Anti-bacterial activity of *Corchorus olitorius* L. and *Acmella caulirhiza* Del. on *Streptococcus mutans*, a cariogenic bacteria in dental caries

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September 2017
DECLARATION

I Namwase Hadijja, hereby declare that the work in this dissertation is entirely my own and that it has not previously been submitted to any other academic institution for any other academic award. I have made no use of sources, materials or assistance other than those which have been openly and fully acknowledged in the text.

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Signature. .......................... Date. 14th sep , 2018

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DEDICATION

I dedicate this work to my son Katabira Ukasha.
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<tr>
<td>A. caulirhiza Del</td>
<td>Acmella caulirhiza Del.</td>
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<td>ATCC</td>
<td>American Type Culture Collection</td>
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<tr>
<td>CFU</td>
<td>Colony-forming unit</td>
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<td>C. olitorius L.</td>
<td>Corchorus olitorius L.</td>
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<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
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<td>Hr</td>
<td>Hour</td>
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<tr>
<td>MBC</td>
<td>Minimum bactericidal concentration</td>
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<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
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<td>Mg</td>
<td>Milligram</td>
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<tr>
<td>MI</td>
<td>Milliliter</td>
</tr>
<tr>
<td>S. mutans</td>
<td>Streptococcus mutans</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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OPERATIONAL DEFINITIONS

Cariogenic bacteria: “These are micro-organisms that produce or promote the development of tooth decay” (Purkait, 2011).

Dental caries: WHO defines it as localized post eruptive pathological process of external origin involving softening of the hard tissue of the tooth and proceeding to the formation of a cavity (WHO, 1962).

Traditional medicine: WHO defines this as the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health as well as to prevent, diagnose, improve or treat physical and mental health (Gureje et al., 2015).

Minimum inhibitory concentration (MIC): it was defined as the least concentration of the solvent plant extract that showed zone of inhibition using the agar well diffusion method.

Minimum bactericidal concentration (MBC): it was defined as the least concentration of the solvent plant extract at which no bacterial growth was observed.
ABSTRACT

Background: Dental caries remain a global oral health challenge with the prevalence reported as high as 66.7% in adults. The problem is due to increased consumption of refined sugars and poor oral health hygiene. This has favored the multiplication of cariogenic *Streptococcus mutans* that produces lactic acid leading to localized cavitation of the enamel hence causing dental caries. However, despite the use of modern medicines, the prevalence of dental caries has remained a challenge. In rural communities of Uganda, medicinal plants have been utilized in the management of dental caries. Among them include *Corchorus olitorius* L. and *Acmella caulirhiza* Del. However, their anti-cariogenic activity against *S. mutans* has not been scientifically evaluated and documented.

Objective: To determine the anti-bacterial activity of *Corchorus olitorius* L. and *Acmella caulirhiza* Del. on *Streptococcus mutans*, a cariogenic bacteria in dental caries.

Methods: It was an experimental laboratory based study. Plant materials of *C. olitorius* L. and *A. caulirhiza* Del. were collected, cleaned and dried according to the standard methods and procedures. Extraction was done by cold maceration using distilled water, methanol and diethyl ether. Agar well diffusion method was used to determine the zone of inhibition and MIC value against *S. mutans* (ATCC 6519). The results were analysed using Statistical Package for Social Science (Spss) version 16 and Analysis of Variance (ANOVA) and Tukey’s (HSD) test.

Results: All the solvent extracts of *C. olitorius* L. and *A. caulirhiza* Del. showed antibacterial activity against *S. mutans*. The aqueous extract of *C. olitorius* L. and the diethyl ether extract of *A. caulirhiza* Del. had the highest zone of inhibition of 16.10mm and 12.03mm respectively. The diethyl ether extract from both plants had the lowest MIC value of 62.5mg/ml. All extracts of *C. olitorius* and the aqueous extract of *A. caulirhiza* Del. had the lowest MBC value of 250mg/ml.

Conclusion: Both *C. olitorius* L. and *A. caulirhiza* Del. as used in oral health practices have been found to have antibacterial activity against the cariogenic *S. mutans*. *C. olitorius* L. has been found to have a better antibacterial activity against *S. mutans*. Further studies should be conducted to isolate bioactive compounds against *S. mutans*. 
CHAPTER ONE: INTRODUCTION

1.1 Background

Diseases of the oral cavity are a common problem globally with dental caries and periodontitis ranking the top most, mainly due to the disruption of normal flora of the cavity by other bacteria. Normal flora of the oral cavity include; *Streptococcus sanguinis*, *S. gordonii*, *Rothia dentocariosa*, *Gemella hemolysans*, *G. adiacens* and these are normally found on the tooth surface (Aas, Paster, Stokes, Olsen, & Dewhirst, 2005).

Dental caries is a pathological, infectious, transmissible, localized and multifactorial disease that leads to the destruction of the enamel (Junaid, Dileep, Rakesh, Pavithra, & Vinayaka, 2013). It is formed as a result of fermentation of carbohydrates like sugary snacks by specific bacterial species such as *Streptococcus mutans* which is the major initiator in the pathogenesis of dental caries. Considerable epidemiologic evidence has linked *Streptococcus mutans* to the pathogenesis of these dental caries (Becker et al., 2002). These microorganisms produce lactic acid through fermentation of sucrose and fructose found in the sugary snacks which dissolves the enamel matrix. The most affected teeth are mostly the molars and premolars since they bear many grooves, nooks, and crannies that can collect food particles leading to plaque, tooth decay and dental cavities.

Of all the diseases of the oral cavity, dental caries is still the most prevalent disease even in most industrialized countries as it affects up to 100% of both school going children and adults (Bagramian, Garcia-Godoy, & Volpe, 2009). In 2010, untreated caries in permanent teeth was reported to be the most prevalent condition worldwide, affecting 2.4 billion people, while the untreated caries in deciduous teeth was reported to be the 10th-most prevalent condition, affecting 621 million children worldwide (Kassebaum et al., 2015). Current data shows that untreated decay of permanent teeth has a global prevalence of over 40% for all individual ages combined and is the most prevalent condition among the 291 diseases affecting humans (FDI World Dental Federation, 2015).

Similarly, in the Ugandan population, dental caries is a serious public health problem with a reported overall dental caries prevalence estimated to be 32.5% in children and 66.7% in adults (Kutesa et al., 2015). The increasing burden of dental caries has been attributed to the current
lifestyle changes especially due to the increased consumption of sugars and refined carbohydrates, combined with poor oral hygiene which have led to invasion of *S. mutans*. However, inadequacy of resources such as oral health personnel and poor dental health care services globally and most especially in developing countries of the African region like Uganda have made people to seek for an alternative form of oral health care in form of traditional medicine mostly using medicinal plants (Agbor & Naidoo, 2016; Kutesa et al., 2015).

Cariogenic bacteria have also developed resistance against the most commonly used antibiotics thus necessitating increased research and extensive screening of natural products, particularly from plants, for anti-cariogenic activity (Junaid et al., 2013). The finding of new antimicrobial compounds in plants has become one of the remarkable alternatives for treatment of dental caries since they are rich in various phytochemical compounds such as alkaloids, flavanoids, tannins, antharaquinones and phenolic compounds with antimicrobial properties (Sylvester, Son, Lew, & Rukayadi, 2015).

Globally, different medicinal plants containing various phytochemicals are being used for relieving dental ailments. Among these medicinal plants include; *Andrographis paniculata* (Acanthaceae), *Cassia alata* (Leguminosae) and *Camellia sinensis* (Simaroubaceae) (Palombo, 2011). Some of these plants are used as chewing sticks like *Salvadora persica* (Salvadoraceae) in the prevention of dental caries in Asia, Africa and Islamic countries (Sukkarwalla, Ali, Lundberg, & Tanwir, 2013).

In Uganda, different medicinal plants have been reported to exist and currently they are being used in the prevention and treatment of dental caries such as *Lantana trifolia* (Verbenaceae), *Draceana fragrans* (Agavaceae) (Odongo, Musisi, Waako, & Obua, 2011), *Corchorus olitorius* L and *Acmella caulirhiza* Del. However, there is insufficient scientific evidence and documentation on their antibacterial activity against *S. mutans* that is one of the important organisms implicated in causing dental caries.

*C. olitorius* L. (Tiliaceae) is a green leafy vegetable in tropical areas of Asia, Africa, South America, Cyprus (Ilhan, Savaroğlu, & Çolak, 2007). Besides the plants being used traditionally to treat dental caries, it is also used in folk remedy in the management of pains, dysentery and enteritis (Islam, 2013). On the other hand, *A. caulirhiza* Del. (Asteraceae) is a flowering creeping
plant commonly found near springs. It has been used traditionally to treat mouth ulcers, sore throat, toothache and earache (Sinei, Okalebo, Mugob, & Mwalukumbi, 2013). C. olitorius L. and A. caulirhiza Del. have been found to have antibacterial activity against Escherichia coli, Staphylococcus aureus and Bacillus pumilus (Ilhan et al., 2007; Sinei et al., 2013). Therefore, it was important to determine the antibacterial activity of these plants against S. mutans since no study had been done despite being used by the local communities in Uganda in the management of oral health diseases like dental caries.

1.2 Problem statement
Dental caries remain a public health challenge worldwide including Uganda. The burden of dental caries is further exacerbated by poor oral health hygiene, changes in dietary lifestyle and the high cost of regular dental checkup. In an attempt to prevent dental caries, different techniques have been used including; tooth brushing, flossing, use of antimicrobial mouth washes such as chlorhexidine and antibiotics like erythromycin. These have resulted into change of the oral and intestinal flora, tooth staining, taste alteration or oral cancer and development of resistant bacterial strains. Among the bacteria implicated in the initiation of the pathogenesis of dental caries as a result of normal flora disturbance is S. mutans, which has become a serious problem in disease development. As a result of these problems leading to dental caries, many local communities and traditional healers have continued to use alternative methods especially traditional medicinal plants in the management of various ailments including dental caries. The use of medicinal plants has become of increasing interest among various communities of the world. Different medicinal plants are being used for prevention and treatment of dental caries including Corchorus olitorius L. and Acmella caulirhiza Del. in various communities of the world as well as in Uganda. However, their antibacterial activity against cariogenic bacteria, Streptococcus mutans had not yet been scientifically evaluated and documented prior to this study.

1.3 Justification of the study
In Uganda, there is increasing burden of dental caries and this problem is further exacerbated by the lack of dental care health services, trained personnel, bacterial resistance and the high cost of treatment especially to the poor individuals and communities. In addition, bacterial resistance has affected the use of modern medicines. This has therefore, led the population to seek for
alternative forms of treatment especially from medicinal plants. As a result, there is increasing use and consumption of different types of medicinal plants in form of herbal medicines in the prevention and treatment of dental caries. Among the medicinal plants commonly used include \textit{C. olitorius} L. and \textit{A. caulirhiza} Del. These have been reported to have antibacterial activity against different microorganisms. However, \textit{C. olitorius} L. and \textit{A. caulirhiza} Del. have commonly been used in the management of dental caries in various communities of Uganda without any scientific evidence to justify their use. Therefore, this study has established the antibacterial activity of \textit{C. olitorius} L. and \textit{A. caulirhiza} Del. against \textit{Streptococcus mutans}, a bacteria that is implicated in causing dental caries.

1.4 Significance of the study
This study has provided evidence of the antibacterial activity of \textit{C. olitorius} L. and \textit{A. caulirhiza} Del. against the cariogenic \textit{Streptococcus mutans}. Knowledge on how important these plants are may also promote their conservation, ensure their continued and proper utilization by the different communities. This study has also generated baseline information from which further studies on the effects of \textit{C. olitorius} L. and \textit{A. caulirhiza} Del. on other cariogenic bacteria can be carried out. Hence expanding the scientific body of knowledge that can be added to existing data bases for future use. This study has also provided baseline information which can be used as a basis for isolation of pure compounds that can be used as templates for development of newer drugs.

1.5 Research questions

1) Do the aqueous, methanolic, diethyl ether and total crude extracts of \textit{Corchorus olitorius} L. and \textit{Acmella caulirhiza} Del. have antibacterial activity against \textit{Streptococcus mutans}?

2) What is the minimum inhibitory concentration and minimum bactericidal concentration of the different active solvent extracts of \textit{Corchorus olitorius} L. and \textit{Acmella caulirhiza} Del. on \textit{Streptococcus mutans}?

1.6 General objective

- To determine the anti-bacterial activity of \textit{Corchorus olitorius} L. and \textit{Acmella caulirhiza} Del. on \textit{Streptococcus mutans}, a cariogenic bacteria in dental caries.
1.6.1 Specific objectives

1. To screen for antibacterial activity of the aqueous, methanolic, diethyl ether and total crude leaf extracts of Corchorus olitorius L. on Streptococcus mutans.

2. To screen for antibacterial activity of the aqueous, methanolic, diethyl ether and total crude aerial extracts of Acmella caulirhiza Del. on Streptococcus mutans.

3. To determine the minimum inhibitory concentration (MIC) of the active solvent extracts of Corchorus olitorius L. and Acmella caulirhiza Del. on Streptococcus mutans.

4. To determine the minimum bactericidal concentration (MBC) of the active solvent extracts of Corchorus olitorius L. and Acmella caulirhiza Del. on Streptococcus mutans.
1.7 Conceptual framework

- **Environmental factors**
  - Type of soil
  - Climate

- **Age of plant**

- **Modern methods used**
  - Tooth brushing
  - Use of mouth washes
  - Use of antibiotics

- **Plant parts used**
  - Leaves
  - Shoot
  - Roots
  - Whole plant

- **Active phytochemicals of *C. olitorius* L. and *A. caulirhiza* Del.**

- **Antibacterial activity on *Streptococcus mutans***

- **Individual factors**
  - Type of diet
  - Oral hygiene
  - Life style

- **No activity**
  - Dental caries
  - Endocarditis

- **Activity**
  - Prevention of dental caries
  - Treatment of dental caries

- **Genetic makeup, modification and resistance of *Streptococcus mutans***
CHAPTER TWO: LITERATURE REVIEW

2.1 Oral health

Oral health is a state of being free from mouth and facial pain, oral and throat cancer, oral infection and sores, periodontal (gum) disease, tooth decay, tooth loss and disorders that limit an individual’s capacity in biting, chewing, smiling, speaking, and psychosocial wellbeing (WHO, 2012). It is essential to the general health and quality of life of the individuals (WHO, 2012).

2.1.1 Oral diseases

Oral diseases including the dental caries, periodontal disease, tooth loss, oral mucosal lesions and oropharyngeal cancers, human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS)-related oral disease and orodental trauma are major public health problems worldwide (Petersen, Bourgeois, Ogawa, Estupinan-Day, & Ndiaye, 2005). The most common oral diseases are dental caries and the periodontal diseases. Globally, about 30% of people aged 65–74 have no natural teeth (WHO, 2012). Oral disease in children and adults is reported to be higher among the poor and disadvantaged population groups globally (WHO, 2012).

2.1.1.1 Periodontal diseases

The periodontal diseases are infections caused by bacteria in the biofilm (dental plaque) that forms on oral surfaces (NIDCR, 2014). The most common forms of the disease are; gingivitis which affects the gums, and periodontitis which may involve all of the soft tissue and bone supporting the teeth (NIDCR, 2014). These diseases are found in 15–20% of middle-aged (35-44 years) adults (WHO, 2012). However, gingival inflammation does not appear until the biofilm changes from one composed largely of gram-positive Streptococci to one containing gram-negative anaerobes for example; Fusobacterium nucleatum, Veillonella parvula, Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus and Treponema denticola (NIDCR, 2014). The moderately progressive adult form of periodontitis is characterized by a gradual loss of attachment of the periodontal ligament to the gingiva and bone along with loss of the supporting bone (NIDCR, 2014). It is reported that periodontitis results from a mixed infection but that a particular group of gram-negative bacteria are key to the process and markedly increase in the subgingival plaque. The bacteria most frequently cited are Porphyromonas
**gingivalis, Prevotella intermedia, Bacteroides forsythus, Treponema denticola, and Actinobacillus actinomycetemcomitans** (NIDCR, 2014).

### 2.1.1.2 Dental caries and its global burden

Dental caries is a localized, progressive decay of the teeth and it is one of the most common types of plaque-related diseases in the general population. Dental caries is the most widespread chronic disease globally and constitutes a major global public health challenge. And the most widely used measure of tooth decay prevalence is the DMFT (decayed, missing or filled teeth) index, which is used to calculate the number of decayed (D), missing (M) or filled (F) teeth (T) (WHO, 2016). In 2010, the untreated dental caries in permanent teeth were reported to be affecting 2.4 billion people globally and in deciduous teeth, it was reported to be affecting 621 million children worldwide (Kassebaum et al., 2015). Current data shows that untreated decay of permanent teeth has a global prevalence of over 40 percent, for all ages combined (FDI World Dental Federation, 2015). The burden of untreated caries has been reported to shift from children to adults, with 3 peaks in prevalence at ages 6, 25, and 70 years (Kassebaum et al., 2015). Whilst, low-income countries have lower levels of tooth decay, this goes almost entirely untreated, reflecting weak oral healthcare systems. And, even in high-income countries more than half of tooth decay is left untreated (FDI World Dental Federation, 2015). Despite the widespread nature of tooth decay, reliable, standardized global data is limited. This is largely because oral health data is not integrated in various national disease surveillance programs, particularly in low- and middle-income countries (FDI World Dental Federation, 2015). Separate national oral health surveys are complex and costly to conduct, and hence not prioritized. This lack of up-to-date epidemiologic information constrains the development of appropriate approaches in controlling dental caries and hence reduce the disease burden (FDI World Dental Federation, 2015).

In Uganda, the overall dental caries prevalence is reported to be 32.5% in children and 66.7% in adults in Uganda (Kutesa et al., 2015). In Rakai, a rural district in Southern Uganda, the prevalence of dental caries was reported to be 57.3% between the ages of 18-62 years (Rwenyonyi, Muwazi, & Buwembo, 2011). This is an indication of widespread disease, in an epidemic proportion among the population.
2.2 Predisposing factors to dental caries
These factors include; unhealthy diets such as increased consumption of sugary foods that provide nutrients to bacteria such as *Streptococcus mutans* in the oral cavity. Other factors include; tobacco use, harmful alcohol use, rising levels of bottle feeding leading to “baby bottle syndrome” and poor oral hygiene. (WHO, 2012).

2.2.1 Cariogenic bacteria
Dental caries is caused by many factors. Three main factors have been suggested including; host, environment and bacteria. Of the bacteria, Mutans streptococci (MS) and lactobacilli are known as the cariogenic oral bacteria. The Mutans streptococci include *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*) (Nishikawa, Nomura, Imai, Senda, & Hanada, 2007). Of the Lactobacilli genus, the dominant species include *Lactobacillus fermentum*, *Lactobacillus rhamnosus*, *Lactobacillus gasseri*, *Lactobacillus casei/paracasei*, *Lactobacillus salivarius*, *Lactobacillus plantarum* (Caufield, Schön, Saraithong, Li, & Argimón, 2015).

2.2.2 Role of *Streptococcus mutans* in the pathogenesis of dental caries
According to reports by Chhour et al. (2005), Microflora associated with dental caries is dominated by gram-positive bacteria, particularly in the genera of *Actinomyces*, *Lactobacillus*, and *Streptococcus*, and more predominantly by *S. mutans*.

*Streptococcus mutans* is a facultative anaerobic Gram positive coccus (round bacterium) commonly found in the human oral cavity. The cells are spherical or ovoid, 0.5-2.0μm in diameter, occurring in pairs or chains, stain Gram-positive and are catalase negative (Al-Mudallal, Al-Jumaily, Muhimen, & Al-Shaibany, 2008). It is an alpha - hemolytic *Streptococci* which further belongs to serotype C, and F (Al-Mudallal et al., 2008). The optimum temperature for growth is 37°C, and growth is usually restricted to 25-45°C (Al-Mudallal et al., 2008).

*S. mutans* has been implicated as the major initiator of dental caries. *S. mutans* adheres to the enamel surface by hydrophobic bonds from where it starts to ferment dietary carbohydrates, notably sucrose that remains in the oral cavity and teeth after chewing food. This sucrose metabolism promotes the firm adherence and cellular aggregation of bacteria to the tooth surface, and together with the acids produced during fermentation become critical in the development of
dental caries and other oral diseases (Chhour et al., 2005). These acids such as lactic acid initiate dissolution of the tooth enamel and accumulation of lactic acid in the dental plaque, subsequently leading to localized decalcification, cavitation and breakdown of calcified dental tissue (Becker et al., 2002; Chhour et al., 2005; Prabu, Gnanamani, & Sadulla, 2006). In addition, the bacteria possess unique enzymes (the glucosyl transferases) which produce a range of complex polysaccharides such as dextrans, levans and glucans that facilitate the primary colonization of clean dental tissues. The polysaccharides also form matrix which on maturation supports invasion by other micro-organisms from the genera of Actinomyces, Selenomonas, Leptotrichia, Campylobacter, and Capnocytophaga (Chhour et al., 2005; Odongo et al., 2011). And in most cases, this complex of microorganism requires management using both the conventional and traditional forms of medicine depending on the health care facilities available to the individuals and population in the communities.

2.3 Modern and Traditional management of dental caries

2.3.1 Modern medicines for prevention and treatment of dental caries

Different methods are used among the population to prevent dental caries including; tooth brushing with fluoride tooth paste, use of fluoridated water, use of other topical fluorides found in mouth washes and vanishes, flossing and dietary changes such as; restriction of sugar consumption especially in between meals, drinking only milk or water between meals and consumption of sugar free snacks (Ramos-Gomez, Crystal, Man Wai, Tinanoff, & Featherstone, 2010). In addition, use of fissure sealants and regular visits to the dentists have been of help and generally recommended (Anusavice, 2005; Ramos-Gomez et al., 2010). In the pipeline of drug development, an anti-caries vaccine is being developed. The vaccine contains genetically engineered alkali producing Streptococci that will be important in prevention of dental caries (Ramos-Gomez et al., 2010).

In treatment of dental caries, the decisions for therapeutic nonsurgical or surgical/restorative care is normally based on the extent of caries lesion and presence of a cavity (Slayton et al., 2016). These methods of treatment include; extraction of first permanent molars of poor prognosis at the optimum time to enable second permanent molars to occupy their spaces in children (Cobourne, Williams, & Harrison, 2014), partial or complete removal of the infected tooth and restoration with plastic restorative material as well as the use of antibacterial or antimicrobial agents.
(Anusavice, 2005). However, all these treatment measures are quite expensive especially to the poor communities if they can be accessed. Therefore, most of the people utilize other alternatives in form of traditional medicines mainly medicinal plants.

2.3.2 Traditional medicines for prevention and treatment of dental caries

The use of traditional methods in the treatment of various ailments including dental caries continues to increase in different parts of the world mainly due to their perceived effectiveness, affordability, accessibility and the assumed minimal or lack of side effects (Ephraim-Emmanuel et al., 2015). Traditional medicines have also proved effective when orthodox medicine seemed to have failed especially in cases of drug resistance (Ephraim-Emmanuel et al., 2015). However, despite the efficacy and positive outcomes of the practice of traditional medicine, the practice is accompanied with negativity due to lack of scientific knowledge on their safety, limited approved drug testing; pre- and clinical trials and non-regulation of the activities of the traditional medical practitioners (Ephraim-Emmanuel et al., 2015). The traditional practices for treating dental and periodontal infections include: the use of acupuncture and herbal mixtures from medicinal plants that resolve dental pain, the use of herbal medications and mineral substances (Ashu Agbor & Naidoo, 2015; Ephraim-Emmanuel et al., 2015).

Globally, different medicinal plants are being used in the prevention and treatment of dental caries and they include; Andrographis paniculata (Acanthaceae), Cassia alata (Leguminosae), Camellia sinensis, Psidium guava and Harrisonia perforata (Simaroubaceae) (Palombo, 2011). These medicinal plants have been reported to have anti-adherence properties that prevent binding of S. mutans to the enamel (Palombo, 2011). Helichrysum italicum (Compositae), Mikania laevigata and M. glomerata (Asteraceae), Polygonum cuspidatum (Polygonaceae) and Syzygium aromaticum (Myrtceae) have been reported to inhibit the growth, viability and cariogenic properties of S. mutans (Palombo, 2011). Other different medicinal plants are also used globally as chewing sticks in the treatment of dental caries and they include; Coptidis rhizome (Ranunlaceaes) and Salvadora persica (Salvadoraceae) because of their anti-bacterial properties (Odongo et al., 2011; Sukkarwalla et al., 2013).

In Uganda, different medicinal plants are being used as mouth washes, herbs or chewing sticks in the prevention and treatment of dental caries such as Mangifera indica L (Anacardiaceae),
*Lantana trifolia* L. (Verbenaceae) and *Citrus limonia* (Rutaceae) (Odongo et al., 2011). Among other medicinal plants used include; *Corchorus olitorius* L. and *Acmella caulirhiza* Del. Both plants have proved to have antibacterial activity against *Escherichia coli, Staphylococcus aureus* (Ilhan et al., 2007; Sinei et al., 2013), though their activity against *Streptococcus mutans* has not been scientifically evaluated.

2.3.2.1 Phytochemical compounds present in medicinal plants with antibacterial activity

Phytochemical compounds are nonnutritive plant chemicals that have protective or disease preventive properties (Murugan, Wins, & Murugan, 2013). These phytochemicals are usually considered to play a role in defense of plants against infections by pathogenic microorganisms. However, many of these phytochemicals can protect humans against diseases (Murugan et al., 2013). These include; alkaloids, flavonoids, tannins, phenols, saponins, quinones, lectins, polypeptides and several other aromatic compounds (Cowan, 1999; Lagnika, Amoussa, Adjileye, Laleye, & Sanni, 2016; Murugan et al., 2013). The different phytochemicals have different mechanisms by which they exert their antimicrobial activity. For example;

**Flavonoids** are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection (Cowan, 1999; Murugan et al., 2013). Their activity is probably due to their ability to; disrupt microbial membranes, complex with extracellular and soluble proteins and to complex with bacterial cell walls (Cowan, 1999). In addition, flavonoids can also act through inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function and energy metabolism (Lagnika et al., 2016).

Antimicrobial activity of *saponin* is due to its ability to cause leakage of proteins and certain enzymes from the cell (Murugan et al., 2013).

**Tannins** bind to proline rich proteins and interfere with the protein synthesis (Cowan, 1999). They can also inhibit the growth of microorganisms by precipitating the microbial protein and thus depriving them of nutritional proteins needed for their growth and development (Lagnika et al., 2016).
The **phenolics and polyphenols** inhibit growth of microorganisms through enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Cowan, 1999).

Plants also contain **quinones** that complex irreversibly with nucleophilic amino acids in proteins often leading to inactivation of the protein and loss of function (Cowan, 1999).

Some plants also contain **lectins and polypeptides** that are often positively charged and contain disulfide bonds. Their mechanism of action may be due to the formation of ion channels in the microbial membrane or competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors (Cowan, 1999).

The antimicrobial activity of **alkaloids** is attributed to their ability to intercalate with DNA, inhibit nucleic acid protein and membrane phospholipid biosynthesis (Cowan, 1999; Wotoyitide, 2012)

### 2.4 Corchorus olitorius L. and Acmella caulirhiza Del. medicinal plants

#### 2.4.1 Corchorus olitorius L.

It is an annual or biennial herb, erect, stout and branched up to 1.5 m high. It belongs to the family of Tiliaceae and Corchorus genus. It is an important green leafy vegetable in many tropical areas including Egypt, Sudan, India, Bangladesh, Philippines, Malaysia, Uganda, Kenya etc. (Ilhan et al., 2007). *C. olitorius* L. requires a plain alluvial soil and standing water which are offered by the monsoon climate (Islam, 2013).
2.4.1.1 Common names of *Corchorus olitorius* L.

The common names of *C. olitorius* L. include; jew's-mallow, nalta jute, tossa jute (English), meloukhia (Arabic), corète potagère (French) Langkapseljute (German), juta (Italian) tossa jute (Swedish), chang shuo huan ma (Chinese) (Ilhan et al., 2007). In Uganda, it is locally called *Muteere* (lusoga), *Otigo* (Acholi).

2.4.1.2 Taxonomic classification of *Corchorus olitorius* L.

Kingdom: Plantae                        Family: Tiliaceae
Phylum: Tracheobionta                    Genus: *Corchorus* L.
Class: Magnoliopsida                     Species: *Corchorus olitorius* L.
Order: Malvales

2.4.1.3 Medicinal uses of *Corchorus olitorius* L.

*C. olitorius* L. is reported to have demulcent, diuretic, lactagogue, purgative, and tonic properties. It is also a folk remedy for aches and pains, enteritis, fever, dysentery, pectoral pains,
and tumors (Islam, 2013). In Ayurvedic medicine, the leaves are used for ascites, pain, piles, and tumors (Osawaru, Ogwu, Ogbeifun, & Chime, 2013). The leaves are also used in cystitis, dysuria and gonorrhea. The cold infusion, is reported to restore appetite and strength (Osawaru et al., 2013). *C. olitorius* L. also has nutritional uses and therefore used as food for example; the seeds are being used as a flavor in herbal tea that is made from dried leaves (Islam, 2013). In North Africa and the Middle East, the young leaves are used as green leafy vegetables and sometimes as condiment (Islam, 2013).

**2.4.1.4 Phytochemistry of Corchorus olitorius L.**

Jute leaves are now reported to contain as many as 17 active nutrient compounds including protein, fat, carbohydrate, fiber, ash, calcium, potassium, iron, sodium, phosphorous, beta-carotene, thiamine, riboflavin, niacin, ascorbic acid, sterols and triterpenes, carotenoids, alkaloids and coumarins (Islam, 2013). The phytochemical analysis of *C. olitorius* L. has also revealed that it contains hydrocyanin and cardiac glycosides in large quantities and some traces of tannins, appreciable quantities of flavonoids, anthraquinones and saponins (Adegoke & Adebayo-Tayo, 2009).

**2.4.1.5 Antimicrobial activity of Corchorus olitorius L.**

The leaves of *Corchorus olitorius* L. were reported to have antimicrobial activity against Gram positive (*Enterrococcus fecealis* and *Bacillus subtilis*) and Gram negative bacteria (*Escherichia coli*, *Proteus vulgaris*, *Klebsiella* spp., *Serratia marcescens*) (Mohammed, 2016). Ilhan et al. (2007), also reported that the leaves of *C. olitorius* L. presented a good antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Yersinia enterocolitica*. In addition, the leaves of *C. olitorius* L. also showed good antifungal activity against *Geotrichum candidum* and *Botrytis cinerea*.

**2.4.1.6 Toxicity profile of Corchorus olitorius L.**

Acute toxicity tests were performed using mice and even at the highest dose of a methanolic extract of *C. olitorius* L, there were no changes in the behavioral patterns and mortality was not observed (Parvin et al., 2015). In addition, there were no detectable signs of either hepatic or renal toxicity, as well as biochemical and histological analysis in both the treated group or the control group of rats (Al Batran et al., 2013).
2.4.2 *Acmella caulirhiza* Del.

It is an annual or perennial flowering herb in the plant family Asteraceae. It is also often referred to as toothache plant. It is a small creeping and ascending plant which grows quickly and bears golden and yellowish flowers (Sinei et al., 2013).

![Acmella caulirhiza Del.](image)

**Figure 2:** *Acmella caulirhiza* Del. (taken by the principal investigator, Namwase Hadijja)

2.4.2.1 Common names of *Acmella caulirhiza* Del.

The common names of *A. caulirhiza* Del. include; Yeux de Poule, Etsegne andewu (Flatie, Gedif, Asres, & Gebre-Mariam, 2009). In Uganda, it is locally called *Mukasa omusajja* (Luganda).

2.4.2.2 Taxonomic classification of *Acmella caulirhiza* Del.

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
<th>Family</th>
<th>Asteraceae</th>
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<tr>
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<td>Eudicots</td>
<td>Genus</td>
<td><em>Acmella</em></td>
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<tr>
<td>Class</td>
<td>Asterids</td>
<td>Specie</td>
<td><em>Acmella caulirhiza</em> Del.</td>
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<tr>
<td>Order</td>
<td>Asterales</td>
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2.4.2.3 Medicinal uses of *Acmella caulirhiza* Del.

Traditionally, the flowers or sometimes stems or leaves of *A. caulirhiza* Del. are chewed or used as a decoction made with water and then used as mouth wash to treat decayed teeth, gingivitis or wounds in the mouth, toothache, sores on the tongue and sore throat (Dubey, Maity, Singh, Saraf, & Saha, 2013; Sinei et al., 2013). In addition, the plant is also claimed to treat stomachache and earaches (Sinei et al., 2013). It has also been well documented for its uses as; spices, antiseptic, antibacterial, antifungal, and antimalarial, an aphrodisiac, antinoceptive, antioxidant treatment, and as remedy for flu, cough, rabies diseases, and tuberculosis (Dubey et al., 2013). It is also noted for its bio-insecticidal activity (Dubey et al., 2013).

2.4.2.4 Phytochemistry of *Acmella caulirhiza* Del.

*A. caulirhiza* Del. is known to contain the following phytochemical compounds and these include; flavonoids, triterpenoids, alkylamides, alkaloids, amino acids, tannins and phenolic compounds (Dubey et al., 2013). In addition, some other active metabolites found in the plant include: spilanthol, Undeca-2E,7Z,9E-trienoic acid isobutylamide, Undeca-2E-en-8,10-diynonic acid isobutylamide, $\beta$-Sitosterol, Stigmasterol, $\alpha$ and $\beta$-Amyrin, Limonene, $\beta$-Caryophyllene 3-Acetylaleuritolic acid, Vanillic acid, $\beta$-Sitostenone Scopoletin and trans-Ferulic acid (Dubey et al., 2013).

2.4.2.5 Antimicrobial activity of *Acmella caulirhiza* Del.

*A. caulirhiza* Del. has been reported to possess antibacterial activity against *Escherichia coli, Staphylococcus aureus* and *Bacillus pimulus* (Sinei et al., 2013). In addition, *Acmella uliginosa* a plant specie from the same genus of *Acmella* has also been reported to possess antibacterial activity against *Staphylococcus aureus*, *S. epidermidis, Enterococcus faecalis, Staphylococcus aureus Methicillin Resistant* bacteria and *Pseudomonas aeruginosa* (Lagnika et al., 2016). The presence of flavonoids, coumarin, triterpene, naphtoquinone and tannin in *Acmella uliginosa* were reported to attribute to its antibacterial activity (Lagnika et al., 2016).

2.4.2.6 Toxicity of *Acmella caulirhiza* Del.

Different adverse effects or mortality have been reported and were detected in albino rats, up to a dose of 3 g/kg administered orally in form of an aqueous extract of *A. caulirhiza* Del. during the 24 hour observation period (Paulraj, Govindarajan, & Palpu, 2013). The hexane extract of *A.
*caulirhiza* Del. in male Wistar rats was injected intraperitoneally at 50 to 150 mg/kg of the extract and they were observed for periods up to 2 hours. The lower doses (50 and 75 mg/kg) only elicited minor behavioral changes, such as grooming and wet dog shakes. Higher doses (100 to 150 mg/kg) induced full tonic clonic convulsions in a dose-dependent manner, which were accompanied by typical electrographic seizures in the electroencephalogram (Paulraj et al., 2013).
CHAPTER THREE: MATERIALS AND METHODS

3.1 Study design
This was an experimental laboratory based study with quantitative methods of data collection and analysis.

3.2 Study setting
The plants were identified at the National herbarium, Department of Botany, Makerere University and later extracted at the experimental laboratory at the Department of Pharmacology and Therapeutics, Makerere University. The analysis of the antibacterial activity of C. olitorius L. and A. caulirhiza Del. was done at the Department of Microbiology, College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB), Makerere University.

3.3 Selection criteria of the medicinal plants
The plants studied were selected basing on local communities’ claims that they were effective in treating dental caries and also through previous ethnobotanical surveys (Jiofack et al., 2010). The leaves of C. olitorius L. and the aerial (leaves, stems and flowers) parts of A. caulirhiza Del. are commonly used by the communities in Uganda namely; Komamboga, Wakiso district and in this study, the leaves and the aerial parts were used respectively to assess the antibacterial activity of these plants against Steptococcus mutans.

3.4 Collection and identification of the plants
C. olitorius L. was collected from Komamboga, Wakiso district from a domestic environment and A. caulirhiza Del. was picked from Binzali, Kampala district from bushes near springs during the rainy season. A voucher specimen was prepared and stored for the two different plants. The specimen were then taken to the National herbarium, at the Department of Botany, Makerere University for identification and copies of the specimen were kept at the herbarium and given accession numbers namely; 48898N for C. olitorius L. and 48899N for A. caulirhiza Del.

3.5 Processing of C. olitorius L. and A. caulirhiza Del
The freshly collected leaves of C. olitorius L. and aerial parts of A. caulirhiza Del. were rinsed separately with clean water and placed on a cloth to drain the excess water from the plant
materials. The plant materials were then placed in a solar drier to dry for 14 days. The plant materials were weighed every week until a constant weight was obtained and this ensured that they were dry. The dried plant materials for each plant were then pounded separately using a mortar and pestle to produce a powder. The powders were stored in airtight containers in a cool dry place awaiting extraction.

3.6 Extraction of *C. olitorius* L. and *A. caulirhiza* Del

3.6.1 Extraction of *C. olitorius* L. and *A. caulirhiza* Del using diethyl ether and methanol

The diethyl ether and methanolic extracts of *C. olitorius* L. and *A. caulirhiza* Del. were similarly done. About 500 grams of *C. olitorius* L. and 400 grams of *A. caulirhiza* Del. were extracted using serial exhaustive extraction with different solvents of increasing polarity starting with diethyl ether and then methanol. The plant powders were extracted using cold maceration method (Elkhair, Fadda, & Mohsen, 2010). The plant powders of *C. olitorius* L. and *A. caulirhiza* Del. were soaked in 2.5 liters of 96% diethyl ether for 72 hours with intermittent shaking, filtered using a Whatman No. 4 filter paper and concentrated using a rotary evaporator (Rotavapor® R-210/R) to recover the diethyl ether. The remaining concentrated filtrate was dried to form the diethyl ether plant extract for either plants. The remaining marc was re-extracted using the recovered diethyl ether for 48 hours and then filtered. The second filtrate of the diethyl ether was used to make the total crude extract.

The remaining marc of *C. olitorius* L. and *A. caulirhiza* Del. was air-dried and later soaked in 2.5 liters of 96% methanol for 72 hours in the same way as performed for diethyl ether. The remaining concentrated filtrate was dried to form the methanolic extract for either plants. The remaining marc was re-extracted using the recovered methanol for 48 hours in the same way as performed for diethyl ether. The second filtrate of the methanolic extract was also used to make the total crude extract.

The diethyl ether and methanolic filtrates of *C. olitorius* L. and *A. caulirhiza* Del. were mixed in a proportion of 1:1 to form the total crude extract for either plants. 500mls of either solvent filtrates were concentrated using a rotary evaporator (Rotavapor® R-210/R) to a volume of 150mls. The remaining volume from diethyl ether and methanol filtrates were mixed together to
form a volume of 300mls. The total volume was then concentrated using a rotary evaporator to form *C. olitorius* L. and *A. caulirhiza* Del. total crude plant extracts respectively.

### 3.6.2 Extraction of *C. olitorius* L. and *A. caulirhiza* Del. using distilled water

For the aqueous extracts, varied temperature conditions of the distilled water were used. About 100 grams of new dried plant material of *C. olitorius* L. was mixed and soaked in 1 liter of boiled distilled water for 6 hours, filtered and concentrated using a freeze drier to form an aqueous leaf extract of *C. olitorius* L. The plant material was soaked for only 6 hours to avoid microbial contamination and growth in the extract.

About 100 grams of new dried plant material of *A. caulirhiza* Del. was soaked in 1L of distilled water for 6 hours with intermittent shaking, filtered and concentrated using a freeze drier to form aqueous extract of *A. caulirhiza* Del. The different solvent extracts were then left to dry completely and later stored under refrigeration at 4˚C. The percentage (%) yield for each solvent extract was calculated as below:

\[
\text{% yield} = \frac{\text{Weight of dried solvent extract obtained}}{\text{Weight of dried plant material soaked}} \times 100
\]

### 3.7 Preparation of stock solutions of plant extracts for bioassay

About one grams of each of the aqueous, methanol, diethyl ether and total crude extracts of *C. olitorius* L. and *A. caulirhiza* Del. were weighed using a weighing balance (model: Sartorius Werke GMBH 2204). Thereafter, a few drops of dimethylsulfoxide (DMSO) were added until dissolution and then topped up with sterilized distilled water to make 1ml for the diethyl ether, methanolic and total crude extracts to make a stock solution of 1000mg/ml. One ml of sterilized distilled water was used for the aqueous extracts to make a stock solution of 1000mg/ml. The different concentrations of the extracts were prepared from the stock solution depending on the bioassay test that was to be performed.

### 3.8 Preparation of experimental control drugs

#### 3.8.1 Positive control

Ciprofloxacin 2mg/ml manufactured by Abacus parenteral Drugs limited. (Uganda) was used as the positive control for the experiment.
3.8.2 Negative control
Sterilized distilled water and DMSO were used as the negative control.

3.9 Microbial strain of Streptococcus mutans
The American Type Culture Collection strain (ATCC 6519) of *S. mutans* was obtained from the Microbiology laboratory at the Department of Microbiology, College of Health Science, Makerere University.

3.10 Preparation of inoculum for Streptococcus mutans
*S. mutans* (ATCC 6519) was then sub cultured on 5% sheep blood agar (pH 7.3). The plates were placed in an anaerobic jar (Oxoid manufacturer, Britain). An anaerobic gas pack (Biomerieux manufacturer, France) and an indicator (Oxoid manufacturer, Britain) were also placed in the anaerobic jar. When the indicator turned pink, this showed that anaerobiasis (anaerobic environment) inside the anaerobic jar had been achieved. These were then incubated at 37˚C for 24hrs. Colony by colony of *S. mutans* (ATCC 6519) that were 24hrs old were picked and suspended in 2mls of 0.9% sterilized normal saline using the direct colony suspension method. The forming turbidity was adjusted to be equivalent to pre-prepared 0.5 Mcfarland standard. The turbidity of the two suspensions (*S. mutans* (ATCC 6519) and the 0.5 Mcfarland standard) were compared by placing the tubes in front of a white paper (Cavalieri et al., 2005). The adjusted inocula equivalent to $1.5 \times 10^8$ cfu/mL (0.5 Mcfarland standard) was used in the different bioassay tests.

3.11 Bioassay procedures
The agar well diffusion method was used in determining MIC and MBC due to technical limitations where we did not have a spectrophotometer that measures turbidity when using the broth dilution method. Therefore, the MIC was defined as the least concentration of the solvent plant extract that showed zone of inhibition using the agar well diffusion method while MBC was defined as the least concentration of the solvent plant extract at which no bacterial growth was observed.
3.11.1 Screening of the extracts for antibacterial activity

The different bioassay tests were done in triplicates. The activity of the aqueous, methanolic, diethyl ether and total crude extracts of *C. olitorius* L. and *A. acmella* Del. against *S. mutans* (ATCC 6519) were assessed using the agar-well diffusion method.

3.11.1.1 Preparation of the extracts for screening

One gram of the diethyl ether, methanol and total crude extracts were carefully weighed using a top load weighing balance and there after dissolved with a few drops of 10% DMSO until dissolution and then topped up with sterilized distilled water to make 1ml to form a concentration of 1000mg/ml. One gram of the aqueous extract was also weighed and dissolved in 1ml of sterilized distilled water to form a concentration of 1000mg/ml.

3.11.1.2 Preparation of media for agar well diffusion assay.

Mueller Hinton Agar (MHA) (Conda laboratories limited, South Africa), was used because it is suitable for growth of *S. mutans*. 22.8 grams of agar powder was dissolved in 600mls of distilled water. The solution was autoclaved using an autoclave Baujahrs 1995 model at 121°C for 15 minutes and then waited to cool to 45°C. It was then transferred into sterile petri dishes where it cooled and solidified under sterile conditions. The petri dishes with the MHA media were incubated for 24 hours at 37°C to ensure that there was no contamination by any microorganisms.

3.11.1.3 Agar well diffusion assay

Sterile plates of solidified Mueller Hinton Agar were inoculated with two loopfuls of freshly prepared inocula equivalent to 1.5 x 10⁸ cfu/mL and streaked to form a mat of the test microorganism. Each plate was divided into four quadrats and each quadrat was labelled. Two quadrats were labelled with the respective solvent plant extract. For example one quadrat was labelled *Corchorus olitorius* L. methanol and the other *Acmella caulirhiza* Del. methanol. For the other two quadrats, one was labelled as negative control and the other as positive control. Four wells (2-solvent plant extracts, a positive and negative control) of 6 mm diameter were bored in the agar using a sterilized cork borer. About 50µL of 1000mg/ml of the different solvent plant extracts were introduced into each well using a micropipette. Ciprofloxacin was used as the
positive control, DMSO was used as the negative control for diethyl ether, methanol, total crude extracts and sterilized distilled water was used as the negative control for aqueous extracts. Ciprofloxacin is a broad spectrum, potent antibacterial drug against both gram positive and gram negative bacteria. It was used as the positive control on the basis that the local communities commonly use it for treatment of dental caries. The plates were inserted in an upright position in an anaerobic jar. An anaerobic gas pack and an indicator were also placed in the anaerobic jar. When the indicator turned pink, this showed that an anaerobic environment inside the anaerobic jar had been achieved. These were then incubated at 37°C for 48hrs as described by Jain et al. (2015), with modification. Antibacterial activity of the plant extracts was determined by measuring the zones of inhibition in millimeters using a Vernier caliper and read off against a millimeter ruler and recorded immediately. The tests were done in triplicates.

3.11.2 Determination of minimum inhibitory concentration (MIC)
The MIC was determined as the least concentration of the solvent plant extract that showed zone of inhibition using the agar well diffusion method. Only the active solvent plant extracts that showed antibacterial activity against Streptococcus mutans were used to determine MIC. However, all the solvent plant extracts were active against S. mutans and were used to determine MIC.

3.11.2.2 Preparation of media for determination of MIC
About 2.1 grams of Trypticasein soy broth (TSB) powder (Conda laboratories limited, South Africa), was dissolved in 71mls of distilled water and there after 1ml of TSB solution was transferred into test tubes. The test tubes having broth were autoclaved at 121°C for 15 minutes and then waited to cool to 45°C.

About 18.24 grams of Mueller Hinton Agar (MHA) powder (Conda laboratories limited, South Africa), was dissolved in 480mls of distilled water. Thereafter, the solution was autoclaved at 121°C for 15 minutes and left to cool to 45°C. It was then transferred into sterile petri dishes where it cooled and solidified under sterile conditions. The petri dishes with the MHA media were incubated for 24 hours at 37°C to ensure that there is no contamination by any micro-organisms.
### 3.11.2.2 Preparation of extracts in determination of MIC

Sterilized labelled test tubes having 1ml of trypticasein soy broth (TSB) were arranged so that 8 dilutions could be made for each solvent such as; aqueous, methanolic, diethyl ether and total crude extracts of both plants. The test tubes were labelled as 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256 to indicate the different serial dilutions. About 2 grams of the methanolic, diethyl ether, total crude extracts were weighed separately. A few drops of DMSO were added to each extract until dissolution and then topped up with sterilized distilled water to make 2ml with a final concentration of 1000mg/ml. About 2 grams of the aqueous extract were also weighed and dissolved with 2mls of sterilized distilled water to make a stock solution with a concentration of 1000mg/ml. Using double dilution method, 1ml from the stock solution of 1000mg/ml was pipetted using a micropipette and added to the 1ml of broth in a test tube labelled as 1/2 and mixed to make 2mls of the resultant solution of 500mg/ml concentration. 1ml was then pipetted from the test tube having 500mg/ml concentration and mixed with 1ml of broth in a test tube labelled as 1/4 to get 250mg/ml concentration which was quarter of the original stock concentration. The same procedure was repeated until 1ml of the final resultant solution of the least concentration in the test tube labelled 1/256 was pipetted and discarded off. Therefore, the stock solution of 1000mg/ml concentration was used to prepare concentrations of 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.625mg/ml, 7.8125mg/ml and 3.906mg/ml of *C. olitorius* L. and *A. caulirhiza* Del. (Wotoyitide, 2012) with modification.

### 3.11.2.3 Agar well diffusion assay in determination of MIC

Sterile plates of solidified Mueller Hinton Agar were inoculated with two loopfuls of freshly prepared inocula (24 hours old) equivalent to 1.5 x 10⁸ cfu/mL to form a cross and streaked to form a mat of the test microorganism. Each plate was divided into four quadrats and each quadrant was labelled. The quadrats were labelled depending on the serial dilution. Each quadrant was labelled as 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256 to indicate the different concentration. Four wells (each with a different concentration) of 6 mm diameter were bored in the agar using a sterilized cork borer. About 50µL of each concentration of the different solvent plant extracts were introduced into each well using a micropipette. The plates were inserted in an upright position in an anaerobic jar. An anaerobic gas pack and an indicator were also placed in the anaerobic jar. When the indicator turned pink, this showed that an anaerobic environment inside the anaerobic jar had been achieved. These were then incubated at 37°C for 48hrs as
described by Jain et al. (2015), with modification. MIC of aqueous, methanol, diethyl ether and total crude extracts of *C. olitorius* L. and *A. cailrhiza* Del. was determined as the concentration that showed the least zone of inhibition. The tests were done in triplicates.

3.11.3 **Determination of the minimum bactericidal concentration (MBC)**

The MBC was the minimum concentration at which no bacterial growth was observed. Only the concentrations of the different solvent plant extracts that showed zone of inhibition were used to determine MBC.

3.11.3.1 **Preparation of the media, extract and inoculate for determination of MBC**

The different dilutions of the solvent plant extracts used in MIC were obtained from the refrigerator and allowed to thaw to room temperature. 0.06g of trypticasein soy broth (TSB) were weighed and dissolved in 5mls of distilled water to make a solution. Thereafter, 100µl of TSB was transferred into different test tubes, autoclaved at 121˚C for 15 minutes and allowed to cool. 100µl of freshly prepared inoculate was pipetted into the test tube having TSB. 200µl were pipetted from the different diluted labeled solvent plant extracts of a higher concentration and added to the inoculum and TSB to obtain the desired final concentration of the plant extract (Andrews, 2001) with modification. For example, 200µl of 1000mg/ml of a plant extract was added to 200µl of inoculum and TSB resulting into a final concentration of 500mg/ml due to the dilution. Therefore 1000mg/ml, 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.625mg/ml, 7.8125mg/ml were used to prepare 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25 mg/ml, 15.625mg/ml, 7.8125mg/ml and 3.906mg/ml respectively. The tubes with inoculate, broth and solvent plant extract were incubated anaerobically at 37˚C for 24 hours.

3.11.3.2 **Preparation of media and inoculation of broth, inoculate and extracts in determination of MBC**

About 9.12 grams of MHA powder was weighed and dissolved in 240mls of distilled water. The resultant solution was autoclaved at 121˚C for 15 minutes and left to cool to 45˚C. It was then transferred into sterile petri dishes where it cooled and solidified under sterile conditions. The petri dishes with the MHA media were incubated for 24 hours at 37˚C to ensure that there was no contamination by any micro-organisms.
The petri dishes were labelled starting with the MIC value of the different solvent plant extracts until the highest concentration. For example, the MIC for diethyl ether extract of both plants was 62.5mg/ml. Therefore, concentrations starting with 62.5mg/ml up to 500mg/ml were used in the determination of MBC. The petri dishes with MHA were then inoculated with 2 loopfuls of a combination of the broth, inoculate and extract that had previously been incubated for 24 hours. The petri dishes were later incubated anaerobically at 37˚C for 24 hours. The plate with the least concentration of the herbal extract that had no growth of bacteria was taken as the MBC for the different solvent plant extracts.

3.12 Quality control

- The identity of the plants to be investigated was determined by an experienced botanist and they were independently confirmed by the team at the National Herbarium in the Department of Botany, Makerere University.
- A voucher specimen of each plant was also kept at the National Herbarium for future reference. Each plant was given an accession number namely; 48898N for *C. olitorius* L. and 48899N for *A. caulirhiza* Del.
- Diethyl ether, methanol, DMSO and ciprofloxacin were checked for potency and their shelf life was confirmed.
- Standard laboratory measures of asepsis and safety were observed before, during and after the experiments.
- Standard calibration of the weighing scale used were assessed to ensure accurate results.
- Results for the MIC and MBC were assessed by the investigator and a microbiologist and immediately photographed and recorded.
- Sterility of TSB, 5% sheep blood agar and MHA were ensured.

3.13 Data management and analysis of results

The data collected was checked for completeness and correctness, validated and exported to Microsoft office excel. The different zones of inhibition were measured to indicate activity of the different concentrations of the diethyl ether, methanol, aqueous and total crude plant extracts. The mean of the duplicated MIC and MBC results were calculated and eventually used to obtain the standard deviation. Standard error of deviation was calculated and used to determine the 95% confidence interval using Statistical Package for Social Science version 16, Analysis of Variance
ANOVA) and Tukey’s (Honest significant difference) test. The results of the zone of inhibition, MIC and MBC obtained were used to determine whether Streptococcus mutans was susceptible or resistant to the antibacterial properties of C. olitorius L and A. caulirhiza Del. This information was used to prove whether the claims that people have are true.

3.14 Ethical consideration

Ethical approval was obtained from Makerere University College of Health Sciences, School of Biomedical Sciences Higher Degrees Research and Ethics Committee and given an approval number which was SBS-HDREC-444.
CHAPTER FOUR: RESULTS

4.1 Percentage yield of Corchorus olitorius L. and Acmella caulirhiza Del. solvent extracts

Extraction of Corchorus olitorius L. and Acmella caulirhiza Del. with the different solvents shows that the aqueous extract of C. olitorius L. and A. caulirhiza Del. had the highest percentage yield. The diethyl ether extracts of both plant had the lowest percentage yield though the diethyl ether extract of A. caulirhiza Del. had the lower percentage yield of the two (Table 1).

Table 1: Percentage yields of the aqueous, methanolic, diethyl ether and total crude extracts of Corchorus olitorius L. and Acmella caulirhiza Del.

<table>
<thead>
<tr>
<th>Solvent extract</th>
<th>% yield of Corchorus olitorius L</th>
<th>% yield of Acmella caulirhiza Del.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>14.4</td>
<td>5.61</td>
</tr>
<tr>
<td>Methanol</td>
<td>3.32</td>
<td>5.04</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>2.02</td>
<td>0.86</td>
</tr>
</tbody>
</table>

The study reports that the aqueous, methanolic, diethyl ether and total crude extracts of A. caulirhiza Del. were light green and oily while those of C. olitorius L. were dark green and not oily.

4.2 Antibacterial activity of the plant extracts of Corchorus olitorius L. and Acmella caulirhiza Del against S. mutans

Using the agar well diffusion method, all the solvent extracts of C. olitorius L. and A. caulirhiza Del. showed antibacterial activity against S. mutans. The aqueous extract of C. olitorius L. and the diethyl ether extract of A. caulirhiza Del. had the highest antibacterial activity as observed from their zones of inhibition at a concentration of 1000mg/ml. Using 6mm diameter of inhibition as the cut off since it was the diameter of the well (Mounyr, Moulay, & Saad, 2016), there was significant inhibition of S. mutans from the aqueous, methanolic, diethyl ether and total crude extracts of both C. olitorius L. and A. caulirhiza Del. Ciprofloxacin which was used as the positive control also showed activity. DMSO and sterilized distilled water which were used as negative controls did not have any antibacterial activity (Table 2).
Table 2: Antibacterial activity of *Corchorus olitorius* L. and *Acmella caulirhiza* Del. against *Streptococcus mutans* at 1000mg/ml concentration for each solvent

<table>
<thead>
<tr>
<th>Medicinal plant</th>
<th>Zone of inhibition at 1000mg/ml concentration for each solvent extract (mm)</th>
<th>ANOVA p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous</td>
<td>Methanol</td>
</tr>
<tr>
<td><em>C. olitorius</em> L.</td>
<td>22.10±0.17&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>15.03±0.05&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>A. caulirhiza</em> Del.</td>
<td>15.03±0.05&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>10.03±0.05&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Using Tukey’s HSD test at p<0.05 to detect difference between two groups, <sup>a</sup> difference detected compared to ciprofloxacin, <sup>b</sup> difference detected compared to either DMSO or distilled water.

**4.3 Minimum inhibitory concentration (MIC) of *Corchorus olitorius* L. and *Acmella caulirhiza* Del. solvent extracts against *Streptococcus mutans***

Using agar well diffusion method, only the plant solvent extracts that showed antibacterial activity in terms of zone of inhibition were used to determine MIC. The minimum concentration of each solvent plant extract that showed the least zone of inhibition was determined as the MIC. The diethyl ether extracts of both *C. olitorius* L. and *A. caulirhiza* Del. had the lowest MIC of 62.5mg/ml. This was followed by the aqueous extract of *C. olitorius* L. and *A. caulirhiza* Del. along with the total crude extract of *C. olitorius* L. at 125mg/ml. The methanolic extract of *C. olitorius* L. and *A. caulirhiza* Del. together with the total crude extract of *A. caulirhiza* Del. had the highest MIC values of 500mg/ml as shown (Table 3).
### Table 3: Minimum inhibitory concentration (MIC) of *Corchorus olitorius* L. and *Acmella caulirhiza* Del. solvent extracts against *Streptococcus mutans*

<table>
<thead>
<tr>
<th>Medicinal plant</th>
<th>Minimum inhibitory concentration (mg/ml) for each solvent extract</th>
<th>ANOVA p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous</td>
<td>Methanol</td>
</tr>
<tr>
<td><em>C. olitorius</em> L.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>125.0±0.057&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>500.0±0.057&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>A. caulirhiza</em> Del.</td>
<td>125.0±0.005&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>500.0±0.057&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Using Tukey’s HSD test at p<0.05 to detect difference between two groups, *a* difference detected compared to ciprofloxacin, *b* difference detected compared to either DMSO or distilled water.

#### 4.4 Minimum bactericidal concentration (MBC) of *Corchorus olitorius* L. and *Acmella caulirhiza* Del. solvent extracts against *Streptococcus mutans*

Only the concentrations that showed zones of inhibition were used to determine MBC. Therefore, from the least to the highest concentration of a particular solvent plant extract with zones of inhibition were used to determine MBC. All solvent extracts of *C. olitorius* L. had a similar MBC of 250mg/ml and the aqueous extract of *A. caulirhiza* Del. had the lowest MBC of 250mg/ml (Table 4).

### Table 4: Minimum bactericidal concentration (MBC) of *Corchorus olitorius* L. and *Acmella caulirhiza* Del. solvent extracts against *Streptococcus mutans*

<table>
<thead>
<tr>
<th>Medicinal plants</th>
<th>Minimum bactericidal concentration (mg/ml) for each solvent extract</th>
<th>ANOVA p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous</td>
<td>Methanol</td>
</tr>
<tr>
<td><em>C. olitorius</em> L.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>250.0±0.00&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>250.0±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>A. caulirhiza</em> Del.</td>
<td>250.0±0.00&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>500.0±0.05&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Using Tukey’s HSD test at p<0.05 to detect difference between two groups, *a* difference detected compared to ciprofloxacin, *b* difference detected compared to either DMSO or distilled water.
CHAPTER FIVE: DISCUSSION

This study set out to determine the antibacterial activity of *Corchorus olitorius* L. and *Acmella caulirhiza* Del. against *Streptococcus mutans*. High percentage yield was obtained from the aqueous extracts of both *C. olitorius* L. and *A. caulirhiza* Del. However, in comparison, the diethyl ether extract of *A. caulirhiza* Del. which had the lowest percentage yield had the highest antibacterial activity in terms of zone of inhibition. This therefore, indicates that the inhibitory activity did not depend on the percentage yield.

In this study, it was observed that the aqueous, methanolic, diethyl ether and total crude extracts of both *C. olitorius* L. and *A. caulirhiza* Del. had antibacterial activity against *S. mutans* at a concentration of 1000mg/ml (Table 2). The difference in the antibacterial activity in terms of zone of inhibition of all solvent extracts of both *C. olitorius* L. and *A. caulirhiza* Del., was significantly greater than that of DMSO and distilled water. However, the antibacterial activity of the solvent extract of *C. olitorius* L. and *A. caulirhiza* Del., was less than that of ciprofloxacin. In addition, both plants had bacteriostatic and bactericidal effects as portrayed by their MIC and MBC values respectively. The difference in the MIC of the aqueous and total crude extracts of *C. olitorius* L. was not significant. For *A. caulirhiza* Del., the difference in the MIC of the methanolic and total crude extracts was also not significant. However, there was a difference in the MIC of the aqueous, diethyl ether, ciprofloxacin, DMSO and distilled water. In the determination of MBC, the difference in the MBC of all solvent plant extracts of *C. olitorius* L. was not significant. For *A. caulirhiza* Del., the aqueous extract had an MBC which was lower than the other plant solvent extracts. This difference was statistically significant. In addition, the difference in the MBC of the plant solvent extracts, negative and positive controls was also statistically significant.

Using the 6mm diameter of inhibition as the cut off, all the solvent extracts of *C. olitorius* L demonstrated antibacterial activity against *S. mutans* where the aqueous extract had the highest zone of inhibition of 22.10mm (Table 2). This result correlates with reports by Ilhan et al. (2007), in which it was demonstrated that *C. olitorius* L. had good antimicrobial activity against *E. coli*, *S. aureus*, *Y. enterocolitica*, *Geotrichum candidum* and *Botrytis cinerea*. The antibacterial activity of *C. olitorius* L. is due to the presence of phytochemical compounds as
reported by Mohammed (2016). According to Islam (2013) and Adegoke & Adebayo-Tayo (2009), *C. olitorius* L. is reported to contain a number of phytochemicals including triterpenes, alkaloids, coumarins, flavonoids and anthraquinone that possess antibacterial activity. This therefore, supports the antibacterial activity of *C. olitorius* L. against *S. mutans* in this study.

In addition, all the solvent extracts of *A. caulirhiza* Del. were also active against *S. mutans*. However, the methanolic and crude extracts of *A. caulirhiza* Del. had the lowest antibacterial activity (Table 2). *A. caulirhiza* Del. has also been reported to possess antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus pimulus* by Sinei et al. (2013). In addition, closely related *Acmella uliginosa* was reported to have antibacterial activity against both gram positive and gram negative bacteria (Lagnika et al., 2016). This is due to the presence of phytochemical compounds such as; flavonoids, naphtoquinone, triterpenes, tannins and coumarins (Lagnika et al., 2016). *A. caulirhiza* Del. is reported to contain flavonoids, triterpenoids, alkylamides, alkaloids, amino acids, tannins and phenolic compounds (Dubey et al., 2013). These phytochemicals present in *A. caulirhiza* Del. influence its antibacterial activity as is reported by Lagnika et al. (2016), in *Acmella uliginosa*. *A. caulirhiza* Del. is also reported to contain spilanthol, Undeca-2E,7Z,9E-trienoic acid isobutylamide, Undeca-2E-en-8,10-diionic acid isobutylamide, β-Sitosterol, Stigmasterol, α and β- Amyrin. The phytochemical compounds present in *A. caulirhiza* Del. could be responsible for its antibacterial activity against *S. mutans*.

Of the plant extracts that had the highest zone of inhibition, the aqueous extract of *C. olitorius* L. was more active as compared to the diethyl ether extract of *A. caulirhiza* Del. (Table 2).

Ciprofloxacin which was the positive control was highly active against *S. mutans* as compared to the plant extracts (Table 2). The difference in the antibacterial activity between ciprofloxacin and the plant extracts is due to the difference in purity since the plant extracts were used in their crude form. Solvents such as; DMSO and distilled water were used as the negative controls. These solvents that were used in dissolution and dilution of the plant extracts did not have any antibacterial activity and therefore did not affect the effect of the plant extracts against *S. mutans*.

For each solvent extract, both plants had similar MIC values for instance; the diethyl ether extract of both plants had 62.5mg/ml as their MIC except for the total crude extract where *C.
C. olitorius L. had a lower MIC compared to that of A. caulirhiza Del. (Table 3). In addition, the methanolic and total crude extracts of A. caulirhiza Del. further portrayed their weak antibacterial activity against S. mutans by having the highest MIC values. The diethyl ether extracts of both plants had the highest ability to inhibit growth of S. mutans possibly because the diethyl ether could have extracted phytochemical compounds with higher bacteriostatic properties.

C. olitorius L. had a higher ability to kill S. mutans as compared to A. caulirhiza Del. as portrayed by its MBC of 250mg/ml hence having higher bactericidal properties (Table 4). Only the aqueous extract of A. caulirhiza Del. was comparable to C. olitorius L.’s bactericidal activity. The aqueous extracts of both C. olitorius L. and A. caulirhiza Del. had good antibacterial activity where by S. mutans was susceptible to both plant extracts in terms of zone of inhibition. In addition, the aqueous extract in both plants had similar MIC and MBC values hence had similar bacteriostatic and bactericidal activities respectively. Therefore, the aqueous extract had the best ability to efficiently extract antibacterial phytochemicals with bactericidal activity against S. mutans. The results in this study are in line with those reported by Gilbert et al. (2014), where the aqueous extract of Dissotis thollonii Cogn. demonstrated better antibacterial activity against S. aureus, Shigella flexneri, E. coli, Salmonella typhi and Enterobacter aerogenes as compared to the methanolic extract. This therefore, supports the traditional use of decoctions of these plants in dental caries.

Generally, C. olitorius L. had a better antibacterial profile in terms of zone of inhibition, minimum inhibitory effects and bactericidal properties against S. mutans as compared to A. caulirhiza Del. This could possibly be due to C. olitorius L. having shown a higher number of phytochemical compounds with better antibacterial properties as described by Islam (2013), and Adegoke & Adebayo-Tayo (2009).

The therapeutic effects of plant materials generally results from a combination of secondary metabolites. Different phytochemical compounds found in C. olitorius L. and A. caulirhiza Del. exhibit different antibacterial mechanisms. For instance, flavonoids are known antimicrobial agents with various mechanisms like inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function and energy metabolism. Tannins are also known antimicrobial
agents that could inhibit the growth of microorganisms by precipitating the microbial protein and thus depriving them of nutritional proteins needed for their growth and development (Lagnika et al., 2016). Alkaloids are reported to inhibit nucleic acid protein and membrane phospholipid biosynthesis (Wotoyitide, 2012). The Phenolic compounds found in A. caulirhiza Del. inhibit growth of microorganisms through enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Cowan, 1999). Therefore, the existence of these different phytochemical compounds in C. olitorius L. and A. caulirhiza Del. contributes to their antibacterial activity against S. mutans, a cariogenic bacteria.

β-Sitosterol found in A. caulirhiza Del. has been reported to have antibacterial activity (Sen, Dhavan, Shukla, Singh, & Tejovathi, 2012). Sen et al. (2012), showed that β-sitosterol has antibacterial activity against different bacterial species including S. aureus and E. coli. This could partly explain the antibacterial activity of A. caulirhiza Del. against S. mutans which is also a gram positive bacteria as S. aureus. In addition, A. caulirhiza Del. contains different alkylamides including spilanthol (N-isobutyldecatriene-2,6,8-amide) (Crouch, Langlois, Mulholland, Nair, & Houghton, 2005). These amides may directly inhibit bacterial pathogens and also provide localised pain relief to patients (Crouch et al., 2005). As reported by Dubey et al. (2013), that A. caulirhiza Del. is used traditionally in treating toothache, stomachache and earache, this could also be attributed to the alkylamides that provide an analgesic effect.

Therefore, the observed antibacterial activity against S. mutans validates the traditional use of C. olitorius L. and A. caulirhiza Del. in treatment of decayed teeth, gingivitis, toothache or wounds in the mouth. As a result, these plants could be potential sources of new antibiotic compounds.
CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study has proved that C. olitorius L. and A. caulirhiza Del. possess antibacterial activity at a concentration of 1000mg/ml against the cariogenic bacteria S. mutans which is implicated in the initiation of dental caries. C. olitorius L. has been found to have a better antibacterial profile in terms of susceptibility, bacteriostatic and bactericidal properties against S. mutans as compared to A. caulirhiza Del. The aqueous extracts of both C. olitorius L. and A. caulirhiza Del. generally had the best antibacterial profile as compared to other solvent extracts. This therefore, supports the use of water locally in the extraction of these plants for prevention and treatment of dental caries.

This study is therefore in agreement with the use of these traditional medicinal plants in the prevention and treatment of dental caries.

6.2 Recommendations

- Further studies should be conducted to isolate bioactive compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.
- Antibacterial activity of C. olitorius L. and A. caulirhiza Del. against other cariogenic bacteria should be performed to establish their antimicrobial spectrum.
- Antimicrobial activity of C. olitorius L. and A. caulirhiza Del. on wild type organisms should be performed

6.3 Limitations

- The effect of different strains in the Streptococcus mutans on extract activity was not determined.
- The plant extracts used were in their crude form and this could have affected the antibacterial activity.
- The agar well diffusion method was used instead of the broth dilution method due to lack of a spectrophotometer.
REFERENCES


APPENDIX I: *Streptococcus mutans*

**Taxonomical Classification of *Streptococcus mutans***

- **Kingdom**: Bacteria
- **Phylum**: Firmicutes
- **Class**: Bacilli
- **Order**: Lactobacillales
- **Family**: Streptococcaceae
- **Genus**: *Streptococcus*
- **Species**: *mutans*

**Characteristics of *Streptococcus mutans***

<table>
<thead>
<tr>
<th>Test</th>
<th>Characteristic exhibited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram staining</td>
<td>• Gram positive</td>
</tr>
<tr>
<td>Microscopy</td>
<td>• Individual cocci are spherical or ovoid</td>
</tr>
<tr>
<td></td>
<td>• Arranged in chains</td>
</tr>
<tr>
<td>Fermentation of carbohydrates</td>
<td>• Positive</td>
</tr>
<tr>
<td>(mannitol, sorbitol, inulin, raffinose,</td>
<td></td>
</tr>
<tr>
<td>melibiose)</td>
<td></td>
</tr>
<tr>
<td>Hydrolysis of esculin</td>
<td>• Positive</td>
</tr>
<tr>
<td>Tolerance to 4% NaCl</td>
<td>• Positive</td>
</tr>
<tr>
<td>Tested with 10% mannitol and 4% 2,3,5-</td>
<td>• Dark pink color shown</td>
</tr>
<tr>
<td>triphenyltetrazolium chloride</td>
<td></td>
</tr>
<tr>
<td>Catalase test</td>
<td>• Negative</td>
</tr>
<tr>
<td>Specific type of exopolysaccharide produced</td>
<td>• polyglucan</td>
</tr>
<tr>
<td>Hemolysis on blood agar</td>
<td>• alpha - hemolytic coccus</td>
</tr>
<tr>
<td>Lancefield grouping</td>
<td>• Serotype C and F</td>
</tr>
</tbody>
</table>

Adopted from (Al-Mudallal et al., 2008; Vildósola Grez et al., 2013).
APPENDIX II: Plates showing zones of inhibition for methanol, diethyl ether, positive and negative controls for *Corchorus olitorius* L. and *Acmella caulirhiza* Del.
APPENDIX III: Plates used in the determination of MIC for diethyl ether extract for Corchorus olitorius L. and Acmella caulirhiza Del.

Plate 1: Muller Hinton agar plates after 48 hours of incubation showing different inhibition zones depending on the concentration of the diethyl ether plant extract of *Corchorus olitorius* L. and *Acmella caulirhiza* Del. against *Streptococcus mutans* in determination of MIC.

Key:

MHA : Muller Hinton agar

C.O : *Corchorus olitorius* L.

A.C : *Acmella caulirhiza* Del.

1/16 : 62.5mg/ml of the diethyl ether plant extract

1/8 : 125mg/ml of the diethyl ether plant extract

1/4 : 250mg/ml of the diethyl ether plant extract

1/2 : 500mg/ml of the diethyl ether plant extract
APPENDIX IV: Pictorial profile of some of the bioassay procedures

Picture 1: Broth for preparation of the different dilutions.

Picture 2: Different dilutions of the different solvent plant extracts with broth.
Picture 3: inoculated plates placed in an anaerobic jar having an anaerobic gas pack and an indicator that has turned pink to show anaerobiosis.

Picture 4: Principle investigator carrying out the dilutions of the different plant extracts.