radiation therapy.

Methods: The experimental mouthrinse contained a mixture of 30% glycerine (antimicrobial agent), 7% potassium nitrate (analgesic) and 0.025% sodium fluoride (anticaries agent). The minimal inhibitory concentration (MIC) of these ingredients was tested against *Candida albicans, Staphylococcus aureus* and *Streptococcus mutans* over 24 hours at different concentrations. MICs of commercially available mouthrinses containing chlorhexidine digluconate (Corsodyl®) and fluoride with triclosan (Plax®) were also determined using the same organisms. All mouthrinses were then tested to determine the percentage kill over 1, 2, and 3 minutes. The costs of these mouthrinses were also compared.

Results: The MICs for glycerine were 10%, 25% and 10% for *C. albicans, S. aureus* and *S. mutans* respectively. Potassium nitrate, sodium fluoride and alum did not show any antimicrobial effects. The MIC of Corsodyl® was <0.02 mg/ml for all the test organisms. The MIC for Plax was 0.02 mg/ml, <0.002 mg/ml and 0.005 mg/ml for *C. albicans, S. aureus* and *S. mutans* respectively. Combining glycerine, potassium nitrate and sodium fluoride into a mixture did not affect the antimicrobial properties of these constituents. The mixture killed 99.78%, 99.88% and 99.98% of *C. albicans*, 61.74%, 70.64% and 85.09% of *S. aureus* and 91.72%, 99.47% and 99.99% of *S. mutans* after 1, 2 and 3 minutes respectively. Two percent chlorhexidine digluconate killed 98.98%, 99.97% and 99.99% of *C. albicans*, 95.83%, 99.68% and 99.97% of *S. aureus* and 99.98%, 99.96% and 99.99% of *S. mutans* after 1, 2 and 3 minutes respectively. Plax® killed 99.99% of *C. albicans* in one minute, 99.66%, 99.99% of *S. aureus* in 1 and 2 minutes respectively; and 99.89%, 99.96% and 99.99% of *S. mutans* in 1, 2 and 3 minutes respectively. The costs of similar amounts of the experimental mouthrinse, Corsodyl® and Plax® were R5.24, R30.00 and R10.00 respectively.

Conclusion: A mouthrinse effective in relieving oral symptoms in patients receiving radiation therapy needs to show some antimicrobial activity against in particular, *C. albicans* and *S. mutans*, whilst at the same time having a palliative effect. This study has shown that the experimental mouthrinse will fulfil these requirements. The experimental mouthrinse was found to be the cheapest in comparison to Corsodyl® and Plax®.

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LIST OF ABBREVIATIONS

ALUM	Aluminium potassium sulphate
BA	Blood agar
C. albicans	*Candida albicans*
Chx	Chlorhexidine
Conc.	Concentration
Gy	Gray
cGy	Centigray
Gly	Glycerine
KNO_3	Potassium nitrate
M	Molar
mg	milligram
ml	millilitre
NaF	Sodium fluoride
NB	Nutrient broth
Nm	nanometre
%	Percentage
^oC	Degree celsius
S. aureus	*Staphylococcus aureus*
S. mutans	*Streptococcus mutans*
SAB	Sabouraudes dextrose broth
Salt	Sodium chloride
SDA	Sabouraudes dextrose agar
Solu	Solution
TA	Tryptone agar

1 LITERATURE REVIEW

1.1 Introduction

Saliva comprises 99% water and 1% organic and inorganic components. Saliva modulates the oral flora by favouring the attachment and proliferation of certain microorganisms and promoting the clearance of others. Saliva also protects the oral tissues from dessication and exogenous insults from acids and degradative enzymes (Almstahl, Wikstrom and Groenink, 2001).

The level of radiation necessary to destroy malignant cells ranges from 40-70 Gy. This dosage is delivered directly to the tumour and surrounding tissues. The salivary tissue is sensitive to radiation at a dosage greater than 30Gy (Cassolato and Turnbull, 2003) and the degree of destruction of glandular tissue is dose related. Radiation of 45Gy to the head and neck field will result in irreversible salivary gland destruction and xerostomia (Rhodus and Bereuter, 2000).

It has been reported that after just five radiation treatments at a dose of 200cGy per day, the salivary rate decreased by up to 57 per cent (Dreizen, 1977 cited by Garg and Malo, 1997).

In addition, drugs used to treat cancer can cause a thickening of saliva inducing a dry feeling and contributing to impairment of salivary function. The following complications are really the sequelae of salivary dysfunction: i) oral-pharyngeal candidiasis, ii) xerostomia, iii) mastication problems and poor

nutritional intake, iv) gingivitis and traumatic oral lesions v) dental caries and halitosis and vi) poorly fitting prostheses (Cohen-Brown and Ship, 2004).

1.1.1 Oral candidiasis

Complications of salivary gland dysfunction and the reduced immunity resulting from cancer therapy may give rise to opportunistic infections such as oral candidiasis caused by *Candida albicans* (Toljanic 1996 cited by Silverman 1999; Epstein, *et al*., 1998). Salivary histamines are the key components of the non-immune host defence system of the oral cavity (Baev, Rivetta and Vylkova, 2004). Histatin 5 kills pathogenic *Candida albicans* at physiological concentrations of 15-30μm (Xu and Levine 1991 cited by Baev *et al.* 2004), therefore when histamines are compromised as in patients where there is salivary dysfunction, C*andida albicans* is over produced. Candidiasis is usually painful and needs treatment, as untreated candidiasis can lead to oropharyngeal and systemic candidiasis.

1.1.2 Xerostomia

Xerostomia is one of the major side effects of radiation therapy for head and neck malignancies. Exposure of the major salivary glands to the field of ionising radiation induces fibrosis, fatty degeneration, acinar atrophy and cellular necrosis within glands. The serous acini appear to be more sensitive than the mucinous acini. Recovery of adequate saliva for oral comfort and function may take up to 12 months (Silverman, 1999).

The xerostomic patient has mucosa that is dry and sticky, and saliva with a stringy or foamy consistency. The normal moist appearance of the oral cavity

is often replaced with a thin, pale and cracked appearance that is more susceptible to gingivitis and bleeding. The patients experience increased problems with tasting, speaking, chewing and swallowing.

1.1.3 Mastication problems and poor nutritional intake

Normally patients present with painful mucositis, loss of taste and partial xerostomia, all of which lead to a lack of desire or frank inability to eat; this results in weight loss and malnutrition (Isenring, Capra and Bauer, 2004) and the quality of life is negatively affected (Epstein, Robertson and Emerton, 2001). A resultant weight loss tends to produce weakness, inactivity and susceptibility to infection.

1.1.4 Dental caries and halitosis

Patients who have not shown any degree of caries activity for years may develop dental decay after irradiation. The cervical areas are most affected. This condition appears to be due to the lack of saliva as well as to changes in salivary chemical composition (Epstein, *et al*., 1996). Radiation caries may develop because of a shift in oral flora to a more cariogenic flora (*S. mutans*) or because of a decreased clearance of carbohydrates and acids, and/or a reduced ability to buffer acids.

1.2 Mucositis and soft tissue necrosis

Most cancer therapies are designed to act on rapidly reproducing cells. The cells that line the gastrointestinal tract from the mouth to the rectum are especially vulnerable (Velez, Tamara & Mintz, 2004). Mucositis is an early

effect of radiation and results from mitotic death of the basal cells of the oral epithelium. It usually appears two weeks after initiation of radiotherapy. Oral mucositis presents with erythema accompanied by dryness, pain and burning symptoms. Soft tissue necrosis usually presents itself as ulceration of the mucosa, is usually painful and optimal hygiene is required.

1.3 Treatment currently prescribed

` After briefly looking at the complications that are experienced by patients that have undergone radiation of the head and neck to treat head and neck cancer, the treatment modalities that are available will be reviewed.

Table 1.1 shows therapies that have been used in the treatment of radiation-induced and chemotherapy-induced mucositis, though some reports demonstrate little evidence supporting the effectiveness of the corresponding interventions (Velez, *et al*. 2004).

Table 1.1
Therapies that deal with specific problems associated with head and neck radiotherapy

Therapy	Dose	Reference
1. Saliva production		
Pilocarpine hydrochloride	5 mg tablet 3 or 4 times per day for 90 days	Cohen-Brown and Ship, 2004; Cassolato and Turnbull, 2003; Porter, Scully and Hegarty 2004;
2. Saliva substitutes		
Buffered solution of glycerine and water	20ml of 4% sol of methyl cellulose, 10ml of glycerine and 1 drop of lemon oil	Cassolato and Turnbull, 2003
3. Mucosal coating agents		
Milk of magnesia		Andrews and Griffiths 2001; Garg and Malo, 1997;
4. Antifungal agents		
Systemic-Fluconazole Ketoconazole Topical - Nystatin-oral suspension Nystatin-lozenge	100mg suspension, rinse then swallow daily 200mg suspension 100,000units/ml, rinse with 5ml for 2min then swallow 4x/day Dissolve 1 lozenge in the mouth 5x/day	Epstein, Gorsky and Caldwell, 2002 Silverman, 1999 Andrews and Griffiths, 2001 Cohen-Brown and Ship, 2004
5. Antibacterial agents		
Chlorhexidine		Hancock, Epstein and Sadler, 2003
6. Topical anaesthetic or analgesics		
Benzydamine hydrochloride.	0.15% oral rinse 15ml every 2 hr and spit	Scully, Epstein and Sonis, 2004; Velez *et al*., 2004
7. Fluoride		
Sodium flouride	1.1% sodium fluoride Put in trays for 5-10 min-spit out excess	Garg and Malo, 1997

- ***Sialogogues:*** Pilocarpine is the only agent approved by the FDA for radiation-induced xerostomia (Chambers, Garden and Kies, 2004).
- ***Saliva substitues:*** Commercially produced artificial saliva can be used but Visch, Gravenmade and Schaub (1986) showed that about one-third of the post-radiation patients did not benefit from saliva substitutes. Also it has been shown that lemon-based saliva substitutes are potentially erosive to enamel when used frequently (Smith *et al*. 2001 cited by Cassolato and Turnbull, 2003).
- ***Antifungal medication:*** Topical therapy (Nystatin, Miconazole, and Clotrimazole) is generally effective in controlling low-grade uncomplicated mucosal candidiasis. In cases of severe oral-pharyngeal candida infection, topical therapy in conjuction with systemic therapy may ensure a lower systemic dose and shorten the duration of the systemic antifungal therapy (Epstein, 2002).
- ***Chlohexidine:*** Chlorhexidine is a potent antimicrobial agent effective against both gram-negative and gram-positive bacteria and fungal organisms but a variety of studies (Feretti *et al*. 1990 cited by Andrews and Griffiths 2001; Epstein, 1992; Dodd, Larson and Dibble 1996; and Pitten *et a*l. 2003) have shown that there was no difference in oral mucositis between the control and chlorhexidine groups of high dose radiotherapy patients. It is thought that the chlorhexidine molecule binds to the negatively charged salivary mucins or glycoproteins rather than directly to the epithelial tissues. The rapid development of xerostomia in radiotherapy patients deprives the oral epithelium of its usual salivary fluid coating, thereby diminishing the mucosal protective effect of chlorhexidine.

- ***Fluoride treatment:*** There is no one universally accepted protocol for the management of radiation caries, however the importance of fluoride is well recognised. Recommended fluoride preparations contain 0.4 per cent stannous fluoride, 1.1 per cent sodium fluoride, 1.23 per cent sodium fluoride or 1.23 per cent phosphate fluoride. Patients are instructed to spread several drops of the fluoride gel into customised trays. The tray is then seated in the mouth and left in place for 5-10 minutes (Andrews and Griffiths, 2001). Patients are encouraged not to drink or eat for one hour, so compliance can be a problem, as reported by Epstein, van der Meij and Emerton (1995) when only 43 per cent of patients reported using fluoride in custom trays daily.

Oral care protocols should strive to maintain the integrity of the oral mucosa and lips, prevent caries and periodontal disease, alleviate oral discomfort and prevent or treat infectious complications. The oral hygiene practices that have been advocated by various authors are largely empirically based and the optimal frequency of cleansing and mouth washing is questionable (Andrews and Griffiths, 2001). Visch *et al.* (1986) concluded that about one-third of post-radiation patients gained no benefit from salivary substitutes. To prevent or at least minimize post-radiation complications, oral hygiene must be maximal and a mouthrinse is essential.

1.4 The use of Mouthrinses

1.4.1 Efficacy

The use of mouthrinse has been indicated in a number of situations, such as as an adjunct to mechanical control of plaque build up (Quirynen *et al*. 1999); as a secondary prevention measure after oral surgery including periodontal therapy (Chandu *et a*l. 2002); and as a plaque growth inhibitor (Charles *et al*. 2004).

Antiseptic mouthrinses have been broadly classified by Newbrun (1985) cited by Mandel (1988) in the following manner: a) phenolic compounds, b) quaternary ammonium compounds, c) oxygenating agents d) Bis-biguanides and e) antibiotics.

An essential oil mouthrinse which is a mixture of thymol, eucalyptol, menthol and methylsalicylate has been shown to be more effective than an amine (stannous fluoride) mouthrinse in inhibiting the development of supragingival plaque over a 4-day period *in vitro* (Mandel 1988, Pan *et al*. 1999). A 4-day plaque regrowth study compared the efficacy of a triclosan mouthrinse to that of an essential oil when the allocated rinse was the only method of oral hygiene, and found that the essential oil rinse produced a plaque reduction of 50%, whilst the triclosan rinse produced a 45% reduction (Addy and Newcombe 1997).

A meta-analysis of six-month studies of antiplaque and antigingivitis agents showed that essential oil and chlorhexidine mouthrinses were effective as both antiplaque and antigingivitis agents (Gunsolley, 2006).

Epstein, *et al*. (2002) studied the effect of fluconazole mouthrinses on oral candidiasis in postirradiation and transplant patients. They found complete symptomatic and clinical relief in 94% of the patients. They concluded that an oral rinse with fluconazole mouthrinse may be useful to manage patients with dry mouth and those who have difficulties in swallowing due to candidiasis.

1.4.2 Chemicals used in mouthrinses

1.4.2.1 Alum (Aluminium potassium sulphate)

A number of investigations have been conducted to study the effect of aluminium salts on human oral flora and dental plaque. In 1940 Henke (cited by Kleber and Putt, 1984) showed that 0.04% aluminium acetate and 0.25% aluminium formate decreased the amount of acid production in a saliva-sucrose mixture by 43%. Kleber *et al*. (1996) evaluated the effect of an alum mouthrinse on dental caries incidence in caries-susceptible children. Their study demonstrated clinically that a daily rinse with alum mouthrinse inhibited dental caries development at least as effectively as home use of a fluoride dentifrice.

1.4.2.2 Glycerine

Some patients who have undergone radiation therapy may have developed a lowered salivary output and the quality of saliva may also have changed. Glycerine is used both as an humectant and as an antimicrobial agent. Kinnunen and Koskela (1991) showed that 10% hexylene glycol killed *Candida albicans, Staphylococcus aureas* and *Streptococcus pyogenes in vitro.*

1.4.2.3 Salt (NaCl)

Salt is known to have some antifungal effect. Fan, Whitway and Shen (2005) showed that the central component of the osmoregulation pathway in *Candida albicans* is MAP Kinase and salt has been shown to interrupt that pathway. Saline solution mouthrinses are safe and economical and have been used in cancer populations (Daeffler 1981, Seglman 1977 cited by Dodd *et al*. 2003).

1.4.2.4 Potassium nitrate

Radiation therapy causes cell death not only of cancerous lesion but also of the surrounding oral tissues. Toxic effects including cell death can be blocked by an elevated extra-cellular calcium or by an elevated external potassium (Baev *et al.* 2004).

Potassium nitrate promotes pain control and healing by changing the resting membrane potential of nerves in, for example an ulcer, to zero or positive, preventing an action potential from taking place and inhibiting these nerves from emitting and conducting pain; this in turn inhibits the autonomic nerves from initiating an inflammatory tissue response. The classic inflammatory response of histamines, bradykines, leukotrines, prostaglandins and oedematous fluid is thus not stimulated to bring inflammatory components to the ulcer lesion (Hodosh, Hodosh and Hodosh, 2004).

1.4.2.5 Sodium fluoride

Fluoride has protective effects on enamel by decreasing demineralization induced by *S. mutans* and by inhibition of bacterial metabolism (Van Loveren,

Buijs and ten Cate, 1993). A study to evaluate the effectiveness of topical fluoride in reducing *S. mutans* in healthy populations concluded that a daily rinse with 0.025% sodium fluoride provided enamel protection against *S. mutans* (Inaba *et al*. 2002). Epstein *et al*. (1998) studied the relationship between fluoride, cariogenic oral flora and salivary flow rate during radiation therapy. They found that cariogenic flora were suppressed when daily topical 1.1% neutral sodium fluoride was used.

1.4.2.6 Triclosan

Triclosan is a broad spectrum antimicrobial agent developed for use in oral products. It is effective against both gram negative and gram positive bacteria (Panagakos et al. 2005; Xu *et al*. 2005). Triclosan is normally recommended for controlling plaque (Pizzo *et al*. 2006) and as an anti-inflammatory agent (Wara-aswapati *et al*. 2004).

1.4.2.7 Chlorhexidine digluconate

Chlorhexidine digluconate is normally given in a concentration of 0.2%. Various studies (McGaw and Belch, 1985; Epstein *et al*. 1992) showed that chlorhexidine gluconate can control oral candidiasis in cancer patients. This mouthrinse is not ideal since it irritates the mucosa and discolours the tongue and teeth especially the interproximal surfaces (Ernst, Prockl and Willershausen, 1998). It also affects the taste quality and intensity of sodium chloride, and the taste quality of sucrose (Helms *et al*. 1995). Pitten *et al.* (2003) showed that cancer patients with chemotherapy-induced leukopenia did not benefit from a chlorhexidine based mouthrinse, instead they developed

severe mucositis when compared to the control group. There was a clear microbial count reduction in the chlorhexidine based group as compared with the control group.

1.5 Summary and conclusions

The optimal care of many patients with oral tumours is a multidisciplinary effort that combines surgery, radiation therapy and or chemotherapy; postoperatively it may involve oral surgeons, periodontists and prosthodontists. Hille, Shear and Sitas (1996) have shown that in South Africa 3.4 per cent of all patients diagnosed with cancer had oral cancer. The gender distribution was 1.8 per cent for females and 5.0 per cent for males. From data collected from Johannesburg General Hospital there were at least 171 patients in 2001 and 161 patients in 2002 who received radiation, chemotherapy, surgery or surgery together with radiation to treat oral carcinoma. More recent data are unavailable.

Patients who have received radiation therapy present with the following major complications: salivary dysfunction; mucositis, soft tissue necrosis; oral infection; and dental caries (figure 1.1). The Prosthodontist normally is called upon to provide obturators and partial/complete dentures so that patients are able to speak or swallow properly while experiencing these complications. At Wits Dental Hospital patients are normally referred from the Oncology unit, JHB hospital. On average 5 patients per week are referred after having had and/or whilst receiving radiation treatment.

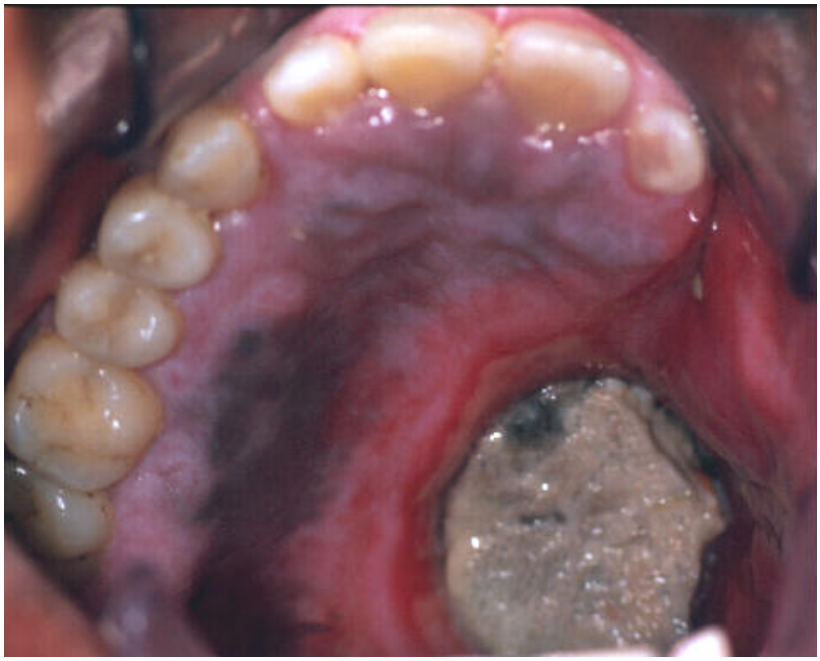

Fig 1.1: Intra-oral complications of patients undergoing radiotherapy treatment showing plaque accumulation, mucositis, buccal mucosal ulceration, and an oro-antral opening

The microbial environment of the mouth may be subjected to change under the influence of various factors such as surgical removal of neoplasm and or the consequence of wearing post-surgical prostheses with obturators. Wiekiewicz, Byzynska, Panek (2003) showed that more pathogenic bacterial flora were found on the obturators than in the post-surgical cavities.

Almastahl, *et al*. (2003) analysed and compared the oral microbial flora in radiation therapy, primary Sjögren´s and neuroleptic patients. They found a marked increased level of *Lactobaccillus* species and *Candida albicans* in the radiation therapy group. *Lactobacillus* is perpetuated by *Streptococcus mutans*. *C. albicans* is the causative agent of oral candidiasis whereas *S. mutans* and *Lactobacilli* are implicated in dental caries. Some infections in the circum-oral region are caused at least in part, by *Staphylococcus aureus*. These include angular cheilitis, osteomyelitis of the jaw and parotitis (Smith, Jackson and Begg, 2001).

Management of these patients is a major problem as there is currently no standard protocol. It is known that mouthrinses can be of assistance, but the currently available commercial mouthrinses are expensive and have side effects (Hodosh, *et al*. 2004). There is a need for a cheaper antimicrobial mouthrinse containing anticariogenic and palliative compounds. Currently patients in the Wits unit are given Chlorhexidine digluconate, Daktarin gel and Paracetamol tablets.

It was therefore decided to investigate a mouthrinse containing readily available and inexpensive agents that would in combination, have the properties desired in a mouthrinse for patients undergoing radiation therapy.

1.6 Aims and Objectives

1.6.1 Aim

The aim of the study was to investigate the antimicrobial properties of salt, glycerine, alum, and a combination of these with potassium nitrate, and to compare the combination with two commercially available mouthrinses.

1.6.2 Objectives

The objectives were to:

- Test the antimicrobial properties of each of the chemicals against *S. mutans*, *S. aureus* and *C. albicans.*
- Compare a mouthrinse containing the agents with the commercially available mouthrinses Corsodyl® containing Chlorhexidine digluconate

(Group Laboratories (Pty) Ltd. Cape Town) and Plax® containing sodium fluoride and triclosan (Colgate and Palmolive (Pty) Ltd. Boksburg)

2 METHODS AND MATERIALS

2.1 Test organisms and preparation of inoculum

Candida albicans ATCC 90028, *Staphylococcus aureus* ATCC 29213 and a clinical strain of *Streptococcus mutans* were used in this study. Sabouraudes dextrose broth (SAB) and Sabouraudes dextrose agar (SDA) were used to culture *C. albicans*. Nutrient broth (NB) and Tryptone agar (TA) were used to culture *S. aureus*. Nutrient broth and Blood agar (BA) were used to culture *S. mutans.*

Cultures were plated on an appropriate agar plate (figure 2.1) and incubated at 37^{o}C for 48 hours. *C. albicans* and *S. aureus* were incubated aerobically whereas *S. mutans* was incubated under CO_2. Strains were further subcultured to ensure purity.

Inoculums were prepared by suspending *C. albicans* in SAB, *S. aureus* and *S. mutans* in NB. The optical densities of the inoculums were adjusted to 0.2 at 620 nm which contained approximately 10^6 organisms per millilitre of suspension.

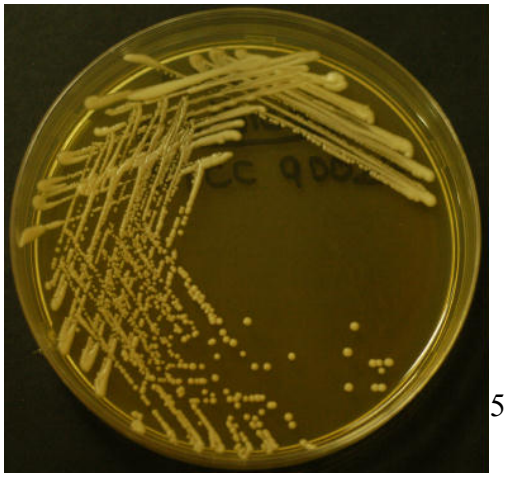

Fig 2.1 Pure culture of test organism *C. albicans*

2.2 Test compounds

Alum (Aluminium potassium sulphate), Glycerine (Hexylene glycol) and Potassium nitrate were purchased from Minema chemicals (South Africa). Sodium fluoride and Salt (Sodium chloride) were purchased from Merck chemicals (South Africa).

2.2.1 Alum (Aluminium potassium sulphate)

A stock solution of Alum was prepared by mixing 11.85g of Alum with 100ml of sterile distilled water which made 0.25M solution. Seven serial ¼ dilutions were prepared and eight concentrations including the stock solution were used in the study. In each dilution the concentration of Alum was calculated using the formula:

Mole/4 X 3 = Concentration in Moles

The final concentrations in each dilution are listed in table 2.1.

Table 2.1 Test concentrations of Alum

	1	2	3	4	5	6	7	8
Alum	Stock solution	3ml of stock + 1ml H_2O	3ml of conc. 2 + 1ml H_2O	3ml of conc. 3 + 1ml H_2O	3ml of conc. 4 + 1ml H_2O	3ml of conc. 5 + 1ml H_2O	3ml of conc. 6 + 1ml H_2O	3ml of conc. 7 + 1ml H_2O
Molar	0.25	0.188	0.141	0.106	0.079	0.059	0.044	0.033

2.2.2 Glycerine (Hexylene glycol)

Various percentage solutions of glycerine were prepared by mixing it with sterile distilled water. Eight concentrations including the stock solution were used in the study. The concentrations are shown in table 2.2.

Table 2.2 Test concentrations of glycerine

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Glycerine	Stock Solution	4ml stock + 1ml H_2O	3.5ml stock + 2.5ml H_2O	3ml stock + 2ml H_2O	2.5ml stock + 2.5ml H_2O	2ml stock + 3ml H_2O	1.5ml stock + 3.5ml H_2O	1ml stock + 4ml H_2O
Percentage	100%	80%	70%	60%	50%	40%	30%	20%

2.2.3 Sodium Chloride

A stock solution of sodium chloride was prepared by mixing 36.1g with 100ml of sterile distilled water which made a 6M solution. Seven serial ¼ dilutions were prepared and eight concentrations including the stock solution were used. In each dilution the concentration of sodium chloride was calculated using the formula: Mole/4 X 3 = Concentration in Moles

The final concentrations in each dilution are listed in table 2.3.

Table 2.3 Test concentrations of Sodium Chloride

	1	2	3	4	5	6	7	8
	Stock solution	3ml of stock + 1ml H_2O	3ml of conc. 2 + 1ml H_2O	3ml of conc. 3 + 1ml H_2O	3ml of conc. 4 + 1ml H_2O	3ml of conc. 5 + 1ml H_2O	3ml of conc. 6 + 1ml H_2O	3ml of conc. 7 + 1ml H_2O
Molar	6	4.5	3.375	2.531	1.898	1.424	1.068	0.801

2.2.4 Potassium nitrate (KNO_3)

A stock solution of Potassium nitrate was prepared by mixing 7g of KNO_3 with 100ml of sterile distilled water to make a 7% solution. Seven serial ½ dilutions were prepared, and eight concentrations including the stock solution were used in the study. In each dilution the concentration of Potassium nitrate was calculated.

The final concentrations in each dilution are listed in table 2.4.

Table 2.4 Test concentrations of Potassium nitrate

	1	2	3	4	5	6	7	8
	Stock solution	3ml of stock + 3ml H_2O	3ml of conc. 2 + 3ml H_2O	3ml of conc. 3 + 3ml H_2O	3ml of conc. 4 + 3ml H_2O	3ml of conc. 5 + 3ml H_2O	3ml of conc. 6 + 3ml H_2O	3ml of conc. 7 + 3ml H_2O
Percentage	7	3.5	1.75	0.875	0.438	0.219	0.109	0.054

2.2.5 Sodium Fluoride (NaF)

A stock solution of Sodium Fluoride was prepared by mixing 0.025g with 100ml of sterile distilled water which made a 0.025% solution. Seven serial ½ dilutions of 0.025% were prepared, and eight concentrations including the stock solution were used.

The final concentrations in each dilution are listed in table 2.5.

Table 2.5 Test concentrations of Sodium fluoride

	1	2	3	4	5	6	7	8
	Stock solution	3ml of stock + 3ml H_2O	3ml of conc. 2 + 3ml H_2O	3ml of conc. 3 + 3ml H_2O	3ml of conc. 4 + 3ml H_2O	3ml of conc. 5 + 3ml H_2O	3ml of conc. 6 + 3ml H_2O	3ml of conc. 7 + 3ml H_2O
Percentage	0.025	0.012	0.006	0.003	0.001	0.0008	0.0004	0.0002

2.2.6 Corsodyl (Chlorhexidine digluconate)

Seven double dilutions of commercially available Corsodyl were prepared. The concentrations of chlorhexidine digluconate in each dilution are shown in table 2.6. Eight concentrations including the stock solution were studied.

Table 2.6 Test concentrations of Corsodyl

	1	2	3	4	5	6	7	8
	Stock solu.	5ml of stock + 5ml H_2O	5ml of conc. 2 + 5ml H_2O	5ml of conc. 3 + 5ml H_2O	5ml of conc. 4 + 5ml H_2O	5ml of conc 5 + 5ml H_2O	5ml of conc. 6 + 5ml H_2O	5ml of conc. 7 + 5ml H_2O
Chlorhe-xidine digluconate mg/ml →	2	1	0.5	0.25	0.125	0.062	0.0315	0.016

2.2.7 Plax (sodium fluoride and triclosan)

Seven double dilutions of commercially available Plax were prepared. The concentrations of fluoride in each dilution are shown in table 2.7. Eight concentrations including the stock solution were studied.

Table 2.7 Test concentrations of Plax

	1	2	3	4	5	6	7	8
	Stock solu.	5ml of stock + 5ml H_2O	5ml of conc. 2 + 5ml H_2O	5ml of conc. 3 + 5ml H_2O	5ml of conc. 4 + 5ml H_2O	5ml of conc. 5 + 5ml H_2O	5ml of conc. 6 + 5ml H_2O	5ml of conc. 7 + 5ml H_2O
Fluoride mg/ml →	0.025	0.012	0.006	0.003	0.001	0.0008	0.0004	0.0002
Triclosan mg/ml →	0.03	0.015	0.008	0.004	0.002	0.001	0.0005	0.0002

2.3 Minimum inhibitory concentration (MIC) test procedure

The minimum inhibitory concentration test was done for all the chemicals using microtitre plates and the test organisms described in section 2.1, in the test concentrations described in tables 2.1 to 2.7. One hundred microlitres of each test concentration was added to 100 µl inoculum (section 2.1) and was placed into 8 wells. Control wells were prepared by adding 100 µl of inoculum to 100 µl of sterile distilled water.

The minimum concentration of glycerine that inhibited microbial growth was mixed with sodium fluoride and potassium nitrate to make up the experimental mouthrinse. 7g of Potassium nitrate, 0.025g of sodium fluoride was added into 70ml of sterile distilled water. Once the solutes were dissolved, 30ml of glycerine was added. This mixture was taken as the stock solution. Seven serial ½ dilutions were then prepared, and eight concentrations including the stock solution were used, table 2.8.

Table 2.8 Test concentrations of the experimental mouthrinse

Mixture	Stock	5ml + 5ml H_2O	5ml + 5ml H_2O	5ml + 5ml H_2O	5ml + 5ml H_2O	5ml + 5ml H_2O	5ml + 5ml H_2O	5ml + 5ml H_2O
Gly*	30	15	7.5	3.25	1.625	0.812	0.406	0.203
NaF	0.025	0.0125	0.0063	0.0032	0.0016	0.0008	0.0004	0.0002
KNO3	7	3.5	1.75	0.875	0.4375	0.2188	0.1094	0.0547

*Percentage

For the commercial mouthrinses (Corsodyl and Plax) and the experimental mouthrinse the first wells were prepared by adding 180 µl of the concentration and 20 µl of inoculum. The final test concentration in each well was calculated in the following manner:

Concentration added into the well ÷ 2 = final concentration into the well (table 2.9).

Table 2.9 Final concentrations of test chemicals into each well after adding the inoculum of *C. albicans (*in *SAB), S. aureus* and *S. mutans (in NB)* respectively.

Test chemical	**Well 1**	**Well 2**	**Well 3**	**Well 4**	**Well 5**	**Well 6**	**Well 7**	**Well 8**
Alum Inoculum	100ul 0.25M + 100ul	100ul 0.19M + 100ul	100ul 0.14M + 100ul	100ul 0.11M + 100ul	100ul 0.08M + 100ul	100ul 0.06M + 100ul	100ul 0.04M + 100ul	100ul 0.03M + 100ul
Conc*	**0.125**	**0.094**	**0.071**	**0.053**	**0.040**	**0.030**	**0.022**	**0.017**
Glycerine Inoculum	100ul 100% solu. + 100ul	100ul 80% solu. + 100ul	100ul 70% Solu. + 100ul	100ul 60% Solu. + 100ul	100ul 50% Solu. + 100ul	100ul 40% Solu. + 100ul	100ul 30% Solu. + 100ul	100ul 20% Solu. + 100ul
Conc**	**50**	**40**	**35**	**30**	**25**	**20**	**15**	**10**
Salt Inoculum	100ul 6M + 100ul	100ul 4.5M + 100ul	100ul 3.38M + 100ul	100ul 2.53M + 100ul	100ul 1.90M + 100ul	100ul 1.42M + 100ul	100ul 1.07M + 100ul	100ul 0.80M + 100ul
Conc.*	**3**	**2.25**	**1.69**	**1.27**	**0.95**	**0.71**	**0.53**	**0.40**
KNO_3 Inoculum	100ul 7% solu. + 100ul	100ul 3.5% solu. + 100ul	100ul 1.75% Solu. + 100ul	100ul 0.875% Solu + 100ul	100ul 0.438% Solu + 100ul	100ul 0.219% Solu + 100ul	100ul 0.109% Solu + 100ul	100ul 0.054% Solu + 100ul
Conc**	3.5	1.75	0.875	0.438	0.219	0.109	0.054	0.027
NaF Inoculum	100ul 0.025% solu. + 100ul	100ul 0.012% solu. + 100ul	100ul 0.006% Solu. + 100ul	100ul 0.003% Solu + 100ul	100ul 0.001% Solu + 100ul	100ul 0.0008 %Solu + 100ul	100ul 0.0004 %Solu + 100ul	100ul 0.0002 %Solu + 100ul
Conc**	**0.012**	**0.006**	**0.003**	**0.001**	**0.0008**	**0.0004**	**0.0002**	**0.0001**
Commercial (Corsodyl) Inoculum	180ul 2 mg/ml + 20ul	100ul 2 mg/ml + 100ul	100ul 1 mg/ml + 100ul	1 100ul 0.5 mg/ml + 100ul	100ul 0.25 mg/ml + 100ul	100ul 0.13 mg/ml + 100ul	100ul 0.06 mg/ml + 100ul	100ul 0.03 mg/ml + 100ul
Conc. †	**2**	**1**	**0.5**	**0.25**	**0.13**	**0.06**	**0.03**	**0.02**
Commercial Plax Fluoride Triclosan Inoculum	180ul 0.025 mg/ml 0.03 mg/ml + 20ul	100ul 0.025 mg/ml 0.03 mg/ml + 100ul	100ul 0.012 mg/ml 0.015 mg/ml + 100ul	100ul 0.006 mg/ml 0.008 mg/ml + 100ul	100ul 0.003 mg/ml 0.004 mg/ml + 100ul	100ul 0.001 mg/ml 0.002 mg/ml + 100ul	100ul 0.0008 mg/ml 0.001 mg/ml + 100ul	100ul 0.0004 mg/ml 0.0005 mg/ml + 100ul
Conc† **Fluoride** **Triclosan**	 **0.025** **0.03**	 **0.012** **0.015**	 **0.006** **0.008**	 **0.003** **0.004**	 **0.001** **0.002**	 **0.0008** **0.001**	 **0.0004** **0.0005**	 **0.0002** **0.0002**

*Molar; **Percentage; † mg/ml

The microtitre plates of *C. albicans* and *S. aureus* were incubated aerobically and *S. mutans* under CO_2 at 37° C for 24 hours. Each well was subcultured for the presence of surviving test microorganisms using the appropriate medium (section 2.1). The culture plates were further incubated at 37° C for 24 hours. The minimum dilution (concentration) with absence of any test organism was recorded as the MIC. Each experiment was repeated three times.

2.4 Time-Kill study procedure

2.4.1 The number of organisms challenged

Inoculums were prepared by suspending *C. albicans* in SAB, and *S. aureus* and *S. mutans* in NB. The exact number of organisms challenged was calculated in the following way: twenty microlitres of the inoculum was serially diluted ten fold to 10^{-1}, 10^{-2}, and 10^{-3}; 20µl was then taken from these dilutions and was plated on the appropriate agar plate. The culture plates were incubated at 37°C for 48 hours, after which colonies were counted, the number of organisms was determined and this number was taken as the challenged organisms.

2.4.2 Time-kill experimental procedure

In the time and percentage kill experiment, 5ml of the experimental mixture (containing 30% glycerine, 7g potassium nitrate, 0.025g sodium fluoride and 70ml of water), was inoculated with 100µl of the inoculum. Every 60 seconds for 3 minutes, 20µl of the inoculated test compound was removed and mixed with 20µl of the universal neutralizer. 20µl of neutralised mixture was then micro-diluted to 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} and spread onto an appropriate

medium; *C. albicans* on SAB, *S. aureus* on TA and *S. mutans* on BA. Culture plates for *C. albicans* and *S. aureus* were incubated aerobically and *S. mutans* under CO_2 at 37^oC for 48 hours. The colony count for each plate was determined and the percentage kill was calculated using the challenged organism count. The percentage of the challenged minus surviving organisms was taken as the number of microorganisms killed.

$$\text{Percentage killed} = \frac{\text{challenged organisms-surviving organisms} \times 100}{\text{Challenged organisms}}$$

The percentage kill was performed three times with all three strains.

The results of the experimental mouthrinse were compared with Corsodyl and Plax using the Chi-square test. The results of Corsodyl and Plax were also compared using the same test.

2.5 Cost comparison

The cost of the three chemicals included into the experimental mouthrinse were compared with the costs of the equivalent amounts of the commercially available mouthrinses (Corsodyl® and Plax®)

3 RESULTS

3.1 Minimum inhibitory concentration (MIC) of test compounds for *C. albicans*

The results are shown in table 3.1. Alum, Salt and KNO_3 had no effect even at the highest concentrations. Glycerine on the other hand and Corsodyl® inhibited *C. albicans* growth at all concentrations. Plax® which had a mixture of fluoride and triclosan inhibited growth at 0.001 and 0.002 mg/ml respectively.

Table 3.1 Minimum inhibitory concentration (MIC) of test compounds for *C. albicans;* + indicates growth and – indicates inhibition of growth.

Test chemicals	Test runs	Microtitre plate							
		Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7	Well 8
Alum	1	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+
Conc*		**0.125**	**0.094**	**0.071**	**0.053**	**0.04**	**0.03**	**0.022**	**0.017**
Glycerine	1	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-
Conc **		**50**	**40**	**35**	**30**	**25**	**20**	**15**	**10**
Salt	1	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+
Conc *		**3**	**2.25**	**1.69**	**1.27**	**0.95**	**0.71**	**0.53**	**0.40**
KNO_3	1	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+
Conc**		**7**	**3.5**	**1.75**	**0.875**	**0.438**	**0.219**	**0.109**	**0.055**
NaF	1	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+
Conc **		**0.025**	**0.013**	**0.006**	**0.003**	**0.002**	**0.001**	**0.0005**	**0.0003**
Corsodyl®	1	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-
Conc†		**2**	**1**	**0.5**	**0.25**	**0.125**	**0.06**	**0.032**	**0.016**
Plax®	1	-	-	-	-	-	+	+	+
	2	-	-	-	-	-	+	+	+
	3	-	-	-	-	-	+	+	+
Conc† Fluoride Triclosan		**0.025 0.03**	**0.012 0.015**	**0.006 0.008**	**0.003 0.004**	**0.001 0.002**	**0.0008 0.001**	**0.0004 0.0005**	**0.0002 0.0002**

* Molar, ** Percentage, †mg/ml

3.2 MIC of test compounds for *S. aureus*

The results on table 3.2 show that Alum inhibited the growth of *S. aureus* from a concentration of 0.094M and glycerine from 30%. Salt, KNO_3 and NaF had no antimicrobial effect on *S. aureus*. Corsodyl® and Plax® inhibited *S. aureus* at all concentrations.

Table 3.2 MIC of test compounds for *S. aureus;* +indicates growth and – indicates inhibition of growth.

Test Chemicals	Test runs	Microtitre plate							
		Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7	Well 8
Alum	1	-	-	+	+	+	+	+	+
	2	-	-	+	+	+	+	+	+
	3	-	-	+	+	+	+	+	+
Conc *		**0.125**	**0.094**	**0.071**	**0.053**	**0.04**	**0.03**	**0.022**	**0.017**
Glycerine	1	-	-	-	-	-	+	+	+
	2	-	-	-	-	+	+	+	+
	3	-	-	-	-	-	-	+	+
Conc **		**50**	**40**	**35**	**30**	**25**	**20**	**15**	**10**
Salt	1	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+
Conc *		**3**	**2.25**	**1.69**	**1.27**	**0.95**	**0.71**	**0.53**	**0.40**
KNO_3	1	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+
Conc**		**7**	**3.5**	**1.75**	**0.875**	**0.438**	**0.219**	**0.109**	**0.055**
NaF	1	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+
Conc **		**0.025**	**0.013**	**0.006**	**0.003**	**0.002**	**0.001**	**0.0005**	**0.0003**
Corsodyl®	1	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-
Conc†		**2**	**1**	**0.5**	**0.25**	**0.125**	**0.062**	**0.032**	**0.016**
Plax®	1	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-
Conc† Fluoride Triclosan		**0.025 0.03**	**0.012 0.015**	**0.006 0.008**	**0.003 0.004**	**0.001 0.002**	**0.0008 0.001**	**0.0004 0.0005**	**0.0002 0.0002**

*Molar **Percentage †mg/ml

3.3 MIC of test compounds for *S. mutans*

The results from table 3.3 show that Alum, KNO_3 and NaF had no effect on *S. mutans*. Salt inhibited the growth of *S. mutans* from a concentration of 1.69M. Glycerine and Corsodyl® inhibited the growth at all concentrations and Plax (containing fluoride and triclosan) inhibited the growth from 0.0004mg/ml and 0.0005mg/ml respectively.

Table 3.3 MIC of test compounds for *S. mutans;* +indicates growth and – indicates inhibition of growth.

Test chemicals	Test runs								
		Well 1	**Well 2**	**Well 3**	**Well 4**	**Well 5**	**Well 6**	**Well 7**	**Well 8**
Alum	1	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+
Conc *		**0.125**	**0.094**	**0.071**	**0.053**	**0.04**	**0.03**	**0.022**	**0.01**
Glycerine	1	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-
Conc **		**50**	**40**	**35**	**30**	**25**	**20**	**15**	**10**
Salt	1	-	-	+	+	+	+	+	+
	2	-	-	-	+	+	+	+	+
	3	-	-	-	+	+	+	+	+
Conc *		**3**	**2.25**	**1.69**	**1.27**	**0.95**	**0.71**	**0.53**	**0.40**
KNO_3	1	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+
Conc **		**7**	**3.5**	**1.75**	**0.875**	**0.438**	**0.219**	**0.109**	**0.055**
NaF	1	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+
Conc**		**0.025**	**0.013**	**0.006**	**0.003**	**0.002**	**0.001**	**0.0005**	**0.0003**
Corsodyl®	1	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-
Conc†		**2**	**1**	**0.5**	**0.25**	**0.125**	**0.062**	**0.032**	**0.016**
Plax ®	1	-	-	-	-	-	-	-	+
	2	-	-	-	-	-	-	-	+
	3	-	-	-	-	-	-	-	+
Conc† Fluoride Triclosan		**0.025 0.03**	**0.012 0.015**	**0.006 0.008**	**0.003 0.004**	**0.001 0.002**	**0.0008 0.001**	**0.0004 0.0005**	**0.0002 0.0002**

*Molar; **Percentage ; †mg/ml

3.4 MIC of the test compounds on the test organisms

Table 3.4 shows that Alum inhibited the growth of *C. albicans, S. aureus* and *S. mutans* at the median concentrations shown. Glycerine inhibited the growth of *C. albicans* and *S. mutans* at concentration <10%. Salt did not have antimicrobial properties against *C. albicans* and *S. aureus* but it had anitimicrobial properties against *S. mutans* at the concentration of 1.69M. KNO_3 and NaF did not have any antimicrobial properties against any of the test organisms.

Table 3.4 Median MICs of the test compounds on the test organisms

Strain	Chemical	Median (n=3)
C. albicans	Alum (M)	>0.125M
	Glycerine (%)	<10%
	Salt (M)	>3M
	KNO_3 (%)	>7%
	NaF (%)	>0.025%
S. aureus	Alum (M)	0.094M
	Glycerine (%)	25%
	Salt (M)	>3M
	KNO_3 (%)	>7%
	NaF (%)	>0.025%
S. mutans	Alum (M)	>0.125M
	Glycerine (%)	<10%
	Salt (M)	1.69M
	KNO_3 (%)	>7%
	NaF (%)	>0.025%

M= Molar
%= Percentage

3.5 The MICs of the mouthrinses on the test organisms

Table 3.5 Shows the median minimum inhibitory concentration of the experimental mouthrinse compared with the commercially available mouthrinses. These figures were identical for each compound and each test.

Table 3.5 Median MICs of the mouthrinses on test organisms

Strain	Chemical	Median (n=3)
C. albicans	Experimental mouthrinse (Glycerine** KNO_3* + +NaF*)	7.5 0.006 1.75
	Corsodyl® (mg/ml)	<0.016
	Plax – fluoride (mg/ml)- triclosan	0.001 0.002
S. aureus	Experimental mouthrinse (Glycerine** + KNO_3* +NaF*)	15 0.012 3.5
	Corsodyl® (mg/ml)	<0.016
	Plax – fluoride (mg/ml)- triclosan	<0.0002 <0.0002
S. mutans	Experimental mouthrinse (Glycerine** + KNO_3* + NaF*)	7.5 0.006 1.75
	Corsodyl® (mg/ml)	<0.016
	Plax® – fluoride (mg/ml)- triclosan	0.0004 0.0005

* Molar

** Percentage

3.6 Percentage of test organisms killed by the experimental mouthrinse and two commercially available mouthrinses.

Figures 3.1, 3.2 and 3.3 show the percentages of the test organisms killed by the experimental mouthrinse and the two commercially available mouthrinses after 1 min, 2min and 3min respectively. The statistical analysis, however, showed no significant differences between all three mouthrinses.

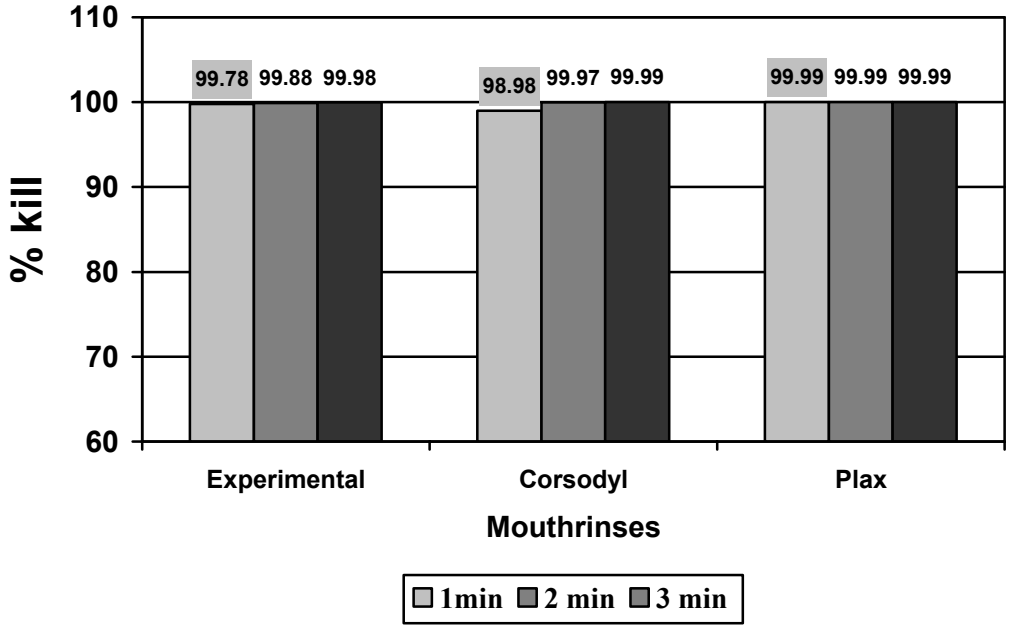

Figure 3.1 Percentage of *C. albicans* killed in 1, 2 and 3 minutes by the experimental mouthrinse, Corsodyl® and Plax®.

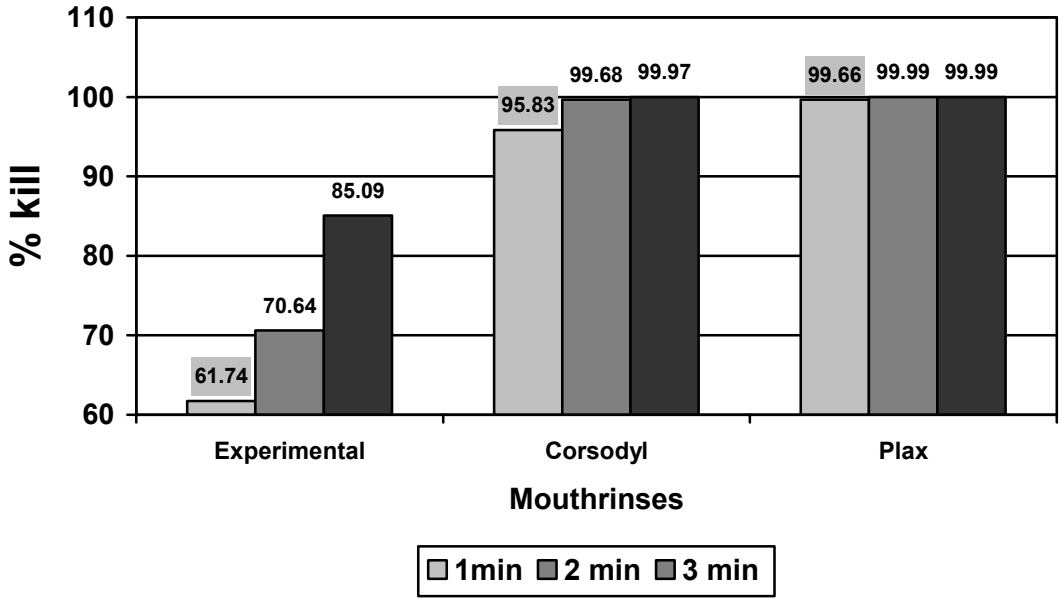

Figure 3.2 Percentage of *S. aureus* killed in 1, 2 and 3 minutes by the experimental mouthrinse, Corsodyl® and Plax®.

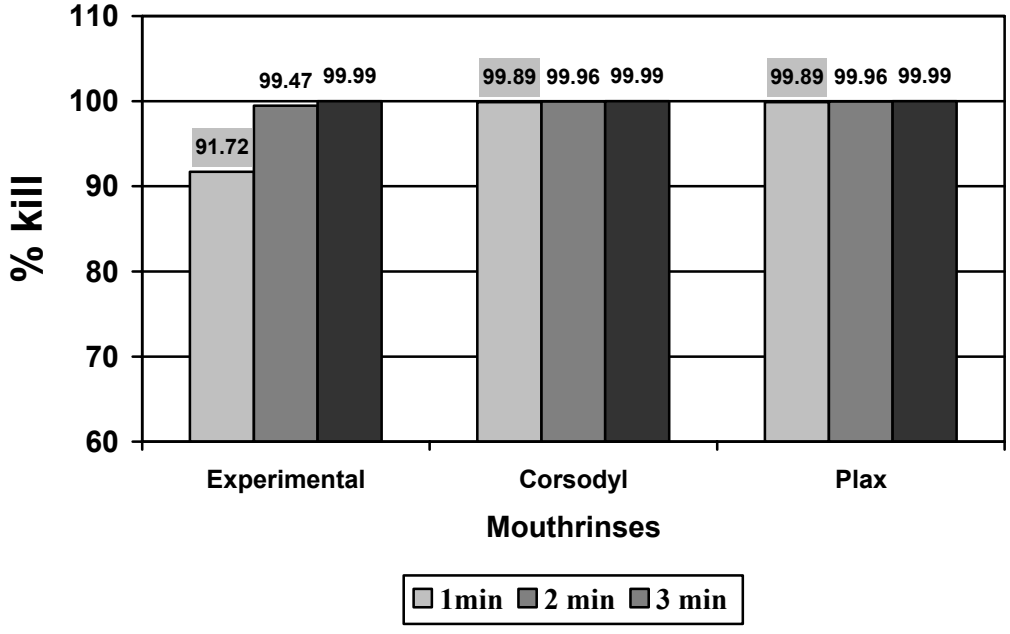

Figure 3.3 Percentage of *S. mutans* killed in 1, 2 and 3 minutes by the experimental mouthrinse, Corsodyl® and Plax®.

3.7 The chi-squared results of the experimental mouthrinse and the two commercially available mouthrinses.

The ability of the experimental mouthrinse to kill the three test organisms after 1 min, 2 min and 3 min respectively was compared to the two commercially available mouthrinses using the chi-squared test and the results showed no significant differences between the mouthrinses (Table 3.6).

Table 3.6 Comparison of percentage kill over time between mouthrinses for all organisms. All values are chi-squared results at P<0.05.

		Experimental mouthrinse	Corsodyl	Plax
Experimental Mouthrinse				
	1min		**0.09**	**0.06**
	2min		**0.18**	**0.17**
	3min		**0.66**	**0.66**
Corsodyl®	**1min**			**0.97**
	2min			**0.99**
	3min			**0.99**
Plax®	**1min**			
	2min			
	3min			

3.8 Cost comparison

Table 3.7 shows that the experimental mouthrinse was cheaper than the commercial mouthrinses. It cost R5.24/100ml including the bottle whereas Corsodyl® cost R30.00/100ml and Plax® cost R10.00/100ml.

Table 3.7 Cost comparison of experimental mouthrinses as at December, 2006

Mouthrinse	Chemical	Cost: R	Cost: R/100ml
Experimental mouthrinse	KNO3	17.50/kg	5.24
	NaF	7.77/kg	
	Glycerine	174.65/l	
Corsodyl®			30.00
Plax®			10.00

4. DISCUSSION

4.1 Selection of test organisms

C. albicans, a normal oral commensal, is the most frequently isolated yeast from oral infections. In South Africa it has been shown that 81% of HIV positive and 63% of HIV negative patients were *C. albicans* carriers (Patel, Shackleton & Coogan, 2006). The most common oral infection caused by *C. albicans* is an oral candidiasis and it affects immunocompromised patients such as those who are HIV positive, organ transplant and cancer patients, particularly those on radiation therapy.

S. aureus is a gram positive coccus, is a potential pathogen, and may be carried asymptomatically in the nasopharynx of up to 40 % of individuals. It does not usually play a significant role in intraoral infections but may cause serious infections associated with accidental or surgically induced wounds (Smith, *et al*., 2001).

S. mutans is implicated in dental caries. It is an acidogenic and aciduric oral commensal which produces acids and extracellular polysaccharides from fermentable carbohydrates. One of the problems encountered by cancer patients with radiation therapy is the development of dental caries. Epstein, McBride and Stevenson-Moore (1991) showed that 66% of their patients treated with radiation therapy had high levels of *S. mutans.* Meng, Liu and Peng, (2005) showed that there is a persistence of *S. mutans* in nasopharyngeal carcinoma patients after radiotherapy, mostly due to the poor oral hygiene

prevalent in cancer patients. This poor oral hygiene may be due to painful open wounds, and this leads to an increase in *S. mutans* levels.

4.2 *C. albicans* and the test chemicals

C. albicans was eliminated by glycerine at a concentration of 10% (table 3.1). Hexylene glycol has been shown to have antifungal properties (Kinnunen and Koskela, 1991), is nontoxic, water soluble, and can be used as an humectant (Hodosh et al. 2004) as well as an antimicrobial compound.

Previous studies have shown that Alum can prevent dental caries (Kleber, et al. 1996) by increasing the salivary pH thus neutralising acids produced by bacteria and acidogenic food. In this study the anticandida activity was investigated, and it was found that there was none.

Salt is known to aid in the formation of granulation tissue and to promote healing (Daeffler, 1981 cited by Dodd et al. 2003). Incorporation of this compound in the experimental mouthrinse was for its mucolytic properties and not as an anticandida compound, so the lack of any anticandida activity was not unexpected.

Similarly potassium nitrate also displayed no anticandida activity, but its inclusion was as an analgesic compound.

Sodium fluoride did not show any anticandida activity. It is known to have antifungal activity at very high concentrations such as 10-20 mg/ml (Ates,

Akdeniz and Sen, 2005) whereas in this study only 0.25mg/ml was used, as a fluoride source for the prevention of dental caries and not as an antifungal agent.

Corsodyl® and Plax® showed good anticandida activity. Corsodyl is commonly used for the treatment of oral candidiasis together with antifungal agents. Its antifungal activity is well established (McGaw and Belch, 1985; Epstein, 1992). Plax, although used mainly as an antiplaque agent, also showed antifungal activity in this study. Plax is a mixture of NaF and triclosan. As the study has shown that NaF did not have any antifungal activity, it is probable that triclosan was responsible. This is confirmed by studies that have shown that triclosan has both antifungal and antibacterial properties (Giuliana et al. 1997).

4.3 *S. aureus* and the test chemicals

Sodium chloride, KNO_3 and NaF did not have any effect on *S. aureus* (table 3.2). These chemicals were included in this study for other purposes as discussed above. Alum on the other hand killed *S. aureus* even at a concentration as low as 0.094 M. A higher concentration of glycerine was required to kill *S. aureus* (25%) compared to *C. albicans* (10%).

Corsodyl® and Plax® gave similar results against for *C. albicans*. Both compounds proved to have good antifungal and antibacterial properties.

4.4 *S. mutans* and the test chemicals

S. mutans proved to be the easiest organism to kill compared with the other two test organisms (table 3.3). Alum and salt even at a very low concentration (0.01M and 1.69M respectively) eliminated *S. mutans*. Potassium nitrate and NaF once again had no effect. Although Plax showed anti *S. mutans* activity, NaF alone did not kill *S. mutans*. Glycerine proved to be the best chemical tested for the elimination of *S. mutans* doing so at a concentration as low as 10%.

Corsodyl® and Plax® once again showed good anti *S. mutans* activity. Chlorhexidine gluconate is an extensively used member of the bisbiguanide group of antimicrobial compounds.

4.5 Selection of chemicals for the experimental mouthrinse

From the above results and from the literature, it was clear that certain chemicals can be used as antimicrobial, lubricant, anticaries, mucolytic, or analgesic agents.

4.5.1 Alum

It was decided to exclude alum from the experimental mouthrinse for a variety of reasons. Kleber and Putt (1994) showed that alum reduced enamel dissolution from approximately 10% after a single 5 min exposure to nearly 90% after a total cumulative treatment time of 100 min. It is not practical to expect patients to hold a mouthrinse in the mouth for 5 minutes at a time. Olmez et al. (1998) showed that the mean plaque and salivary levels of *S.*

mutans were significantly reduced after two weeks. In this study Alum eliminated *S. aureus* and *S. mutans* but so did glycerine. Alum crystallized out when it was added to a solution that had Potassium nitrate and hexylene glycol. The other reason that led to the decision to leave out alum is that the study carried out by Putt and Kebler (1986) showed that alum solution was most effective when used in concentrations greater than 0.005mol/L in the pH range 3.5-4.5 and for exposure greater than 4 min, whereas the recommended exposure time of the experimental mouthrinse is going to be 1 min; it was considered impractical to assume patient compliance for a 4-minute rinse.

4.5.2 Glycerine

Glycerine is used as an humectant and as an antimicrobial agent. Kinnunen and Koskela (1991) showed that 10% hexylene glycol killed *C. albicans, S.aureus* and *S. pyogenes.* In this study similar results were obtained, and so glycerine was selected as a main compound for the experimental mouthrinse.

4.5.3 Sodium Chloride

Salt displayed minimal antibacterial property. It was meant to be used for its mucolytic property and enhancement of taste. Saline solution is also thought to aid in the formation of granulation tissue and to promote healing (Dodd, Dibble and Miaskowski, 2000). However, salt does not mix with hexylene glycol and potassium nitrate; therefore it was excluded from the experimental mouthrinse.

4.5.4 Potassium nitrate

Cancer patients also develop soft tissue necrosis, oral infections and painful mucositis, all of which cause difficulty in talking and eating. An analgesic compound incorporated into their oral care regimen would therefore be beneficial. In this study KNO_3 did not show any antimicrobial activity but it is known to have an analgesic effect and therefore it was decided to incorporate it into the experimental mouthrinse. Gillam et al. (1996) showed that rinsing twice daily with a 3% KNO_3/Silica/NaF mouthrinse compared with Silica/NaF control resulted in reduced discomfort from cervical dentine sensitivity when evaluated by tactile and thermal stimuli.

Potassium nitrate has also been used in a gel form to treat aphthous ulcers (Hodosh, et al., 2004). It is also possible that the compliance of using NaF is decreased because of the pain that is caused by open wounds as inserting custom trays in the mouth with open wounds can be extremely painful, therefore incorporating KNO_3 will also help to solve this problem.

4.5.5 Sodium Fluoride

The use of topical fluoride to reduce caries has become the standard of care for patients undergoing radiotherapy, but compliance has been reported as generally being poor because the patient is expected to apply it using trays which are supposed to be left sometimes for as long as five minutes (Andrews and Griffiths, 2001; Epstein, et al. 1996). Sodium fluoride if incorporated into a mouthrinse will be highly beneficial. Although sodium fluoride did not show any antimicrobial properties, it promotes remineralisation of the enamel,

(Marsh and Martin, 1999) decreases the rate of sugar uptake and acid production by plaque bacteria, as well as reduces extracellular polysaccharide production. This means that sodium fluoride can reduce development of dental caries in many ways without showing any antimicrobial effects. An effective concentration (0.025 mg/ml) of sodium fluoride was therefore incorporated into the experimental mouthrinse.

4.6 The experimental mouthrinse

The experimental mouthrinse contained glycerine as an antimicrobial and humectant, potassium nitrate as an analgesic and sodium fluoride as a caries controlling agent. Mixing these three ingredients did not affect the antimicrobial property of glycerine (table 3.6). MIC results of this experimental mouthrinse showed that even if it is diluted 4 to 8 times it will be effective against all three test organisms.

The efficacy of a mouthrinse depends on the effective concentration of its active ingredients, the effectiveness of all the compounds and the time required to achieve the target factors. In this study the first phase established the first two factors and the second phase established the time required to eliminate the test microorganisms. This study was carried out for one, two, and three minutes to examine the percentage of test organisms killed. However, results of one minute are most relevant because patients normally do not hold the solution in their mouth for long. If they are advised to hold the solution in their mouths for a longer time period, the patient compliance will not be good. In one minute, the experimental mouthrinse was most effective

against *C. albicans* (99.78% kill), which is the most common causative agent of oral candidiasis, especially in cancer patients who undergo radiation therapy.

Sixty two percent of *S. aureus* was killed within a minute of exposure to the experimental mouthrinse. Large numbers of *S. aureus* are found in the saliva of healthy older subjects (Samaranayake, 2002) and in the oral cavity it can infect open wounds. The experimental mouthrinse has the capacity to reduce the numbers of *S. aureus* which can therefore reduce the chances of *S. aureus* infection in cancer patients.

Ninety two percent of *S. mutans* was killed within 1 minute of exposure to the experimental mouthrinse. Reduction in the number of these bacteria in the mouth will reduce plaque formation and hence caries formation. Cancer patients are often in pain and their oral hygiene is not adequate. Reduction of saliva can also make them more susceptible to caries formation.

The experimental mouthrinse demonstrated an appropriate antimicrobial effect, has the capacity to alleviate pain from open mucosa lesions and to decrease carious lesion formation. It will certainly be easier for clinicians who have to construct obturators and dentures for these patients to take impressions without causing too much pain or traumatising the already traumatised and infected mucosa**.** The speedy recovery of the abused tissues and minimising of infection is of paramount importance

4.7 Comparison of the experimental mouthrinse to the commercial mouthrinses

4.7.1 Efficacy

The results showed that after one minute of exposure to both the experimental and commercial mouthrinses, most of the challanged *C. albicans* were killed (Figure 3.1). Corsodyl® killed slightly less *C. albicans* when compared to the experimental mouthrinse and Plax®. In two and three minutes the percentage of killed *C. albicans* was similar for all three mouthrinses. When *S. aureus* was exposed to all three mouthrinses for 1 minute, 2 minutes and three minutes, the percentage killed by the experimental mouthrinse as compared to the two commercially available mouthrinses was far less. Both Corsodyl® and Plax® showed similar results. When *S. mutans* was exposed to the experimental mouthrinse for 1 minute, the percentage kill was lower than the commercial mouthrinses, however after 2 minutes and 3 minutes it killed a similar percentage compared to the commercially available mouthrinses. Both commercially available mouthrinses do not have pain relieving compounds and these mouthrinses can not act as an humectant. Even though the percentage kill of *S. aureus* was far less than both the commercially available mouthrinses after 1 min, the chi squared results showed no statistically significant difference.

4.7.2 Cost

Chlorhexidine digluconate cannot be obtained over the counter without a prescription. Plax® on the other hand can be obtained without a prescription but may be too expensive for the lower income earners of the community. The experimental mouthrinse costs R5.24 for a bottle of 100ml whereas Corsodyl® costs R30.00 and Plax® costs R10.00. It contains an analgesic that both commercial mouthrinses do not have. Separate analgesics are usually prescribed for patients with open wounds thus increasing the cost of treating such patients. The experimental mouthrinse incorporates an analgesic which may obviate the need for additional medication thus further reducing the costs.

5 CONCLUSION

A mouthrinse effective in relieving oral symptoms in patients receiving radiation therapy needs to show some antimicrobial activity against *C. albicans*, *S. mutans* and *S. aureus,* whilst at the same time having an humectant and palliative effect. It would seem that the experimental mouthrinse shows promise in fulfilling these requirements.

Sucralose could be added to enhance the taste, especially for young children. Sucralose is 600 times sweeter than sucrose. It provides no energy, it is poorly absorbed and it is excreted unchanged from the body. It is also heat stable (ADA report, 2004). Bowen, Young and Pearson (1990) showed that sucralose is non-cariogenic in rats. The mouthrinse efficacy will then have to be tested with added sucralose to test whether sucralose will affects its antimicrobial properties. If it does not, then cytotoxicity tests will also be required.

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