DETERMINING THE ANTIBACTERIAL STRENGTH OF COMMERCIALLY AVAILABLE MOUTHRINSES IN UGANDA (KAMPALA) AGAINST Streptococcus mutans

By

MUGANZI EMMY

15/U/7825/PS

SUPERVISOR: Mrs. TEDDY TINDYEBWA

A RESEARCH REPORT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE IN BIOTECHNOLOGY OF MAKERERE UNIVERSITY

March, 2018
DECLARATION

Muganzo Enayo confirm that the work for the following term paper with the title: "DETERMINING THE ANTIBACTERIAL STRENGTH OF COMMERCIALLY AVAILABLE MOUTHBRINES IN UGANDA (KAMPALA) AGAINST Streptococcus mutans" was solely undertaken by myself and that no help was provided from other sources as those allowed. All sections of the paper that use quotes or describe an argument or concept developed by another author have been referenced, including all secondary literature used, to show that this material has been adopted to support my work.

STUDENT
Date 6th Aug 2018 Signature

SUPERVISOR APPROVAL
Date 10th Oct 2018 Signature
ACKNOWLEDGEMENT

I would like to express my sincere gratitude to my supervisor, Ms. Teddy T. who enlightened me with her knowledge, experience and support during this study. I would like thank the entire staff at the department of Plant sciences, Microbiology and Biotechnology, College of Natural sciences, Makerere University.
DEDICATION

I dedicate this to my dear mother Ms. Kalimba Aujeni may God continue to bless you abundantly.
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List of abbreviations

WHO - world health organization

S.mutans - *Streptococcus mutans*

MS - *Mitis salivarius*

MH - Mueller Hinton
EXECUTIVE SUMMARY

This in-vitro experiment was conducted to test the antibacterial activity of different mouthrinses commonly used in the urban centers, on the dental bacteria *Streptococcus mutans*. 6 different brands of mouthrinses were used namely; Toothex, Nana herbal, Tyzorin, Tooth Heal, Mannyo Teethmix and Tooche drops. The antimicrobial activity on *Streptococcus mutans* was determined by using Agar well diffusion method. The study proved that a regular mouthrinse, D was more effective than the herbal mouthrinses. The variations in antimicrobial activity of tested mouthrinses were run simultaneously against ciprofloxacin. Their activity is directly related to the presence of antimicrobial compositions in their formulations. The antimicrobial composition of the different brands determined by their effectiveness provides a better choice to the individual as a way of controlling oral biofilms and other diseases related to the tested organism.
CHAPTER ONE

INTRODUCTION

1.1 Background

Dental decay is common among the young people as it could be five times as common as asthma and seven times as common as hay fever (U.S department of health and human services, 2000). The industrial revolution made cane sugar and refined flour easily obtained, thus tooth decay and caries have still prevailed in the human population (Suddick & Harris 1990). However, Petersen (2003); Touge-Decker & Van Loveren (2000) argue that poor exposure to effective toothpastes and increasing consumption of sugar are the main cause of dental caries in adults, also affected many of the school going children. Policy makers must therefore acknowledge that oral health problems pose a serious concern to the public and oral health goals must be included in the health agenda.

Today over 3 billion people in the whole world are suffering the untreated oral conditions. Deshpande et al. (2014) ague that Microorganisms within biofilms normally occur naturally in the oral environment. These microorganisms adhere to the surface to form a complex which consists of cells, water, intracellular and extracellular material (Sutherland 2001). Dental plaque forms upon their overgrowth (i.e. biofilms). It’s the accumulation of this plaque that leads to decay/caries after interaction with cariogenic bacteria.

![Figure 1: effect of acid production on teeth](image)

*Figure 1: effect of acid production on teeth*
Therefore, dental caries will always depend on biofilms which contain the different microorganisms that feed on dietary carbohydrates. Sucrose is therefore a key factor in initiating and developing caries since it is fermentable by these microorganisms. Newbrun 1967; Yoo et al. (2007) state that sucrose is a requirement for the synthesis of intracellular and extracellular polysaccharides of bacteria in dental plaque. Deshpande et al. (2014) argue that there is need to control these biofilms to prevent overgrowth of oral microbiota that in return promotes plaque development, caries and periodontal diseases. McCracken & Cawson 1983 stated that although \textit{S.mutans} has been linked to plaque and dental caries, it’s still uncertain whether it’s the primary causative agent. However, this coexists in the biofilm micro community with other bacterial strains, fungi and viruses.

Munro & Grap (2004), state that some other microorganisms can move and colonize other body tissue due to dental plaque. These can be \textit{S.aureus, S.epidermis & E.coli}. These can be incorporated in the oral environment from the skin, Nasopharynx or fecal-oral route, and can still cause pathogenesis (Foster 1996; Kamal& Bernard 2015). Together they form an oral cavity ecosystem. We cannot eliminate all the microflora but better to remove the most prominent and most cariogenic from the dental plaque.

Gram positive streptococci developed by gram negative anaerobic bacterial aggregation, accumulates to form dental plaque that can lead to tooth loss in most cases. Many systemic diseases develop including gingival and periodontal diseases that leads to poor oral health. Hence, different mechanisms have to be developed to control plaque formation by reducing bacterial load since brushing alone would remove only 50% of the dental plaque. Mouthrinses have been widely used in the fields of preventive dentistry and periodontics. The use of these has been proposed as a means of reducing the levels of oral bacteria, especially \textit{Streptococcus mutans}. Improvement of oral health can influence the life quality of people, so development of the new preventive and treatment methods and products which are safe, effective and economical is necessary. Different chemical measures with different antimicrobial agents are circulating the market today. Hence, this study has been undertaken to know whether these antimicrobial agents are effective on the most prominent cariogenic bacteria in the oral cavity that contributes to dental plaque formation.
1.2 Problem statement
Uganda is experiencing high rates of dental caries among the young and old most especially in the urban region where there is high consumption of sugar which are substrates to the disease-causing bacteria. This problem is caused mainly by bacteria (i.e. \textit{S.mutans}) which is the prominent one. One of the most accessible treatments in Uganda is the use of mouth rinses apart from the use of toothpastes. To increase benefit to their consumers, manufactures have used different agents in their preference of which some have seemed weak and unable to act against the bacteria leading to oral health problems.

Oral diseases have a profound impact on society in terms of pain, discomfort, social and functional limitations, handicap and the effect on the quality of life, whose combined effect has been largely ignored. However individual treatment options which are simple and cheap to improve oral health status of the population are unavailable since most which have been adopted cannot perform effectively. This is evidenced by a variety of mouthrines most of which cannot stop or reduce the above-mentioned effects hence a concern. Mouthrines for dental caries need to contain the majority of the antimicrobial agents so as to reduce, control and manage the various dental diseases. Many of these mouthrines have claimed to possess the antimicrobial properties but very little research has been done to prove these claims, making most of the antimicrobial mouthrines available on market being counterfeit. Therefore, their activity needs to be evaluated to minimize the persistence of this disease-causing bacteria.

1.3 Research Objectives
1.3.1 General objective
To determine the effectiveness of commercially and commonly available mouthrines on \textit{Streptococcus mutans} and comparing the antimicrobial activity between each material.

1.3.2 Specific objectives
- To evaluate the in-vitro bacterial inhibition of six commercially available mouthrines against \textit{Streptococcus mutans}.
- To compare the antibacterial activity between each material on the organism tested, for both regular and herbal antimicrobial agents.
1.3 Hypotheses

1.3.1 Null hypothesis
There is no significant difference in the antibacterial activity of different mouthrinses against *Streptococcus mutans*.

1.3.2 Alternative hypothesis
There is a significant difference in the antibacterial activity of different mouthrinses against *Streptococcus mutans*.

1.4 Justification of the study

- The results of this study will be used to develop new knowledge for scientists so as to develop new preventive measures of oral health community based on the indigenous knowledge of local plant species so as to reach the WHO expectations.
- This will provide a viable alternative or choice of the antimicrobial mouthrinses used to control and eradicate bacteria responsible for oral infections. This will be of prime importance to consumers.
- It will also create awareness of quality standards of antimicrobial mouthrinses using sensitivity of selected pathogenic bacteria.

1.5 Scope of the study
This study aimed at determining the effectiveness of six commonly used mouthrinses in the urban regions of Uganda (i.e. Toothex, Nana herbal, Mannyo teeth mix, Tyzorin, Tooth Heal and Tooche drops) against the most prominent tooth decay-causing bacteria *S. mutans* in human oral environment. During this study, six different mouthrinses were used of which three will be herbal and the other three regular (i.e. not of plant origin).

**Table 1: List of mouthrinses**

<table>
<thead>
<tr>
<th>Regular mouthrinse</th>
<th>Herbal mouthrinse</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Tyzorin)</td>
<td>B (Toothex)</td>
</tr>
<tr>
<td>D (Tooth Heal)</td>
<td>C (Nana)</td>
</tr>
<tr>
<td>E (Tooche drops)</td>
<td>F (Mannyo Teethmix)</td>
</tr>
</tbody>
</table>
CHAPTER TWO

LITERATURE REVIEW

2.1 Review

Tooth decay can also be known as dental caries or cavities which is the breakdown of teeth due to acids made by bacteria, mainly *streptococcus mutans* which is the most prominent and *streptococcus sobrinus* and *lactobacilli* found in the mouth.

The biggest problem today in the field of oral health is a disease of hard tissue of teeth or dental caries as well as gum disease. Dental caries is an infectious disease which causes progressive demineralization of the hard tissue of the tooth surface due to organic acids derived from sugar containing foods. Dental caries will only be formed when there is interaction between the substrate (food) tooth and/ or saliva, time and microorganisms that cause caries such as *S. mutans*, *S. sobrinus* which are the most prominent. Sugar is the perfect food for cavity- causing microbes. Dental plaque is a sticky film made up of millions of bacteria that build on the teeth. This film consistently forms because there are always bacteria in the mouth. The bacteria continue to multiply rapidly, especially when fed with the right kind of food. If the plaque is left to grow without treating, you may find yourself in a predicament where your oral health is steadily declining.
Oral health has not improved in the past 25 years and oral conditions remained a major public health challenge all over the world in 2015 (Kassebaum, Smith, Bernabe’ Fleming, Reynolds, Vos, Murray, Marcenes & GBD 2015 oral health collaborators). Hobdell et al. (2003) argues that the world dental federation, WHO and international Association for dental research set two general oral health goals for the year 2020. As Hobdell et al. still claims, the first of those goals was to minimize the impact of diseases of oral and craniofacial origin on health and psychosocial development, giving emphasis to promoting oral health and reducing oral disease amongst populations with the greatest burden of such conditions and diseases.

To identify the overall population with unmet normative demand for dental care, people with untreated oral conditions and the Disability- adjusted life year (DALY) estimates are the relevant measures (Kassebaum et al., 2015). Kassebaum et al. (2015) still claims that the number of people with untreated oral conditions reached 3.5 billion in 2015, which is expected to increase as many populations continue to grow. Despite the above problems, Pannuti et al., 2003 argues that brushing teeth with toothpaste is the most commonly practiced form of oral hygiene in most countries, which is supposed to be done twice a day according to the National health service
(2015). Slate (1998) that prior to buying toothpastes two things must be put in mind i.e. clinical significance and aesthetic appeal.

With increasing number of drug resistant microorganisms, many researchers have put much focus on the use of plant extracts (herbal drugs) apart from the existing artificial drugs (regular drugs), to generate drugs against dental caries. Kampala city experiences significantly higher caries than rural districts (Rwenyonyi, 2005) despite the many toothpastes and other mouthrinses used, thus creating an alarm to determine their effectiveness. The aim of this study was to determine the effectiveness of the antimicrobial activity of commonly and commercially available mouthrinses against *Streptococcus mutans* in-vitro.

### 2.2 Antimicrobial mouthrinses

Mouthrinses contain compounds which act to fight against microbial growth, strengthen tooth enamel, whiten teeth, bring good breath and bring comfort to the user. However, depending on the manufacturer of the drug, many of them will have different components of varying concentration. *Streptococcus mutans* and other oral bacteria can produce organic acids at levels that induce demineralization of tooth structure leading to clinically detectable caries.

![Figure 3: Hole in tooth caused by streptococcus mutans](image)

Active compounds within these mouthrinses therefore work by inhibiting demineralization by inhibiting bacterial enzymes due to their bactericidal activity. These inhibit acid production by bacteria.
2.3 Use of mouthrinses

Usually the method of tooth brushing used by many people is most times not sufficient enough over a long period to provide a level of plaque control consistence with oral hygiene. However, incorporation of chemical agents which are effective and pose anti-plaque or antimicrobial activity into different dental products, has been proposed as a potential method to reduce plaque-mediated diseases. Antimicrobial chemotherapeutic agents have been proposed to reduce the increasing levels of oral bacteria, especially *S. mutans*.

Many scientists have advised to brush twice at least every day to cause effective eradication of the sugary material that remains in the oral environment that acts as substrate for the bacteria and other microbes. However, most manufacturers state that their products should be used after brushing to increase effectiveness against the bacteria. Others can however be used at any time of the day depending on the user.
CHAPTER THREE

RESEARCH METHODOLOGY

This in-vitro experiment used reference bacteria from college of veterinary medicine, microbiology lab. This reference bacterium was studied for resistance against the different antimicrobial compounds found in the six different mouthrinses.

3.1 Research design

The study was a laboratory-based experimental study. The research design was aimed at determining the effectiveness of antibacterial activity of different mouthrinses against *S. mutans*.

3.2 Samples

Toothex, Nana herbal, Mannyo teeth mix, Tyzorin, Tooth Heal and Tooche drops were purchased from shops in Kampala town, taking note of the manufacture’s seal and expiry date.

3.2.1 Sample collection

6 mouthrinses of which three are herbal and three regular were selected for in-vitro antimicrobial testing. These were collected from normal shops in Kampala town. Their brand name, active ingredients and their country of origin are listed in the table as well as the companies for the respective mouthrinses taking concern of the expiry date and seal of the manufacturer.

3.3 Procedure

Evaluation of the mouthrinses

The research was aimed at knowing the brands of the commonly used mouthrinses against dental caries around Kampala city. As a result six different mouthrinses were selected for assessment of their in-vitro antimicrobial activities. These were purchased from normal markets in Nansana, Kasubi and Nakulabye.

Nutrient agar was prepared to assess the antimicrobial activity of mouthrinses against the pathogen.
**Antimicrobial assay**

The antimicrobial activity of mouthrinses was determined by agar well diffusion method to determine the susceptibility of the bacteria.

A 24hr broth (MH) culture of *S. mutans* of 1.0ml was measured and seeded in the MH agar and allowed to dry for one hour.

Wells were made in the solid medium by using a sterile 10mm cork-borer.

Each of the three solutions were contained on the agar medium for each sample and arranged equidistantly.

0.2 ml of the mouthrinse was introduced into each of the wells while the same amount of sterile distilled water was used as the negative control.

For a positive control, Ciprofloxacin was used against *S. mutans* and the plates anaerobically incubated at 37°C for 24 hrs.

To determine the antimicrobial activity, zones of inhibition in mm were measured for each solution with respect to the type of sample. The plates were made in triplicates to increase reliability of the results.
CHAPTER FOUR

RESULTS

This was an in-vitro study conducted to assess the anti-microbial efficacy of six commercially available mouthrinses on \textit{S.mutans}.

The effectiveness of the different treatments had statistically significant variation (one way-ANOVA, F=87.95, p< 0.001) against the test organism.

The results of this investigation showed that mouthrinse D had the maximum zones of inhibition and highest efficacy against the test organism, \textit{S.mutans} compared to all other mouthrinses.

The study showed that antimicrobial activity A (17.08±1.01) and F (16.0±0.5) was not statistically significantly different. There is no significant difference in the antimicrobial activity of B (10.17±0.29) and C (12.33±1.77) statistically.

The mean values ± standard deviations of zones of inhibition are given for the test organism. Each sample was repeated thrice (n=3).

\textbf{Table 2: Mean zones of inhibition}

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean ± standard deviation(SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17.083±1.010^{bc}</td>
</tr>
<tr>
<td>B</td>
<td>10.167±0.289^{a}</td>
</tr>
<tr>
<td>C</td>
<td>12.333±1.774^{ab}</td>
</tr>
<tr>
<td>D</td>
<td>42.0±4.359^{c}</td>
</tr>
<tr>
<td>E</td>
<td>18.833±0.289^{cd}</td>
</tr>
<tr>
<td>F</td>
<td>16.0±0.5^{bc}</td>
</tr>
<tr>
<td>POSITIVE</td>
<td>23.083±1.809^{d}</td>
</tr>
</tbody>
</table>
4.1 Data analysis
Data was analyzed using the mean range of zones of inhibition of antibacterial activity against reference organism (i.e. One-way ANOVA as a statistical package).

*Figure 4: Graph representation of results*

4.2 Discussion of results
A wide range of mouthrinses containing different active ingredients is available on the market. It is important to know their antimicrobial activity because they mainly used to control microorganisms. Maintaining a good oral hygiene could be related to proper use of different antimicrobial agents especially mouthrinses that are readily available in the urban centers, in addition to tooth brushing and regular visits to the dentists for checkups. The decision of most consumers, on which mouthrinse to use relies on many factors including social factors, advertising factors and economic factors. This cariogenic bacterium is the leading cause of dental caries in all oral streptococci. It metabolizes sucrose to lactic acid. It can also metabolize dietary sucrose and synthesizes glucan by cell surface and extracellular glucosyltransferase that helps the bacteria to be established in the dental plaque. Therefore the antimicrobial activity of 6
mouthrinses was comparatively evaluated against the most prominent dental cariogenic bacteria *S.mutans*. The data from this study showed all the investigated dental care products greatly varied in their effectiveness against the tested organism, *S.mutans*.

Mouthrinse D (Tooth Heal) have shown maximum zone of inhibition against *S.mutans*. This could be due to the presence of both synthetic and naturally occurring essential oils such as Menthol, Thymol, and Camphor which has excellent antibacterial properties. This observation is similar to the earlier study carried out by L. Galvão, V. Furletti & S. Bersan et al. on the activity of these compounds on *S.mutans*. Thymol has antibacterial activity by making the bacterial membrane more permeable hence impairing the cell membrane structure.

The complex and volatile nature of these compounds is formed by aromatic plants as secondary metabolites. They constitute chemical structures of several groups such as terpenes and terpenoids, aromatic and aliphatic, which are all characterized by low molecular weight. This explains their successful bacteriostatic and bactericidal action.
CHAPTER FIVE

Conclusion and recommendation

Mouthrinse D proved to be the most effective against the tested organism based on mean diameter of zones of bacterial inhibition produced by agar well diffusion method. Mouthrinse B and C (which are herbal drugs), showed the lowest efficacy against the tested organism with the minimum zones of bacterial inhibition. Regular mouthrinses showed higher efficacy against S. mutans than herbal drugs. However, F which is a herbal drug showed effectiveness against the tested organism S. mutans than the rest of the herbal drugs which indicates that more purification and increasing the concentration of the active substances as well as standardizing them would increase its activity. This drug F is a locally made drug without proper standardization. More research should therefore be made on how to support and establish appropriate measures on standardization of these locally made drugs to increase their effectiveness. Also more research should be performed using more mouthrinses on market and tested against other cariogenic bacteria like Escherichia coli, Staph. aureus e.tc.

5.1 References


### 5.2 Appendices

**Appendix A: Ingredients for various mouthrinses to be tested for antimicrobial activity.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ingredients</th>
<th>Country of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nana herbal (C)</td>
<td>Calendula and Ratanhia flavous, Cinnamon, Grapefruit seed extract, Green tea extract, papaya plant extract, Eucalyptus oil, Aloevera, Indian almond, Peelu, Clove, peppermint and strawberry.</td>
<td>Uganda</td>
</tr>
<tr>
<td>Toothex (B)</td>
<td>Green herbs</td>
<td>Uganda</td>
</tr>
<tr>
<td>Mannyo teeth mix (F)</td>
<td>Not stated</td>
<td>Uganda</td>
</tr>
<tr>
<td>Tocoche drops (E)</td>
<td>Menthol BP, Solvent ether BP, Cajuput Oil BPC, Clove Oil BP, Tolu Balsam BPC and</td>
<td>India</td>
</tr>
<tr>
<td>Product</td>
<td>Description</td>
<td>Country</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Tyzorin (A)</td>
<td>Tibezonium iodide 0.05g/100ml</td>
<td>Pakistan</td>
</tr>
<tr>
<td>Tooth Heal (D)</td>
<td>Iodine, potassium iodide, Tannic acid, menthol, Camphor, Thymol, Glycerine, Purified water.</td>
<td>Dapppar</td>
</tr>
</tbody>
</table>