ANTIBACTERIAL ACTIVITIES OF THREE MEDICINAL PLANTS ON ORGANISMS ASSOCIATED WITH DENTAL PLAQUE

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DECLARATION

It is hereby declared that the work in this thesis is original and was carried out by the student and supervised by the supervisors below. Work from other authors where cited have been duly acknowledged. This work has not been concurrently submitted in candidature for any degree.

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ABSTRACT

The antimicrobial properties of plants have shown promise for development of new drugs which might aid to overcome the increasing challenge of resistance and also the toxicity of the currently available antibiotics. Hence, the aim of the study was to investigate the antibacterial activities of *Sida acuta*, *Anthocleista nobilis*, and *Zanthozylum leprieurii* on clinical isolates of *Streptococcus mutans* and *Streptococcus sanguis* and two reference strains: *Streptococcus mutans* (ATCC 700610) and *Streptococcus sanguis* (ATCC 10556) which are known to cause dental caries and periodontal diseases.

The plants were selected based on existing traditional medicine knowledge, usage and interaction with herbal healers. The antibacterial activity of aqueous and ethanol extract was determined by agar-well diffusion method. Subsequently, Minimum Inhibitory Concentration (MIC) was determined by using macro broth dilution method at a concentration ranging between 200mg/ml and 3.125 mg/ml. Minimum Bactericidal Concentration (MBC) was obtained by sub-culturing the test dilution which showed no visible turbidity. Standard antibiotic penicillin (1.5i.u) and gentamicin (10µg) were used for comparison. Both the aqueous and ethanolic extract of the selected medicinal plants showed inhibition zones which did not differ significantly ($P>0.05$) against each tested bacteria. Among the plant parts tried on the microorganisms, the leaves of *Sida acuta* proved to be more potent than the stem bark of *Anthocleista nobilis*, followed by the stem bark of *Zanthozylum leprieurii*. The ethanolic extract of *Anthocleista nobilis* was more efficient in its antibacterial activity as compared to its aqueous extract. The inhibitory activity of *Anthocleista nobilis* stem bark against *Streptococcus mutans* was comparatively more than that of gentamicin (10µg) but less than penicillin (1.5i.u). The aqueous and ethanolic extract of *Zanthozylum leprieurii* did not have inhibitory activity

on *Streptococcus sanguis* strains. The ethanolic extract of *Zanthozylum leprieurii* showed better results as compared to the aqueous ones. The antibacterial activity of all the plant extracts were concentration dependent, increasing with increasing concentration ($P<0.05$). The results from the study support the ethnomedicinal use of the plants and suggest that the plants extracts have compounds with antibacterial properties that can be used, as phytotherapeutic agents in the developments of new drugs. *Sida acuta* and *Anthocleista nobilis* showed promising antibacterial activities and thus can be employed as an effective anti-plaque agent and can be used in the prevention of dental caries.

DEDICATION

This work is dedicated to God without whom I am nothing, and also to my parents, Mr. Helmut K. Nyadroh and Mrs. Lucy Nyadroh, my supervisors, Prof. Mercy J. Newman and Dr. Elizabeth S. Bannermann for their encouragement, invaluable support, prayer, and love.

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LIST OF ABBREVIATIONS

ANOVA	Analysis Of Variance
ATCC	American Type Culture Collection
API	Analytical Profile Index
CLSI	Clinical and Laboratory Standards Institute
CPMR	Centre for Plant Medicine Research
DMSO	Dimethyl Sulphoxide
LSD	Least Significant Difference
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
SPSS	Statistical Package for Social Sciences
UGDS	University of Ghana Dental School
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Traditional medicine may be defined as "the medicine that refers to health practices, approaches, knowledge and beliefs incorporating plant, and animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being" (WHO, 2006). Traditional medicines have been described by the World Health Organization as one of the surest means to achieve total health care coverage of the world's population. In spite of the sidelining of traditional medicine practices in the past, current attention has given it a new drive into research, investments, design of programmes in this field in several developing countries (Bodeker, 1994). The World Health Organization (WHO) estimates that up to 80% of the world's people rely on plants for their primary health care (Fennell *et al*., 2004). Despite the exceptional progress in synthetic organic chemistry of the 20th century, over twenty fifth of prescribed medicines in developed countries are derived directly or indirectly from plants (Newman *et al*., 2000). Ghana today has dual systems of medical practice in law. These are the traditional and modern medical practices and are promoted to co-existence in order to reach the greatest number of citizens (Abbiw, 1990). Traditional medicines play a vital role where approximately 70% of the population uses it services (Abbiw, 1990). These services serve as forerunners in the primary medical care of the population due to their accessibility and affordability to the vast rural populace (Abbiw, 1990). Herbal remedies used in folk medicine provide a remarkable and still largely unexplored source for the development of new drugs for chemotherapy which might aid to overcome the increasing challenge of resistance and also the toxicity of the currently

available antibiotics (Saied *et al*., 2003). It is therefore important to carry out screening of these plants in order to authenticate their use in folk medicine and to reveal the active principles present (Saied *et al*., 2003). It is in this context that the leaves of *Sida acuta* and stem bark of *Anthocleista nobilis* and *Zanthozylum leprieurii* were screened for antibacterial activity against clinical isolates of *Streptococcus mutans* and *Streptococcus sanguis*, which are known to cause dental caries and periodontal diseases (Petersen *et al*., 2005).

Sida acuta belongs to the family Malvaceae. This shrub is 30 to 100cm in height, with a strong taproot, stem and branches flattened at the extremities (Swarbrick, 1997). The leaves are slender, alternate, lancoelate, and acute. The leaves are 1.2 to 9 cm or more long and about 0.5 to 4 cm wide in diameter, with a pair of stipules (Swarbrick, 1997).

Anthocleista nobilis belongs to the family Gentianaceae. It is a small to medium sized tree up to 18m tall; the bark is smooth, pale grey, inner bark cream yellow and glandular. Leaves are opposite and crowed at the end of branchelets (Olatunji, 1983).

Zanthozylum leprieurii is a tree of about 24 metres high with large thorns. It belongs to the family, Rutaceae. The leaves are compound with reddish translucent glands scattered over the surface. The flowers are white in panicle inflorescences with lateral branches spike-like, and its fruits are bright orange to red with glandular pitted surface (Olatunji, 1983).

1.2 PROBLEM STATEMENT

The increasing resistance of pathogenic bacteria to currently used chemotherapeutics and antibiotics, opportunistic infections in immunocompromised individuals and financial considerations in developing countries have necessitated the need for a global alternative prevention treatment options and products of oral diseases that are safe, effective and are affordable (Tichy and Novak, 1998).

Despite several agents being commercially available, these chemicals can change oral micro-biota and have adverse side-effects such as vomiting, diarrhoea and tooth staining (Park, *et al*; 2003). For example, bacterial resistance to most (if not all) of the antibiotics commonly used to treat oral infections (penicillin and cephalosporin, erythromycin, tetracycline and derivatives and metronidazole) has been documented (Bidault and Grenier, 2007). In addition antibacterial agents such as cetylpyridinium chloride, chlorohexidine, and amine fluorides are reported to cause toxicity, teeth staining, and oral cancer (Knoll-Kohler and Stiebel, 2002). Hence, the search for alternative products continues and natural phytochemicals isolated from plants used in traditional medicine are considered as good alternatives to synthetic chemicals (Prabu *et al*; 2006). The three medicinal plants are selected for antimicrobial assay based on their ethnomedicinal and traditional use against infectious diseases, and interaction with herbal healers.

1.3 JUSTIFICATION

The continual emergence of resistance to synthetic antimicrobial agents has necessitated the need for the search of alternative means of treating infectious diseases. Herbal medicine is one of the areas that have shown to be a useful alternative. The study evaluated the antibacterial activities of *Sida acuta*, *Zanthozylum leprieurii*, and *Anthocleista nobilis* against clinical isolates of *Streptococcus mutans* and Streptococcus *sanguis* which are known to cause dental plaque. These would give an insight into their usage pharmaceutically as phytotherapeutic agents for the prevention and treatment of oral diseases, which may provide alternatives to synthetic antibiotics.

1.4 AIM AND OBJECTIVES

To investigate the antibacterial activities of the extracts of *Sida acuta, Zanthoxylum leprieurii* and *Anthocleista nobilis* on *Streptococcus mutans* and *Streptococcus sanguis* isolated from dental plaque of patients at the University of Ghana Dental School (UGDS).

1.5 SPECIFIC OBJECTIVES

- To evaluate and compare the antibacterial activity of the aqueous and ethanolic extracts of *Sida acuta, Zanthoxylum leprieurii* and *Anthocleista nobilis* on *Streptococcus mutans* and *Streptococcus sanguis* isolated from dental plaque of patients at the University of Ghana Dental School.
- To determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts of *Sida acuta, Zanthoxylum leprieurii* and *Anthocleista nobilis* against *Streptococcus mutans* and *Streptococcus sanguis* isolated from dental plaque of patients at the University of Ghana Dental School.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Dental Plaque

Dental plaque found on the tooth surface generally refers to the different microbial community (predominantly bacteria) embedded in a matrix of polymers of bacterial and salivary origin (Scheie, 1994). Dental plaque develops naturally on teeth and helps in preventing the colonization of the enamel by exogenous organisms by forming part of the host defense systems (Scheie, 1994). Dental caries and periodontal diseases are prevalent worldwide. Dental caries affects 60-90% of school-aged children as well as the vast majority of adults in most industrialized countries (Petersen *et al*; 2005). The cost of treating dental caries is very expensive for government of both developing and developed countries. In industrialized countries the cost of treating dental caries cost between 5% to 10% of total health care expenditures exceeding the cost of treating cardiovascular diseases, cancer and osteoporosis (Sheiham, 2001). The prevalence rate of dental caries is high in most developing low-income countries where more than 90% of caries is untreated (Wang, 2002). Oral health is essential to general well-being and relates to the quality of life that extends beyond the craniofacial complex. There is significant evidence linking poor oral health to chronic conditions, systemic diseases, such as cardiovascular diseases, rheumatoid arthritis and osteoporosis. These have also been linked with poor oral health and also lead to pregnancy complications such as preterm low birth weight (Yeo *et al*; 2005). Tooth loss due to poor periodontal health affect about 20% of adult globally, can result in morbidity and premature death. Dental plaque, an example of biofilm, has a greater risk of causing diseases because it is usually found at protected and stagnant surfaces (Scheie, 1994). The microorganisms that form plaque are mainly *Streptococcus mutans, Streptococcus sanguis,* and

anaerobes (Garcia, *et al*; 2008). Dental plaque is primarily composed of microorganisms and one gram of plaque (wet weight) contains approximately 10^{11} bacteria (Petersen *et al*., 2005). More than 500 distinct species of microbial species are found in dental plaque (Scheie, 1994). An individual can harbor 150 or more distinct species of microorganisms (Scheie, 1994). Studies estimate that about 25,000 species of bacteria are found in the oral cavity out of which 1,000 can exist as part of the dental biofilm ecosystem (Donlan and Costerton, 2002). The species abundance and biodiversity of microorganisms is due to the ecological factors provided by the mouth (Marsh *et al*., 2003). The main ecological factors are pH, saliva, Redox reactions and temperature (Marsh *et al*., 2011). The microorganisms prefer a pH of 7 (Neutral pH level). Saliva acts as a buffer, maintaining the pH at a range of 6.75 and 7.25 and also serves as source of nutrients for the microorganisms (Marsh *et al*., 2011). Gingival crevicular fluid also serves as nutrient source for the microorganisms (Marsh *et al*., 2011). The normal temperature in the mouth is 35^{0}C - 37°C. A significant shift in the dominant species in plaque occurs when there is a two degrees change in temperature (Marsh *et al*., 2011). Aerobic bacteria undergo Redox reactions to maintain the oxygen level in the mouth at a semi-stable homeostatic condition (Marsh *et al*., 2011). Acids released from dental plaque as a result of the fermentation of sugars by the microorganisms leads to demineralization of the adjacent tooth surface and consequently dental caries (Scheie, 1994). Dental plaque organisms also cause irritation around the gums which can result in gingivitis, periodontal diseases and tooth loss (Scheie, 1994).

Periodontal diseases may trigger pathways leading to cardiovascular diseases through direct or indirect effects of oral bacteria (Hernichel-Gorbach *et al*., 1996). Oral bacteria such as *Streptococcus sanguis* and Porphyromonas *gingivalis* induce platelet

aggregation, which leads to thrombus formation (Hernichel-Gorbach *et al*., 1996). These organisms have the platelet aggregation-associated protein, which is collagen-like molecule on their surface (Herzberg *et al*., 1998). There are over more than 1,000 reported cases of endocarditis associating it onset with dental procedures or diseases (Drangsholt, 1998). Dental procedures such tooth extraction, endodontic treatment, root scaling, periodontal surgery have been reported to cause bacteremia (Drangsholt, 1998). Persistent dental disease is painful, and most importantly it has been linked to diabetes, high blood pressure, heart diseases and multiple sclerosis later in life (Taylor *et al*., 2004) . Dental caries caused by dental plaque organisms also cause bad breath and foul taste, in some cases the infection can spread from the teeth to surrounding soft tissues which may leads to edentulous mouth (Baelum *et al*., 1997).

2.1.1 Mechanism of Dental Plaque Formation

The formation of dental plaque involves two types of bacterial adherent interactions. Firstly selective attachment of the bacteria to the acquired pellicle which is the inner-layer between plaque and the substratum (such as the tooth surface) and secondly bacterial accumulation through specific adhesive and cohesive interactions involving components of the plaque matrix and direct bacterial cell contact. Organisms are selectively attached to the pellicle due to specific interactions involving their cell surface constituents and macromolecules of the salivary pellicle. Organism such as *Streptococcus Salivarius* which is prominent in the dorsum of the tongue and in saliva do not adsorb well to teeth. Other organisms such as *Streptococcus sanguis* and *A. viscosus* which are numerous in saliva adsorb easily to the pellicle and are prominent in developing new plaque (Scheie, 1994).

In the first phase of bacterial adherance, the organisms are loosely attached to the surface by Van deer Waal forces due to the net negative charge on both the teeth and bacterial surface. There is no strong attraction because of the repulsive effect of the negative charges. The second phase of attachment leads to a firmer bonding which involves the linking of the polymeric substance on the bacterium surface to the target surface by the formation of hydrogen, hydrophobic or ionic bonds. The adsorption of *Streptococcus mutans* involves electrostatic interactions in which the bacterial binds to hydroxyapatite which include calcium and phosphate groups on the mineral surface. It is postulated that the cell wall teichoic acid which gives the bacteria a net negative charge forms bridges with the calcium ions on the enamel or pellicle. The bacteria attachment is via adhesins found on their surface which binds to specific receptors on the pellicle and other host tissues. Protein adhesins called 'lectin' binds to specific sugars. Other adhesins which have hydrophobic moieties binds to hydrophobic receptors. Adhesins aids in the recognition and binding to specific receptors and complex macromolecules (Scheie, 1994).

The second phase, involves bacterial accumulation. Bacterially-derived polymers and salivary components are both essential in this process. *Streptococcus mutans* synthesizes extracellular glucans and fructans from sucrose but not from other carbohydrates and this polymer synthesis helps it multiply in large masses. Recent studies show that certain serovars of *Streptococcus mutans* can form plaque in the absence of sucrose, however, such plaques are less tenacious to enamel than plaque formed in the presence of sucrose. The average range of pH in plaque is about 7.1 in caries free persons and about 5.5 in persons with extreme caries activities. A drop in pH causes the demineralization of the tooth surface. At a critical pH of 5.5 the tooth minerals lose calcium and phosphates into plaque. This buffering activity helps initially

in maintaining the pH at 5.5, a drop in the pH leads to the demineralization of the subsurface. A further decrease in the pH leads to demineralization of the enamel. An important discovery suggests that certain cariogenic and acidogenic species of *streptococcus* especially *Streptococcus mutans* metabolize dietary sucrose and synthesize glucan by using the enzyme glucosyltransferase. This enzyme is essential in the establishment of *Streptococcus mutans* in dental plaque. Certain cariogenic bacteria have reservoirs of carbohydrates which are obtained from intracellular polysaccharides which serves as a source of carbohydrate for fermentation and maintenance of acid production in plaque during periods when the individual's diet is sugar free (Scheie, 1994).

2.2 *Streptococcus mutans*

Streptococcus mutans belongs to the viridans group of streptococci, and is part of the normal oral flora of man, and is an aetiological agent in smooth-surface dental caries (Hamada *et al*., 1980). Streptococci of the *mutans* group are highly acidogenic; they produce short-chain carboxylic acids which dissolve hard tissues such as dentine and enamel, and are the most cariogenic pathogens (Shaw, 1987). In addition, they produce insoluble extracellular polysaccharides, which improve their adherence to the tooth surface and encourage biofilm formation by fermenting sucrose (Shen *et al*., 2004).

Biofilms can tolerate many antagonistic conditions such as variations in pH, antimicrobial agents, and nutrient and oxygen deprivation (Davey *et al*., 2000). Due to the considerable serological and genetic heterogeneity of human and animal *Streptococcus mutans* strains they are grouped into eight species collectively known as mutans streptococci with the major species in humans being *Streptococcus mutans* (serotype *c,e,f*) and *Streptococcus sopranos* (serotype *d,g*) with occasional isolation of *Streptococcus rattus* (serotype *b*) and *Streptococcus cricetus* (serotype *a*),

Streptococcus mutans serotype *c* account for 70-100% of human isolates of mutans streptococci (Loesche, 1986). *Streptococcus mutans* produces bacteriocin, mutacin, which is active against other streptococcal species and non-streptococcal Gram-positive bacteria (Hamada *et al*., 1975). Mutacin provides an ecological advantage in the adverse microbial community and also facilitate the transmission of species between mother and child (Grönroos *et al*., 1998). The efficient establishment and colonization of *Streptococcus mutans* in the oral cavity is due to the production of bacteriocin (Roger, 1976). The production of the enzyme haemolysin which is a virulence factor is responsible for blood invasion and aids in supplying them with their requirement of iron (Kuramitisu *et al*., 1993).

Virulence factors of *Streptococcus mutans* are mainly adhesion, acidogenicity, and acid tolerance. The adherence by *Streptococcus mutans* to teeth surfaces is by sucrose-dependent and sucrose-independent mechanisms. The former involves extracellular glucosyltransferase (GTFs) for synthesis of glucans (glucose polymers) that mediate bacterial adhesion and contribute to biofilm formation (Van Houte, 1994). *Streptococcus mutans* has three GTFs: GTF B, GTF C, and GTF D. The former two synthesize primarily water-insoluble glucans, while the latter synthesizes only water-soluble glucans; the activity of all three enzymes is required for optimal adherence of *Streptococcus mutans* (Tanzer *et al*., 2001). The enzymes are important virulent factors of *Streptococcus mutans* necessary for the pathogenesis of dental caries (Tanzer *et al*., 2001). *Streptococcus mutans* is also known to cause vaginitis (Rizvi *et al*., 2003).

2.3 *Streptococcus sanguis*

Streptococcus sanguis is a member of the viridans streptococcus group. It is Gram-positive cocci with alpha-hemolytic abilities (Zhu *et al*., 2001). *Streptococcus sanguis*

is an oral commensal involved in dental caries by pioneering bacteria colonization of the oral cavity leading to biofilm formation (Zhu *et al*., 2001).

It survives by metabolizing dietary sugars into organic acids particularly, lactate. The accumulation of bacteria, food particles and salivary constituents around and on the teeth leads to the formation of ecological niche dental plaque (Nikiforuk, 1985). The production of acid from the fermentation of dietary sugars leads to a fall in localized pH levels leading to the corrosion of the enamel and beginning of dental caries (Loesche, 1986). The frequency of *Streptococcus sanguis* is and its incidence is antagonistically related to cariogenic *Streptococcus mutans* by producing the enzyme sanguicin (Zhu *et al*., 2001).

Streptococcus sanguis cause infective endocarditis and other important diseases as atherosclerosis in risk patients, specifically after a trauma when it enters into circulating blood (Okahashi *et al*., 2010). In animal models, the severity of infective endocarditis is associated with the ability of *Streptococcus sanguis* to adhere to and activate platelets (Herzberg *et al*., 1992). About 60% of *Streptococcus sanguis* can induce human platelets to aggregate *in vitro* (Herzberg *et al*., 1992).

2.4 Herbal Medicine

Herbal medicines mainly used in traditional medical systems have been used in medical practice for thousands of years, due to their accessibility and affordability. Herbal medicines could therefore help to meet the health needs of majority of the populace who are unable to afford imported drugs or do not have access to the facilities of a modern hospital (krogsgaard *et al*., 1984). Using of plants for medicinal purposes is an important part of the culture and the tradition in Africa, thus 80% of the population depends directly on traditional medicine for their primary health care (Kirby *et al*., 1996).

Herbal medicines are prepared from a variety of medicinal plants materials which are the leaves, roots, bark, and fruits and they usually contain active biological ingredients used for treating chronic or mild ailments or diseases (De Slmet, 1992). These medicinal plants can be used to manage and treat almost all known diseases of man and animals (Lewington, 1990). The World Health Organization (WHO) estimates that up to 80% of the world's people rely on plants for their primary health care (Fennell *et al*., 2004).

Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants (Newman *et al*., 2000). This highlights the continuous interest in laboratory screening of medicinal plants, not only to determine the scientific rationale for their use, but also discover new active principles (Newman *et al*., 2000). It has been estimated that between 35,000 and 70,000 different species of plants have been used by various people of the world with the commercial value of plant-derived drugs by western drugs standing at about 40 billion United State dollars every year (Lewington, 1990). According to WHO about 74% of the 119 plant derived pharmaceutical medicines are used in modern medicine in ways that correlate directly with their traditional uses as plant medicine in native cultures (WHO, 2003).

The sophistication of herbal remedies varies around the world with technological advancement of countries that produce them and use them. These remedies usually ranges from teas and crude tablets used in traditional medicine to concentrated, standardized extracts produced in modern pharmaceutical industries under a physician's prescription and supervision (Chowa, 1996). In Europe, the herbal medicines are mainly grouped into three (3) categories. The first and the most rigorously controlled

are prescription drugs, which include injectable forms of phytomedicine and those used to treat life-threatening diseases. The second category is the over-the-counter (OTC) phytomedicines and the third category is traditional herbal remedy products that have not been clinically tested but judged safe on the basis of generations of use in the treatment of diseases without serious incidents (Chowa, 1996).

A special feature of plants is their ability and capacity to produce a large number of organic chemicals of high structural diversity called secondary metabolites (Shah, 2005). Screening of compounds obtained from medicinal plants for their pharmacological assay has indeed been the vast source of innumerable therapeutic agents representing molecular diversity engineered by nature (Shah, 2005). The use of plant derived new compounds that are not based on existing synthetic antimicrobial agents is one of the ways to prevent antibiotic resistance of pathogenic species (Shah, 2005). Phytomedicines have shown a great promise in the treatment of infections including viral infections (Cowan, 1999).

A lot more of the plants needs to be scientifically studied, even though some herbal medicines have withstood scientific testing. Most people patronize herbal medicines because they feel they are innocuous and less expensive than orthodox medicines, however, they can be hazardous if they are not used appropriately. For instance, herbal medicines have been reported to be responsible for about 35% of cases of acute renal failures in some African countries (Bull *et al*., 1989).

Addae-Mensah (1992), has reported that even though *Croton membranaceous* has diterpenes and alkaloids which possess anticancer and anti-ulcer properties, the same plant has phorbol esters which are known to be co-carcinogenic and can cause cancer of the oesophagus. He also reports that *Crotalaria*, *Senecio*, *Cynoglossum* and *Heliotropium* genera contains pyrrolizidine alkaloids found to be highly hepato-toxic.

Scientific investigations into herbal medicines must therefore involve the therapeutic effects as well as the conceivable long-term toxic effects which may not manifest themselves until it is too late.

The World Health Organization (WHO) since May 1978 has been making a study of medicinal plants and this has promoted the identification of about 20,000 species of medicinal plants with a more detailed investigation of a shortlist of 200 species. A larger number of these plants have their origins from the world's tropical forest and they are extensively used in traditional medicine, which play a major role in maintaining the health and welfare of both rural and city dwellers in developing countries (Robin, 2005).

Medicinal Plants are vital products found in the forest areas throughout the world especially in South Asia, from the plains to the high Himalayas and the tropical and subtropical belts (Komen 1991; Robin 2005). However, tropical forests are the source of a large proportion of the world's recognized medicinal plants. India has documented more than 2,500 plant species as having medicinal value, Sri Lanka about 1,400 and Nepal around 700. In Ghana, about 2,000-2,500 medicinal plants are used as medicinal plants and are found mostly at high altitudes particularly in stressful environments where they grow very slowly and cannot live elsewhere. Others are however, more largely distributed and adapt easily to different ecological conditions (Abbiw, 1990).

Medicinal plants in Africa and drugs derived from them are the back-bone of several food and pharmaceutical industries or treatment in primary health care and constitute a big fortune of great economic and strategic value (Luc van-puyvelde, 1988). Ghana has a particularly strong tradition in medicinal plants as they play a vital cultural and economic role in poverty alleviation, due to the fact that people of significant status both in villages and urban areas use these plants (Abbiw, 1990). Another reason for the

growing popularity of herbal medicine in Ghana is that many people believe they are safer and "more natural" than pharmaceutical products (Dwuma-Badu, 1986).

Many medicinal plants of Africa are still under investigation for their chemical components and bioactivity against microorganisms. Table 1, indicates the ethnomedicinal uses of *Sida acuta*, *Anthocleista nobilis*, and *Zanthozylum leprieurii* which holds an enormous promise for the development of antimicrobial agents.

Table 1: The Ethnomedicinal Uses of the Selected Plants

Plant Name (Family)	**Local Name**	**Traditional Medicinal Use**	**References**
Sida acuta (Malvaeceae)	Wirewood (Obraneatuto - Akan)	Used to treat asthma, renal inflammation, colds, fever, headache, ulcers, mouth ulcers worms Leaves used to treat diarrhoea, malaria, and dental diseases Haemorrhoids, fevers, impotency, gonorrhoea, and rheumatism. In mixture as aphrodisiac and for boils and eye cataracts	(Coee and Anderson, 1996) Nacoulma *et al*., (1996) (Dash, 1991; Pal and Jain, 1998)
Anthocleista nobilis (Gentianaceae)	(Wudifo kete – Akan)	Leaves used to treat abdominal pains of uterine origin The bark and roots are used to treat constipation, periodontal diseases, menstrual pains, fever, and stomach-ache	Olatunji, (1983)
Zanthozylum leprieurii (Rutaceae)	(Oyaa-Akan)	The bark, leaves, and roots are used for a variety of treatments including toothache, coughs, leprous ulcerations, rheumatism, lumbago, stomach ache, urinary and venereal diseases Stem bark is used to treat dental diseases, kidney pain, arthritis, skin infections, dysentery, intestinal worms, back pain, post-partum pain syphilitic sores, rheumatic pains, bleeding gums, and leprous ulcers	Olatunji, (1983) Oliver-Bever, (1982) Adesina, (1987)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 STUDY SITE AND POPULATION

Clinical sample collection started from February and ended in April 2014. A total of twenty (20) dental plaque samples were collected from the gingival side of the canine and molar teeth of each consented patient using sterile scalers and curettes at the University of Ghana Dental School (UGDS), Korle-Bu. The isolation and identification of organisms from dental plaque was performed at the Department of Microbiology, University of Ghana Medical School (UGMS), Korle-Bu.

The evaluation of the antimicrobial properties of the selected medicinal plants was done at the Microbiology Department of the Centre for Plant Medicine Research (CPMR) at Mampong-Akwapim.

3.1.2 STUDY DESIGN

An experimental study was used in carrying out this research which is aimed at evaluating the antimicrobial properties of leaves of *Sida acuta*, the bark of *Anthocleista nobilis* and *Zanthoxylum leprieurii* from traditional herbal healers who use these herbs to cure various diseases at Koforidua in the Eastern region against *Streptococcus mutans* and *Streptococcus sanguis* that cause dental plaque. A simple random sampling technique was employed in the research in which only patients with dental plaque were recruited. Samples from consented patients who met the inclusion criteria were taken for analysis.

3.1.3 INCLUSION CRITERIA

- Consented patients with dental plaque
- All plaque samples that show growth of organisms.

3.1.4 EXCLUSION CRITERIA

- Patients who did not consent to the study were excluded.

3.1.5 INFORMED CONSENT

The study was approved by the Ethical and Protocol Review Committee, University of Ghana Medical School (UGMS), College of Health Science, Korle-Bu. The protocol identification number of the ethical clearance for this study was MS-Et/M.4 – P 3.3/2013-2014 with reference number MS-AA/C.2/Vol.18^{A}. Consent was also sought from the Dean, University of Ghana Dental School (UGDS), Korle-Bu.

3.1.5 SAMPLE COLLECTION, TRANSPORT, AND STORAGE

The dental plaque samples were collected from the gingival side of the canine and molar teeth of consented patients who attended the University of Ghana Dental School (UGDS) Clinic, Korle-Bu using sterile scalers and curettes. The samples were placed into sterile tubes containing 5ml Brain Heart Infusion Broth (BHI, Difco, Detroit, USA) which was sealed tightly labeled and transported immediately to the laboratory and incubated at 37^{o}C for 18hours.

The medicinal plants included in the study, *Sida acuta*, *Anthocleista nobilis* and *Zanthoxylum leprieurii*, were collected from traditional herbal healers in Koforidua, Eastern region who use these plants to cure various diseases. The plants were authenticated by a Taxonomist from the Plant Development Department (PDD) of the Centre for Plant Medicine Research (CPMR) and voucher specimens (*Sida acuta* −

Voucher No: CPMR 7210, *Anthocleista nobilis* − Voucher No: CPMR 0610, *Zanthozylum leprieurii* − Voucher No: CPMR 50) of each plant kept at the Centre for Plant Medicine Research (CPMR) herbarium.

3.1.6 TEST MICROORGANISMS AND CONTROL ANTIBIOTICS

Six clinical strains were used in the study: *Streptococcus mutans W7, Streptococcus mutans W13, Streptococcus mutans W11, Streptococcus sanguis W14, Streptococcus sanguis W18,* and *Streptococcus sanguis W20.* Another two reference strains of *Streptococcus mutans* (ATCC 700610) and *Streptococcus sanguis* (ATCC 10556) were also tested. Penicillin (1.5i.u) and Gentamicin (10µg) antibiotics (Himedia, India) were used as positive controls.

3.2 METHODS

3.2.1 Extract Preparation

The Plants collected were sun dried for one week and ground in sterile mortar and pestle to fine powder. The powders were kept in transparent sealed plastic containers and stored until used. For the ethanol extraction 500g of the pulverized plant was macerated in 5 litres of 70% ethanol for 24 hours. The extract was then filtered using Whatman No. 1 filter paper and evaporated using a rotary evaporator and then lyophilized at the College of Agriculture, University of Ghana, Legon.

For the aqueous extraction, 500g of the pulverized plants was dissolved in 5 litres of distilled water and boiled using a hot plate at high temperature at 100^{o}C for 30 minutes and then low temperature at 60^{o}C for 15 minutes for concentration. The suspension was then filtered using Whatman No. 1 filter paper and the filtrate was lyophilized at the College of Agriculture, University of Ghana, Legon.

3.2.2 Isolation of bacteria from clinical specimen

The dental plaque samples were cultured in Brain Heart Infusion broth (BHI, Difco, Detroit, USA) and then incubated at 37ºC for 18 hours. After bacterial enrichment, the turbid broth media were cultured onto Blood Agar using streak plate method and incubated at 37ºC in 5% CO_2 for 48 hours.

3.2.3 Culture and Biochemical Identification of the Microorganisms

The cultured bacteria were examined for macroscopic characteristics such as colour, size, morphology and haemolysis on Blood agar. Differential media of Mitis Salivarius agar (MSA) aided in distinguishing viridans streptococcus from other streptococcus. Gram reaction showed Gram positive cocci in short or long chains. The slide catalase test was done using a drop of hydrogen peroxide (H_2O_2) on a microscope slide to distinguish *Streptococcus* species which were catalase negative from *Staphylococcus* species. Subsequently, optochin test was performed to distinguish viridans *Streptococcus* from pneumococci which are optochin (ethylhydrocupreine hydrochloride) sensitive. A disc (5 µg) of optochin was applied to the surface of an inoculated blood agar and incubated aerobically at 35-37^{o}C for 18-24 hours. The viridans streptococci were resistant showing an inhibition zone size of less than 10 mm. For biotyping, Analytical Profile Index 20 Strep (bioMérieux, France), an identification system for Streptococcaceae was used. The API 20 Strep strip contains 20 microtubes containing dehydrated substrates for the demonstration of enzymatic activity or the fermentation of sugars. The enzymatic test was inoculated with a dense suspension of the organism (turbidity greater than 4 McFarland) from a pure culture. During incubation, metabolism produces colour changes that were either spontaneous or revealed by the addition of reagents. The fermentation tests were inoculated with an

enriched medium which rehydrates the sugar substrates. Fermentation of carbohydrates is detected by a shift in the pH indicator. The reactions were read according to the reading table and the identification was obtained by referring to the Analytic Profile Index.

3.2.3 ANTIMICROBIAL ASSAY

3.2.3.1 Agar-well diffusion method

The spreading method of Cruickshank *et al*., (1980) and agar - well diffusion method was used. Each control strain and clinically isolated organism of *Streptococcus mutans* and *Streptococcus sanguis* was inoculated individually into 6ml of peptone broth (Sigma, P0556, Sigma-Aldrich Inc., USA) and the density was adjusted to 0.5 McFarland turbidity standards resulting in a suspension of 1.5×10^8 colony forming units.

Mueller Hinton agar with 5% sheep blood (Oxoid, CM0337, Oxoid Ltd, United Kingdom) was seeded with the test organisms and the plate made to dry for some few minutes. After, which wells were made in the agar using sterile cork borer measuring 6mm in diameter. About 100µl of the prepared 200mg/ml, 150mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/m, 3.125mg/ml of aqueous and ethanolic extracts of *Sida acuta*, *Zanthoxylum leprieurii* and *Anthocleista nobilis* were dispensed into the labeled wells. The plates were then kept in the refrigerator for one hour for the extract to diffuse into the medium. The aqueous extract was dissolved in 10mls sterile distilled water and the ethanolic extract was reconstituted in 10 ml of 20% Dimethyl Sulfoxide (DMSO). Penicillin (1.5i.u) and gentamicin (10µg) antibiotics (Himedia, India) were used as positive controls to compare the zones of inhibition with that of the extracts. Sterile distilled water and 5% DMSO was used as negative control. The plates

were incubated at 37ºC in 5% CO_2 for 24−48 hours. Analysis was done in duplicate. Diameters of the zones of inhibition in the duplicate plates were measured by calculating the difference between cork borer (6mm) and the zone of inhibition (Singh *et al*., 2002).

3.2.3.2 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts

The Minimum Inhibitory Concentration (MIC) was determined using the macro broth dilution method according to methods describe by CLSI, (2006). The plant extracts was diluted from concentrations ranging from 200 to 3.125 mg/ml in Mueller Hinton broth (Liofilchem, Italy). To each dilution tube 0.1 ml of bacterial inoculum was seeded. Control tubes with no bacterial inoculation were simultaneously maintained. Tubes were incubated at 35−37°C for 24 hours. The lowest concentration of the extract that produced no visible bacterial growth (turbidity) was recorded as the Minimum Inhibitory Concentration (CLSI, 2006). The Minimum Bactericidal Concentration was determined by sub-culturing the test dilution (which showed no visible turbidity) on to freshly prepared blood agar media. The plates were incubated for 18-42 h at 37 °C. The highest dilution that yielded no single bacterial colony on the Mueller agar plates was taken as the MBC.

3.2.3.3 Statistical Analysis

Student T-test was used to compare the significant difference between aqueous and ethanolic extracts (Appendix 9 □ 11). One-way ANOVA was used for the comparison of the means followed by post hoc LSD. $P \leq 0.05$ was considered statistically significant. Results were expressed as mean ± SD (Standard Deviation) data, using Statistical Package for Social Scientist (SPSS) version 20.0 statistical analysis software. Tables and graphical displays were also used to illustrate data.

CHAPTER FOUR

4.0 RESULTS

The susceptibility of the test organisms to the aqueous and ethanolic extracts of the individual medicinal plants namely, *Sida acuta, Anthocleista nobilis* and *Zanthozylum leprieurii* was studied. The isolates that showed zones of inhibition equal to or more than 7mm were considered susceptible. Figures 1–3 below summarizes the antibacterial activity of the aqueous and ethanolic extracts of the individual medicinal plants on the test organisms.

The aqueous extract of *Sida acuta* showed significant zones of inhibition against the test organisms. The maximum zone of inhibition was produced against the standard strain *S. sanguis* (ATCC 10556) as shown in Figure 1 with a mean inhibition zone of 21.5mm which was greater than the positive controls; Penicillin and Gentamicin which produced inhibition zones of 20mm and 12mm respectively. The aqueous extract of *Anthocleista nobilis* exhibited diverse antimicrobial activity against all the test organisms. The maximum zone of inhibition was produced against clinical strains of *S. mutans* and *S. sanguis* (ATCC 10556) with zones of inhibition 15.7mm and 15mm respectively which were higher than Gentamicin (positive control) which had mean inhibition zone of 12mm. However the zone of inhibition was lower than that of Penicillin (positive control) with a mean inhibition zone of 20mm as shown in Figure 2 and Table 3. The aqueous extract of *Zanthozylum leprieurii* did not show any inhibitory activity on *S. sanguis* strains (Table 4) although, there was a small zone of inhibition against *S. mutans* strains. The ethanolic extracts of the various plants demonstrated the highest inhibitory effects on most of the organisms. It was observed that the inhibitory activities of the plant extracts increased with increasing concentration. The ethanolic extract of *Sida acuta* leaves inhibited the growth of the clinical strains of *S. mutans* at

inhibition zone size of 22.7mm. This zone size was greater than penicillin and gentamicin (positive control) with inhibition zone sizes of 20mm and 12mm respectively. The ethanolic extract of *Anthocleista nobilis* exhibited inhibitory activity against the test organisms. The highest inhibitory zone of this extract was observed at 200mg/ml with the range of inhibition zone size of 17– 13.3mm as shown in Table 3 and Figure 2. The ethanolic extract *Zanthozylum leprieurii* did not show any inhibitory activity on *S. sanguis* strains (Table 7), however, there were smaller zones of inhibition recorded against *S. mutans* strains. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the extract against the microorganisms are shown in Figures 4 and 5. For instance, the MIC and MBC value of *Sida acuta* leaves for *S. mutans* clinical strains were both 3.125mg/ml. *Anthocleista nobilis*, *Zanthozylum leprieurii*, penicillin and gentamicin had MIC values of 50mg/ml, 100mg/ml, 6.25mg/ml, and 50mg/ml respectively, while their corresponding MBC values were 50mg/ml, 100mg/ml, 6.25mg/ml, and 50mg/ml. The MIC values of 100mg/ml, 100mg/ml, 3.125mg/ml, and 50mg/ml were obtained for *Sida acuta*, *Anthocleista nobilis*, Penicillin, and Gentamicin respectively against *S. sanguis* clinical strains, while their corresponding MBC values were 100mg/ml, 100mg/ml, 6.25mg/ml, and 50mg/ml (Figure 4 and 5).

The MIC values of *Anthocleista nobilis* against the test organisms were found to be greater than 3.125mg/ml. From the experiment it was shown that the *Sida acuta* leaves performed best followed by *Anthocleista nobilis* stem bark and *Zanthozylum leprieurii* stem bark.

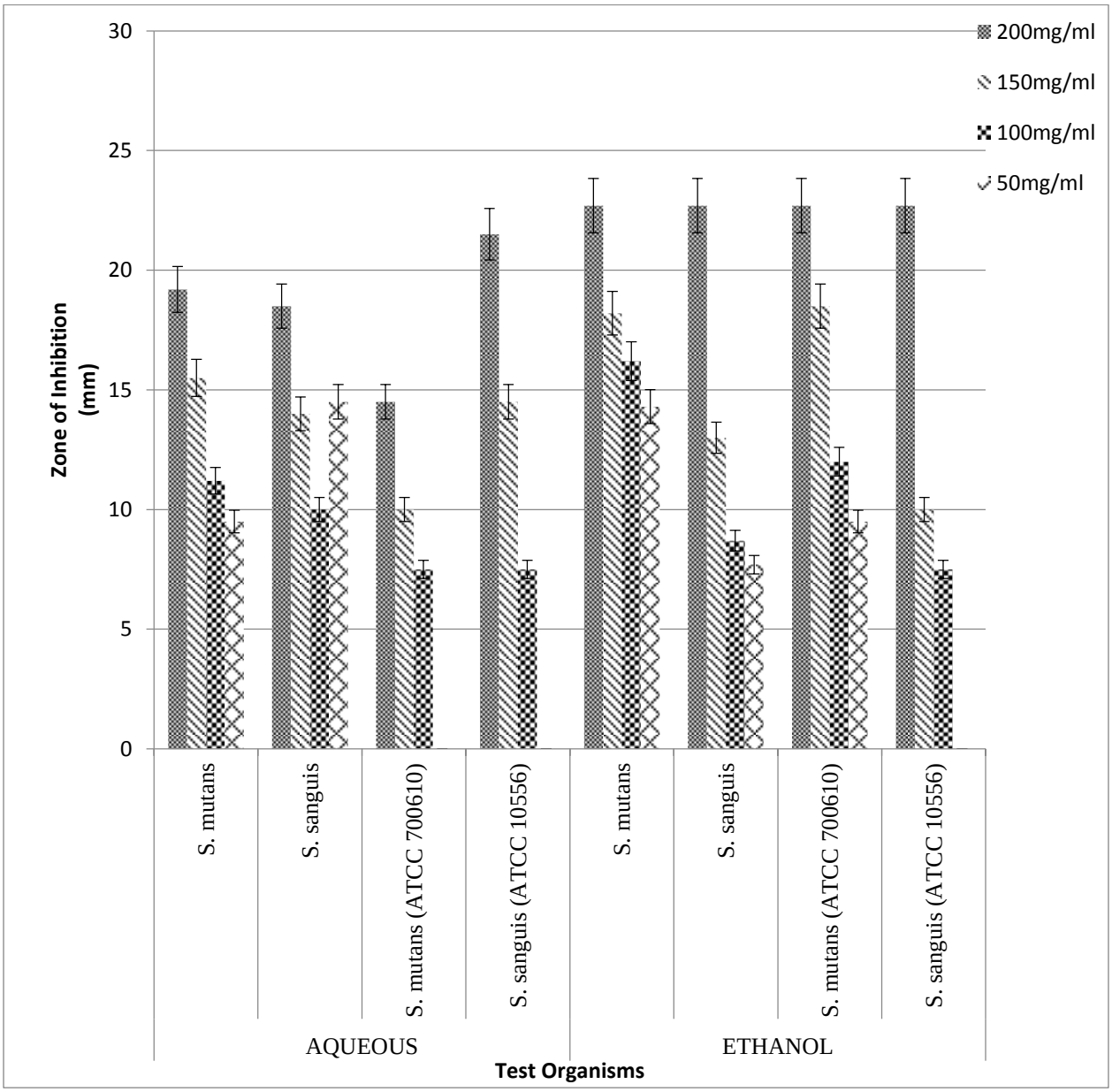

Figure 1: Antimicrobial Activity of *Sida acuta* Leaf Extracts on the Test Organisms

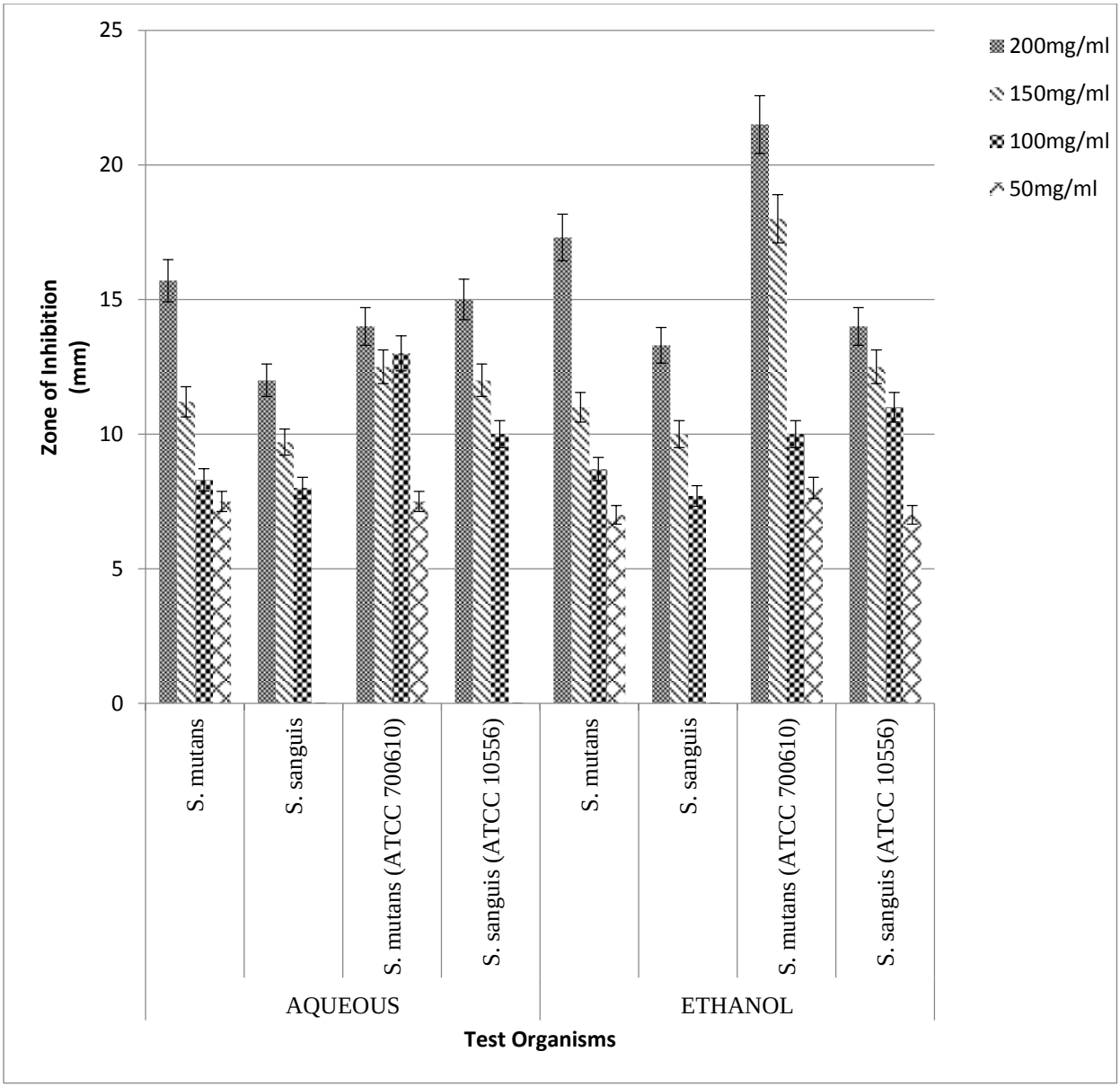

Figure 2: Antimicrobial Activity *Anthocleista nobilis* Stem bark Extract on the Test Organisms

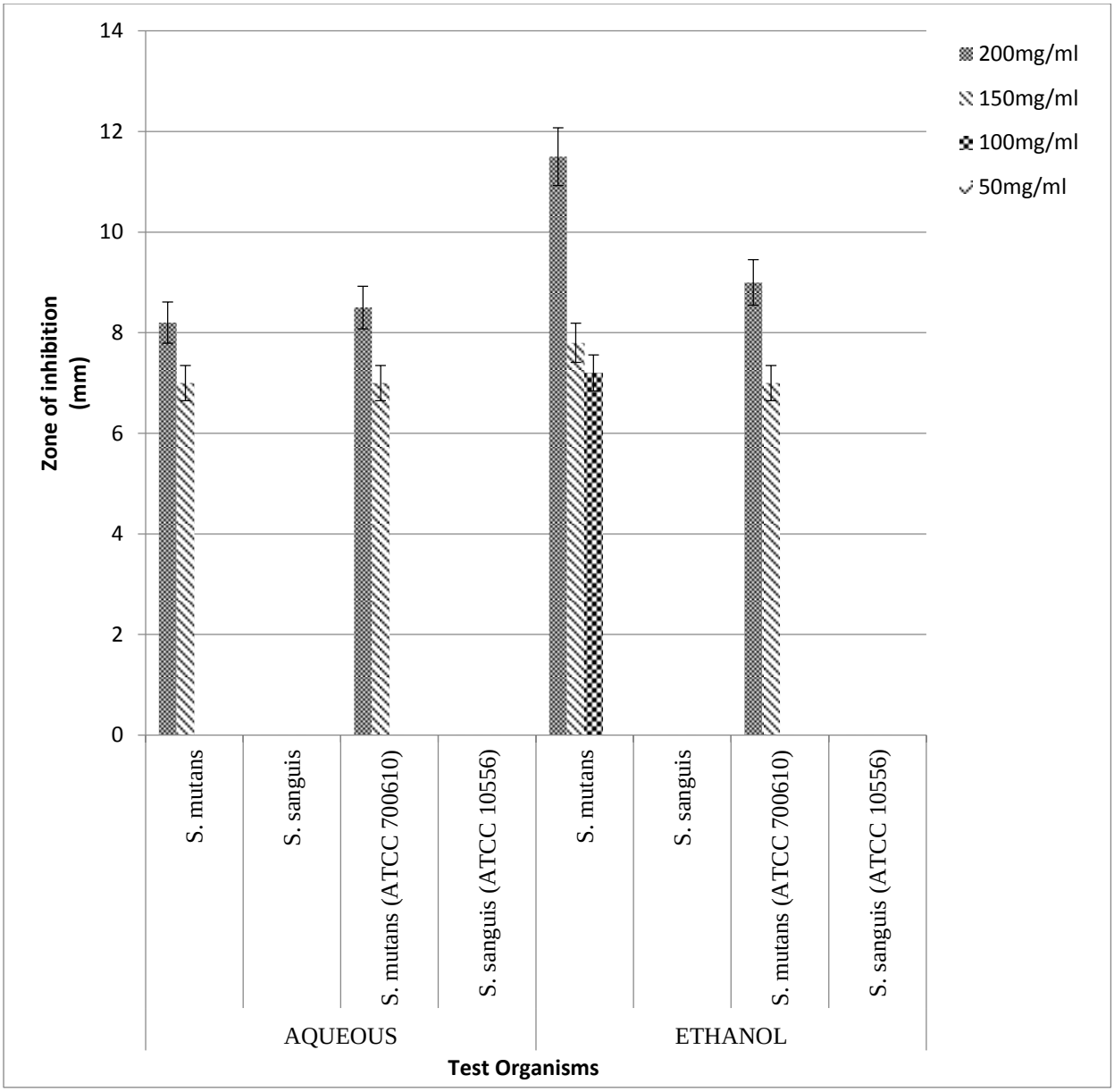

Figure 3: Antimicrobial Activity of *Zanthozylum leprieurii* Stem bark Extract on the Test Organisms

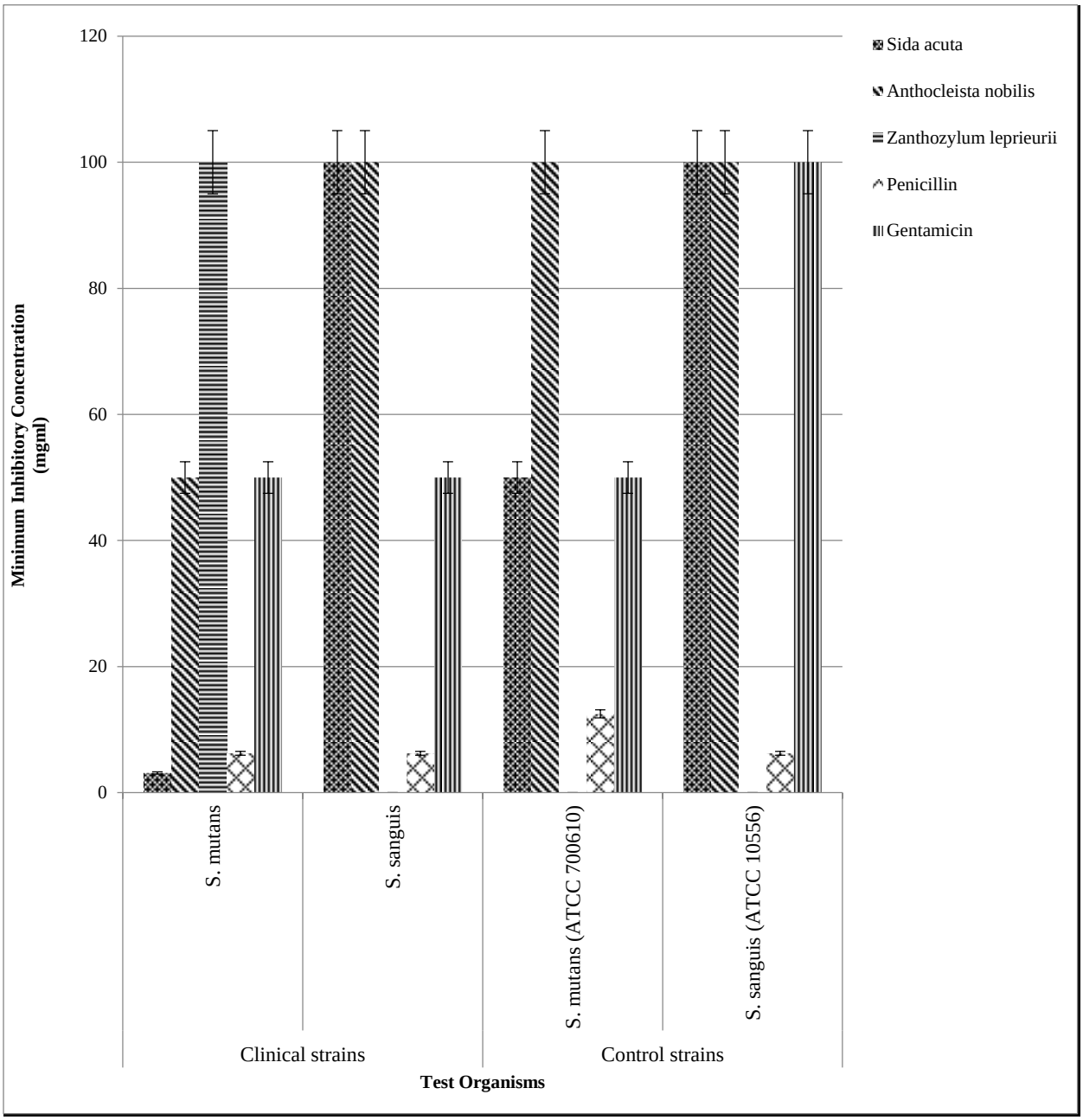

Figure 4: Minimum Inhibitory Concentration (MIC) of the Plant Extracts on Test Organisms

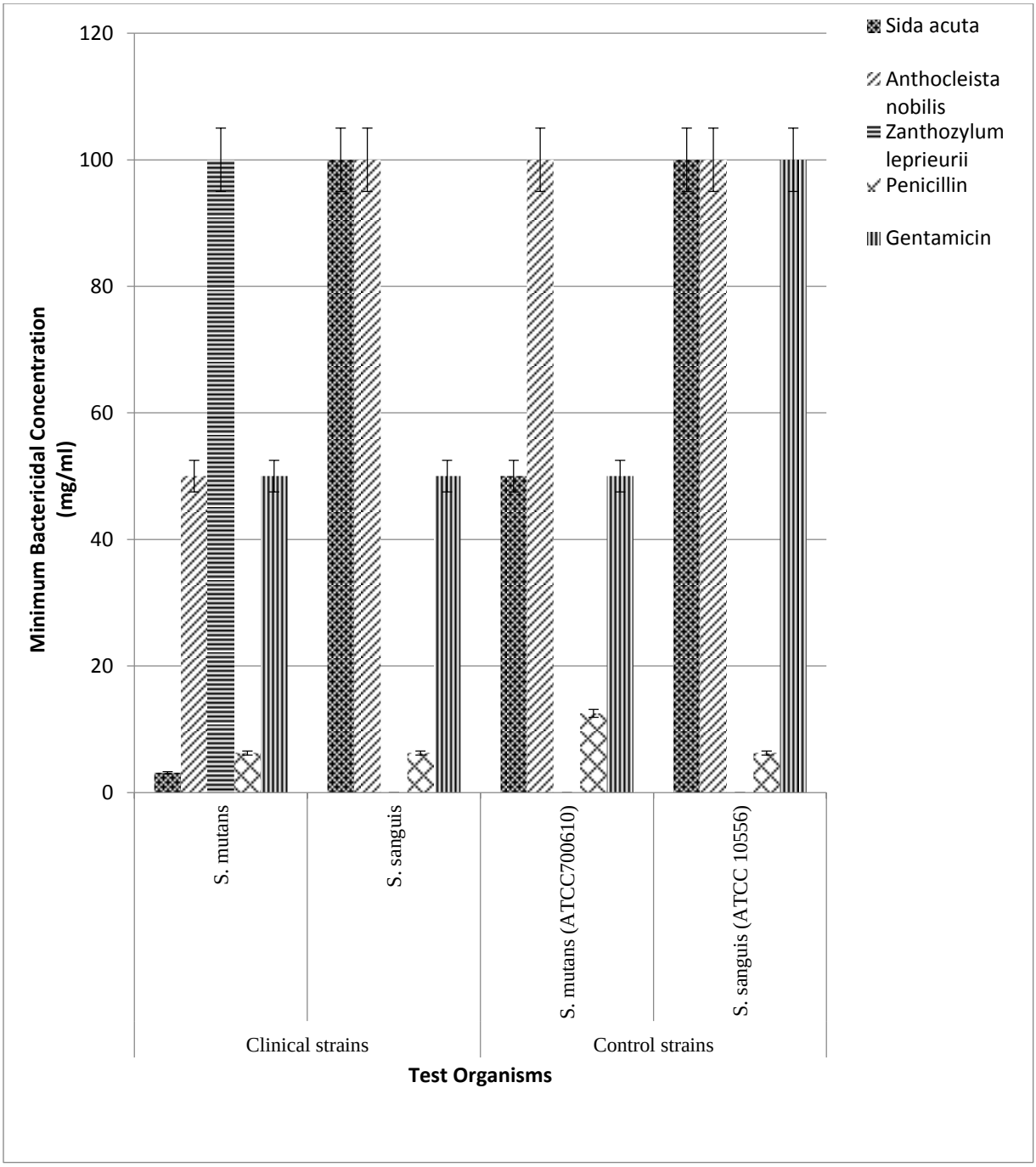

Figure 5: Minimum Bactericidal Concentration (MBC) Values of Respective MIC Concentrations of the Plants extracts on Test Organisms

CHAPTER FIVE

5.0 DISCUSSION

The increasing threat and spread of antibiotic resistance by a wide range of common pathogens has led to increased investigations into traditional medicinal plants as alternatives (Nostro *et al*., 2000). Many published works also show the efficacy of traditional medicinal plants against microorganisms (Evans *et al*., 2002). According to Tshibangu *et al.* (2002), plants have given western pharmacopoeia about 7000 different pharmaceutically essential compounds and a number of top-selling medicines of modern time, examples of which are artemisinin, taxol, camptothecin, and quinine.

In the present study, antibacterial activity of aqueous and ethanolic extracts of *Sida acuta*, *Anthocleista nobilis* and *Zanthozylum leprieurii* was effective against clinically isolated cariogenic bacteria. Among the three plants, the leaves of *Sida acuta* were found to be more active than the stem bark of *Anthocleista nobilis* and *Zanthozylum leprieurii*. This is probably why substantial research has been done on the leaves of *Sida acuta* as compare to the other plants.

The leaves of *Sida acuta* displayed good antibacterial activity against *Streptococcus mutans* and *Streptococcus sanguis* which are both Gram positive microorganisms. These findings were similar to studies done by Oboh *et al*. (2007) in which the leaves of *Sida acuta* was found to be effective against three Gram positive bacteria; *Streptococcus faecalis*, *Staphylococcus aureus*, and *Bacillus subtilis* (MIC values between 5-10 mg/ml). Anani *et al*. (2000) studied the antimicrobial activity of the aqueous and ethanolic extracts of the leaves of *Sida acuta*. They also used the Macro broth dilution method to determine the MIC values and obtained similar MIC (3.125 mg/ml) values against *Staphylococcus aureus* and *Bacillus subtilis* as was obtained in this research. Similar work done by Coee and Anderson (1996), Ekpo and Etim (2009),

and Oboh *et al*. (2007) showed that the extract of *Sida acuta* had preferential and specific activity against Gram positive bacteria such as *Staphylococcus aureus*, *Streptococcus faecalis*, and *Bacillus subtilis*. They also recorded MIC values lower than 50mg/ml. This confirms the present studies in which leaves of *Sida acuta* have significant antibacterial activities against *Streptococcus mutans* and *Streptococcus sanguis*. The high sensitivity of Gram positive bacteria could be attributed to the cell wall structure which has an outer peptidoglycan layer which is not an effective barrier as compared to Gram negative bacteria. The slightly higher potency of the ethanolic extract over the aqueous extract in the agar diffusion assay agrees with the reports of Essien *et al*. (2009). Limited studies have been done on the antimicrobial effects of the plant towards cariogenic bacteria. Studies done by Essien *et al*. (2009) suggest that the antimicrobial property of *Sida acuta* is attributed to the presence of alkaloids and flavonoids. The mechanism of action of the alkaloids is attributed to their ability to exert cytotoxic action via the inhibition of DNA synthesis and stabilization of topoisomerase II DNA covalent complexes (Rabe and Vastoden, 2000). Flavonoids obtained from *Sida acuta* inhibited *in vitro Streptococcus mutans* (Batsista *et al*., 1994). This report provides new evidence for efficacy against *Streptococcus mutans* and *Streptococcus sanguis*.

The stem bark of *Anthocleista nobilis* showed antibacterial activity against *Streptococcus mutans* and *Streptococcus sanguis* with MIC values between 50 – 100 mg/ml. These findings were similar to studies done by (Annan and Dickson, 2008) in which the aqueous and ethanolic stem bark of *Anthocliesta nobilis* was effective against two Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*). They obtained MIC values less than 500 mg/ml.

(Akinyemi and Ogundare, 2012) also detected that another species *Anthocleista djalonensis*, was effective against *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*. Therefore the antibacterial activity of *Anthocleista nobilis* obtained in this research agrees with the antimicrobial activity displayed by other *Anthocleista* species. Studies done by Kraus (1995) suggest that the antimicrobial property of *Anthocleista nobilis* is attributed to the presence polyphenols. Polyphenol is capable of carrying mass destruction to the cell membrane of Gram positive bacteria (Herrera-Arellano *et al*., 2004).

Zanthozylum leprieurii stem bark was the least effective against the test organisms. The aqueous and ethanolic extract had significant activity against *Streptococcus mutans* but did not have any inhibitory activity on *Streptococcus sanguis*. This could be due to the inherent resistant factor of the isolate and the likely previous exposure of the organism to other antimicrobial drugs or agents as a result of drug abuse in the population. The findings were similar with studies done by (Oshomo and Idu, 2012) in which the aqueous and ethanolic extract of *Zanthozylum zanthoxylloides*, another species inhibited the growth of *Streptococcus mutans*, *Bacillus subtilis*, and *Staphylococcus aureus*. Similar studies done by (Agyare *et al*. 2014) have showed that the plant extract is effective against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Bacillus subtilis* which are both Gram positive organisms. Yao *et al*. (2005) reported antibacterial efficacy of ethanol extract from stem bark of *Zanthozylum nitidum* growing in China against three oral pathogenic bacteria (*Streptococcus mutans*, *Actinomyces naeslundii*, and *Actinobacillus actinomycetes micomtans*). Wan *et al*. (2005) also observed that toothpaste containing *Zanthozylum nitidum* decreased the incidence of dental plaque and gingivitis. The efficacy of *Zanthozylum armatum* as used by the local inhabitants in Iran for the management of dental caries was confirmed by Negi and coworkers (Negi

et al. 2012) Therefore, the antibacterial activity displayed by *Zanthozylum leprieurii* obtained in this research falls in line with the antibacterial activity displayed by other *Zanthozylum* species. This infers that *Zanthozylum leprieurii* possesses antibacterial properties. However, none of the previous studies have addressed the antimicrobial effects on *Zanthozylum leprieurii* against *Streptococcus mutans* and *Streptococcus sanguis*. Previous studies attribute the antimicrobial activity of *Zanthozylum leprieurii* to the presence of alkaloids, flavonoids, terpenoids, and saponins (Sodipo *et al*., 1991; Stephen *et al*., 2009).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The study was done to investigate the antibacterial activities of the extracts of *Sida acuta*, *Zanthozylum leprieurii* and *Anthocleista nobilis* on *Streptococcus mutans* and *Streptococcus sanguis*. The result from the study shows that all the medicinal plants possessed antibacterial activities against the test microorganisms. *Sida acuta* and *Anthocleista nobilis* showed promising antibacterial activities and thus can be employed as an effective anti-plaque agent and can be used in the prevention of dental caries. From this study, it was observed that the ethanolic extracts exhibited high inhibitory activities on the test organisms than aqueous extracts. This could be due to the ability of ethanol to extract more of the essential oils and secondary plant metabolites which are believed to exert antibacterial activities on the test microorganisms. The results from the study support the ethnomedicinal use of the plants and suggest that the plant extracts have compounds with antibacterial properties that can be used as antimicrobial agents in the developments of new drugs.

6.2 RECOMMENDATIONS

Based on the findings from this study, it is recommended that:

1. Evaluation of the phytochemical properties and pharmacological studies of the medicinal plants should be conducted to determine their relative safety as a possible antimicrobial agent.
2. Toxicity studies should be carried out on the medicinal plants to determine their safety indices.
3. Clinical trials of the medicinal plants should be explored to determine the potential of these medicinal plants in the treatment of serious human and animal infectious diseases.

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Table 2: Antimicrobial activity of aqueous extract of *Sida acuta* against the test organisms

Concentration (mg/ml)	**Mean Zone of Inhibition (mm) ± SD**			
	S. mutans	*S. sanguis*	*S. mutans* (ATCC 700610)	*S. sanguis* (ATCC 10556)
200	19.2±0.2	18.7±0.0	14.5±0.3	21.5±0.7
150	15.5±0.1	14.0±0.0	10.0±0.0	14.5±0.7
100	11.2±0.1	7.7±0.0	7.5±0.3	7.5±0.7
50	9.5±0.2	0±0.0	0±0.0	0±0.0
25	7.5±0.2	0±0.0	0±0.0	0±0.0
12.5	7.3±0.0	0±0.0	0±0.0	0±0.0
6.25	0±0.0	0±0.0	0±0.0	0±0.0
3.125	0±0.0	0±0.0	0±0.0	0±0.0
Controls				
Penicillin(1.5i.u)	20.3±0.0	20.0±0.0	20.0±0.0	21±0.0
Gentamicin (10µg)	12.3±0.0	17.0±0.0	17.0±0.0	16±0.0
Distilled water	0±0.0	0±0.0	0±0.0	0±0.0

Table 3: Antimicrobial activity of aqueous extract of *Anthocleista nobilis* against the test organisms

	Mean Zone of Inhibition (mm) ± SD			
Concentration (mg/ml)	*S. mutans*	*S. sanguis*	*S. mutans* (ATCC 700610)	*S. sanguis* (ATCC 10556)
200	15.7±0.0	12±0.0	14±0.0	15±0.0
150	11.2±0.3	9.7±0.0	12.5±0.7	12±0.0
100	8.3±0.0	8.0±0.0	13±0.0	10±0.0
50	7.5±0.2	0±0.0	7.5±0.7	0±0.0
25	0±0.0	0±0.0	0±0.0	0±0.0
12.5	0±0.0	0±0.0	0±0.0	0±0.0
6.25	0±0.0	0±0.0	0±0.0	0±0.0
3.125	0±0.0	0±0.0	0±0.0	0±0.0
Controls				
Penicillin(1.5i.u)	20.3±0.0	20.0±0.0	20.0±0.0	21±0.0
Gentamicin (10µg)	12.3±0.0	17.0±0.0	17.0±0.0	16±0.0
Distilled water	0±0.0	0±0.0	0±0.0	0±0.0

Table 4: Antimicrobial activity of aqueous extract of *Zanthozylum leprieurii* against the test organisms

	Mean Zone of Inhibition (mm) ± SD			
Concentration (mg/ml)	*S. mutans*	*S. sanguis*	*S. mutans* (ATCC 700610)	*S. sanguis* (ATCC 10556)
200	8.2±0.1	0±0.0	8.5±0.7	0±0.0
150	7.0±0.0	0±0.0	7±0.0	0±0.0
100	0±0.0	0±0.0	0±0.0	0±0.0
50	0±0.0	0±0.0	0±0.0	0±0.0
25	0±0.0	0±0.0	0±0.0	0±0.0
12.5	0±0.0	0±0.0	0±0.0	0±0.0
6.25	0±0.0	0±0.0	0±0.0	0±0.0
3.125	0±0.0	0±0.0	0±0.0	0±0.0
Controls				
Penicillin(1.5i.u)	20.3±0.0	20.0±0.0	20.0±0.0	21±0.0
Gentamicin (10µg)	12.3±0.0	17.0±0.0	17.0±0.0	16±0.0
Distilled water	0±0.0	0±0.0	0±0.0	0±0.0

Table 5: Antimicrobial activity of ethanolic extract of *Sida acuta* against the test organisms

Concentration (mg/ml)	**Mean Zone of Inhibition (mm) ± SD**			
	S. mutans	*S. sanguis*	*S. mutans* (ATCC 700610)	*S. sanguis* (ATCC 10556)
200	22.7±0.1	18.8±0.2	22.5±0.7	15.5±0.3
150	18.2±0.2	13±0.0	18.5±0.7	10±0.0
100	16.2±0.2	8.7±0.0	12±0.0	7.5±0.3
50	14.3±0.0	7.7±0.0	9.5±0.7	0±0.0
25	12.7±0.2	0±0.0	0±0.0	0±0.0
12.5	10.8±0.0	0±0.0	0±0.0	0±0.0
6.25	9.5±0.0	0±0.0	0±0.0	0±0.0
3.125	7.8±0.1	0±0.0	0±0.0	0±0.0
Controls				
Penicillin(1.5i.u)	20.3±0.0	20.0±0.0	20.0±0.0	21±0.0
Gentamicin (10µg)	12.3±0.0	17.0±0.0	17.0±0.0	16±0.0
Distilled water	0±0.0	0±0.0	0±0.0	0±0.0

Table 6: Antimicrobial activity of ethanolic extract of *Anthocleista nobilis* against the test organisms

Concentration (mg/ml)	**Mean Zone of Inhibition (mm) ± SD**			
	S. mutans	*S. sanguis*	*S. mutans* (ATCC 700610)	*S. sanguis* (ATCC 10556)
200	17.3±0.0	13.3±0.0	21.5±0.7	14±0.0
150	11±0.0	10±0.0	18±0.0	12.5±0.3
100	8.7±0.0	7.7±0.0	10±0.0	11±0.0
50	7.0±0.0	0±0.0	8±0.0	7±0.0
25	0±0.0	0±0.0	0±0.0	0±0.0
12.5	0±0.0	0±0.0	0±0.0	0±0.0
6.25	0±0.0	0±0.0	0±0.0	0±0.0
3.125	0±0.0	0±0.0	0±0.0	0±0.0
Controls				
Penicillin(1.5i.u)	20.3±0.0	20.0±0.0	20.0±0.0	21±0.0
Gentamicin (10µg)	12.3±0.0	17.0±0.0	17.0±0.0	16±0.0
Distilled water	0±0.0	0±0.0	0±0.0	0±0.0

Table 7: Antimicrobial activity of ethanolic extract of *Zanthozylum leprieurii* against the test organisms

Concentration (mg/ml)	**Mean Zone of Inhibition (mm) ± SD**			
	S. mutans	*S. sanguis*	*S. mutans* (ATCC 700610)	*S. sanguis* (ATCC 10556)
200	11.5±0.0	0±0.0	9±0.0	0±0.0
150	7.8±0.2	0±0.0	7±0.0	0±0.0
100	7.2±0.0	0±0.0	0±0.0	0±0.0
50	0±0.0	0±0.0	0±0.0	0±0.0
25	0±0.0	0±0.0	0±0.0	0±0.0
12.5	0±0.0	0±0.0	0±0.0	0±0.0
6.25	0±0.0	0±0.0	0±0.0	0±0.0
3.125	0±0.0	0±0.0	0±0.0	0±0.0
Controls				
Penicillin(1.5i.u)	20.3±0.0	20.0±0.0	20.0±0.0	21±0.0
Gentamicin (10µg)	12.3±0.0	17.0±0.0	17.0±0.0	16±0.0
Distilled water	0±0.0	0±0.0	0±0.0	0±0.0

Table 8: The MIC values in mg/ml of the plant extracts in Mueller-Hinton Broth over a 24 hour period

Ethanolic Extract	*S. mutans*	*S. sanguis*	*S. mutans* (ATCC700610)	*S. sanguis* (ATCC 10556)
Sida acuta	3.125	100	25	100
Anthocleista nobilis	50	100	100	100
Zanthozylum leprieurii	100	0	0	0
Penicillin	6.25	3.125	3.125	6.25
Gentamicin	50	50	50	100

Table 9: The MBC values in mg/ml of the plant extracts on Mueller-Hinton Agar (5% Sheep blood) over a 24 hour period

Ethanolic Extract	*S. mutans*	*S. sanguis*	*S. mutans* (ATCC700610)	*S. sanguis* (ATCC 10556)
Sida acuta	3.125	100	50	100
Anthocleista nobilis	50	100	100	100
Zanthozylum leprieurii	100	0	0	0
Penicillin	6.25	6.25	12.5	6.25
Gentamicin	50	50	50	100

APPENDICES

Appendix 1: Independent sample test comparing the zone of inhibition between aqueous and ethanolic extract of *Sida acuta*

		t-test for Equality of Means					
						95% Confidence Interval of the Difference	
		t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Mean zone of inhibition (mm)	Equal variances assumed	-.747	30	**.461**	-1.28750	-4.80622	2.23122
	Equal variances not assumed	-.747	29.638	**.461**	-1.28750	-4.80802	2.23302

ANOVA comparing the zone of inhibition of the concentration of *Sida acuta*

Mean zone of inhibition (mm)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	491.556	3	163.852	19.593	**.000**
Within Groups	234.152	28	8.363		
Total	725.709	31			

Post- Hoc

Multiple Comparisons

Mean zone of inhibition (mm)

LSD

(I) Concentration (mg/ml)	(J) Concentration (mg/ml)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
50 mg/ml	100 mg/ml	-4.96250*	1.44591	**.002**	-7.9243	-2.0007
	150 mg/ml	-7.48750*	1.44591	**.000**	-10.4493	-4.5257
	200 mg/ml	-10.72500*	1.44591	**.000**	-13.6868	-7.7632
100 mg/ml	50 mg/ml	4.96250*	1.44591	**.002**	2.0007	7.9243
	150 mg/ml	-2.52500	1.44591	**.092**	-5.4868	.4368
	200 mg/ml	-5.76250*	1.44591	**.000**	-8.7243	-2.8007
150 mg/ml	50 mg/ml	7.48750*	1.44591	**.000**	4.5257	10.4493
	100 mg/ml	2.52500	1.44591	**.092**	-.4368	5.4868
	200 mg/ml	-3.23750*	1.44591	**.033**	-6.1993	-.2757
200 mg/ml	50 mg/ml	10.72500*	1.44591	**.000**	7.7632	13.6868
	100 mg/ml	5.76250*	1.44591	**.000**	2.8007	8.7243
	150 mg/ml	3.23750*	1.44591	**.033**	.2757	6.1993

*. The mean difference is significant at the 0.05 level.

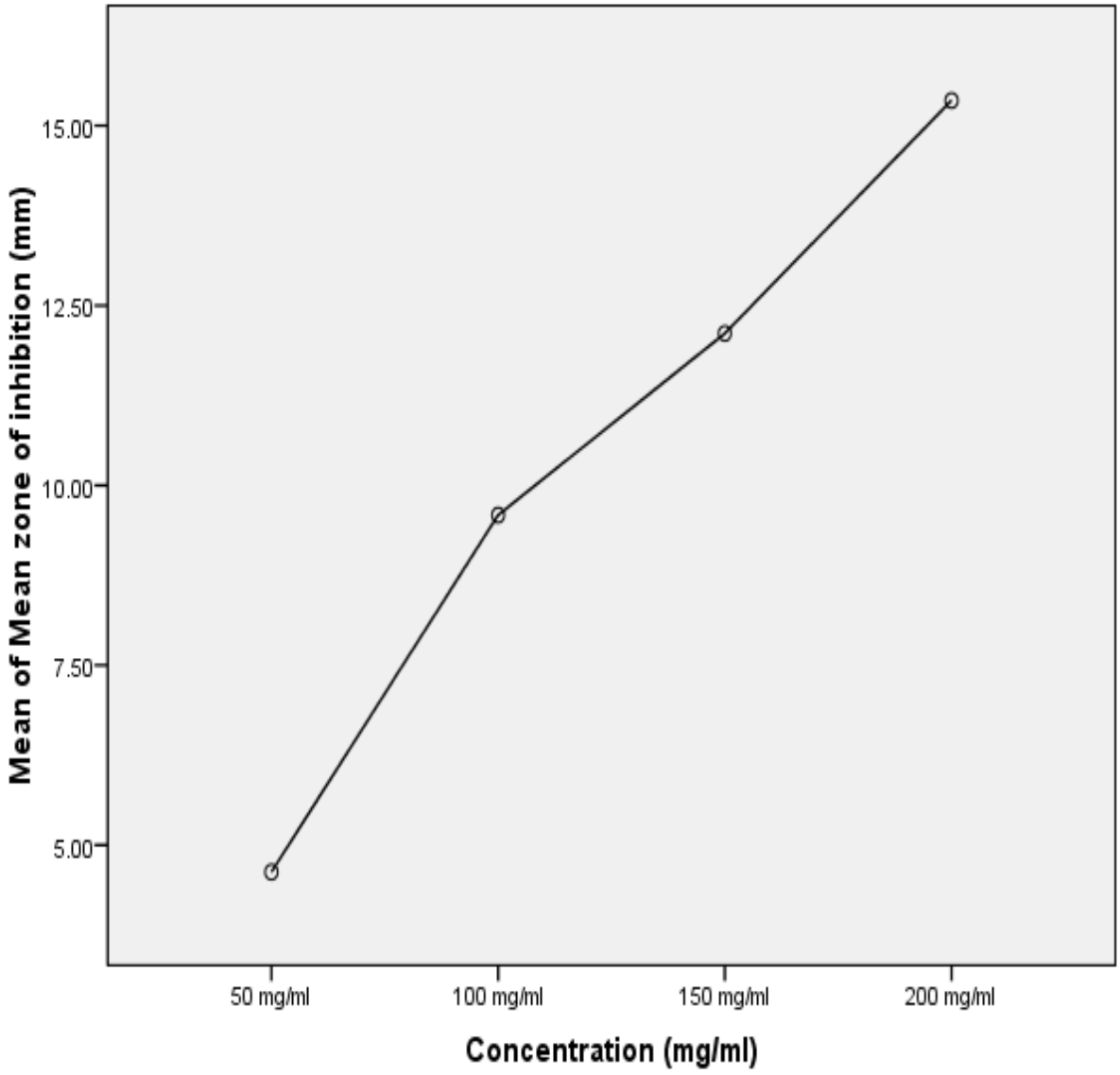

Figure 6: A Mean Plot of Zone of Inhibition against the Concentration of *Sida acuta*

Appendix 2: Independent sample test comparing the zone of inhibition between aqueous and ethanolic extract of *Anthocleista nobilis*

		t-test for Equality of Means					
						95% Confidence Interval of the Difference	
		t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Mean zone of inhibition (mm)	Equal variances assumed	-.747	30	**.461**	-1.28750	-4.80622	2.23122
	Equal variances not assumed	-.747	29.638	**.461**	-1.28750	-4.80802	2.23302

ANOVA comparing the zone of inhibition of the concentration of *Anthocleista nobilis*

Mean zone of inhibition (mm)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	491.556	3	163.852	19.593	.000
Within Groups	234.152	28	8.363		
Total	725.709	31			

Post- Hoc

Multiple Comparisons

Mean zone of inhibition (mm)

LSD

(I) Concentration (mg/ml)	(J) Concentration (mg/ml)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
50 mg/ml	100 mg/ml	-4.96250*	1.44591	**.002**	-7.9243	-2.0007
	150 mg/ml	-7.48750*	1.44591	**.000**	-10.4493	-4.5257
	200 mg/ml	-10.72500*	1.44591	**.000**	-13.6868	-7.7632
100 mg/ml	50 mg/ml	4.96250*	1.44591	**.002**	2.0007	7.9243
	150 mg/ml	-2.52500	1.44591	**.092**	-5.4868	.4368
	200 mg/ml	-5.76250*	1.44591	**.000**	-8.7243	-2.8007
150 mg/ml	50 mg/ml	7.48750*	1.44591	**.000**	4.5257	10.4493
	100 mg/ml	2.52500	1.44591	**.092**	-.4368	5.4868
	200 mg/ml	-3.23750*	1.44591	**.033**	-6.1993	-.2757
200 mg/ml	50 mg/ml	10.72500*	1.44591	**.000**	7.7632	13.6868
	100 mg/ml	5.76250*	1.44591	**.000**	2.8007	8.7243
	150 mg/ml	3.23750*	1.44591	**.033**	.2757	6.1993

*. The mean difference is significant at the 0.05 level.

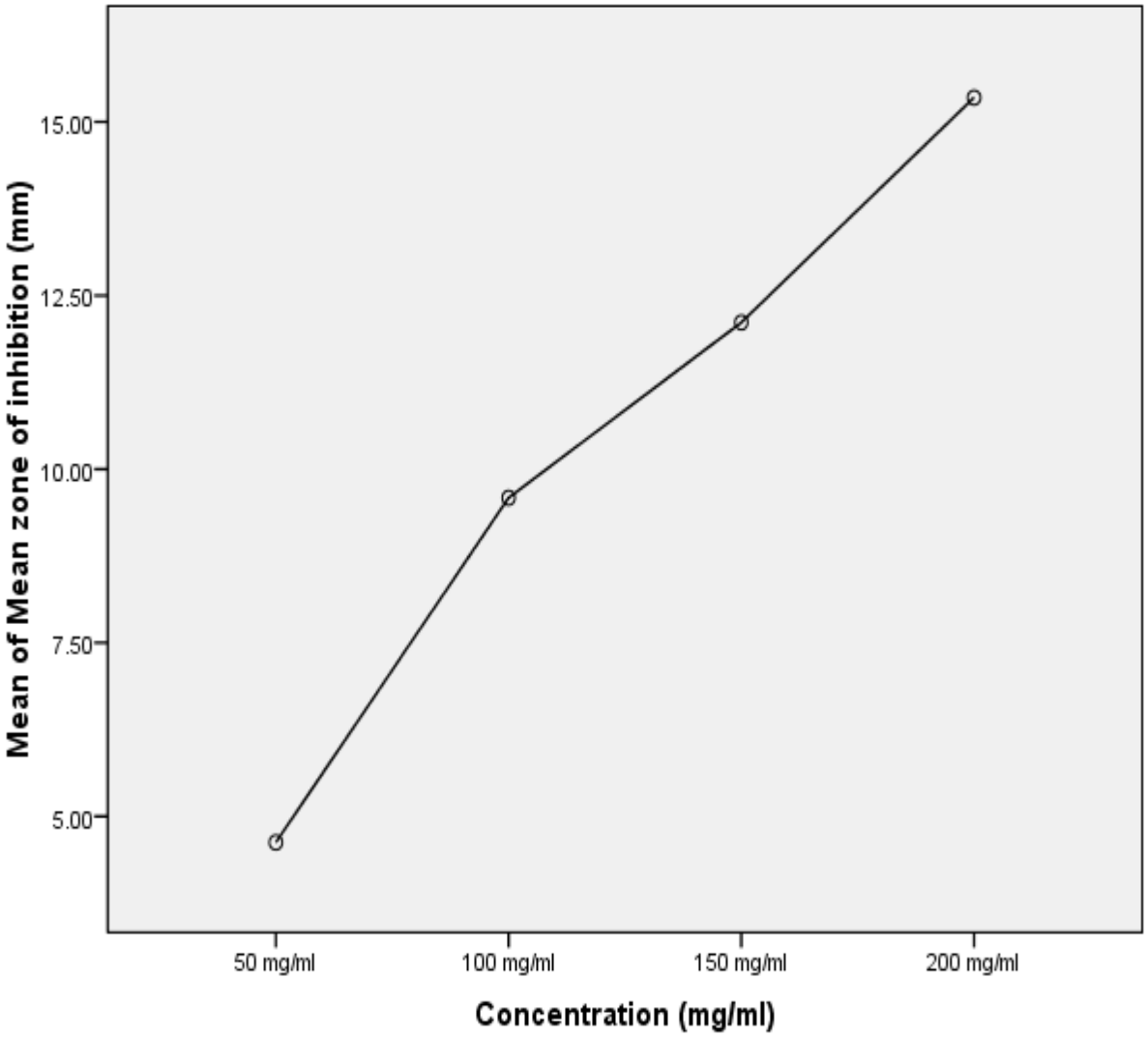

Figure 7: A Men Plot of Zone of Inhibition against the Concentration of *Anthocleista nobilis*

Appendix 3: Independent sample test comparing the zone of inhibition between aqueous and ethanolic extract of *Zanthozylum leprieurii*

		t-test for Equality of Means					
		t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
Mean zone of Inhibition (mm)	Equal variances assumed	-.544	30	.590	-.73750	-3.50471	2.02971
	Equal variances not assumed	-.544	28.961	.590	-.73750	-3.50888	2.03388

ANOVA comparing the zone of inhibition of the concentration of *Zanthozylum leprieurii*

Mean zone of Inhibition (mm)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	115.695	3	38.565	3.279	**.035**
Within Groups	329.280	28	11.760		
Total	444.975	31			

Post- Hoc

Multiple Comparisons

Mean zone of Inhibition (mm)

LSD

(I) Concentration (mg/ml)	(J) Concentration (mg/ml)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
50 mg/ml	100 mg/ml	-.90000	1.71464	.604	-4.4123	2.6123
	150 mg/ml	-3.60000*	1.71464	.045	-7.1123	-.0877
	200 mg/ml	-4.65000*	1.71464	.011	-8.1623	-1.1377
100 mg/ml	50 mg/ml	.90000	1.71464	.604	-2.6123	4.4123
	150 mg/ml	-2.70000	1.71464	.127	-6.2123	.8123
	200 mg/ml	-3.75000*	1.71464	.037	-7.2623	-.2377
150 mg/ml	50 mg/ml	3.60000*	1.71464	.045	.0877	7.1123
	100 mg/ml	2.70000	1.71464	.127	-.8123	6.2123
	200 mg/ml	-1.05000	1.71464	.545	-4.5623	2.4623
200 mg/ml	50 mg/ml	4.65000*	1.71464	.011	1.1377	8.1623
	100 mg/ml	3.75000*	1.71464	.037	.2377	7.2623
	150 mg/ml	1.05000	1.71464	.545	-2.4623	4.5623

*. The mean difference is significant at the 0.05 level.

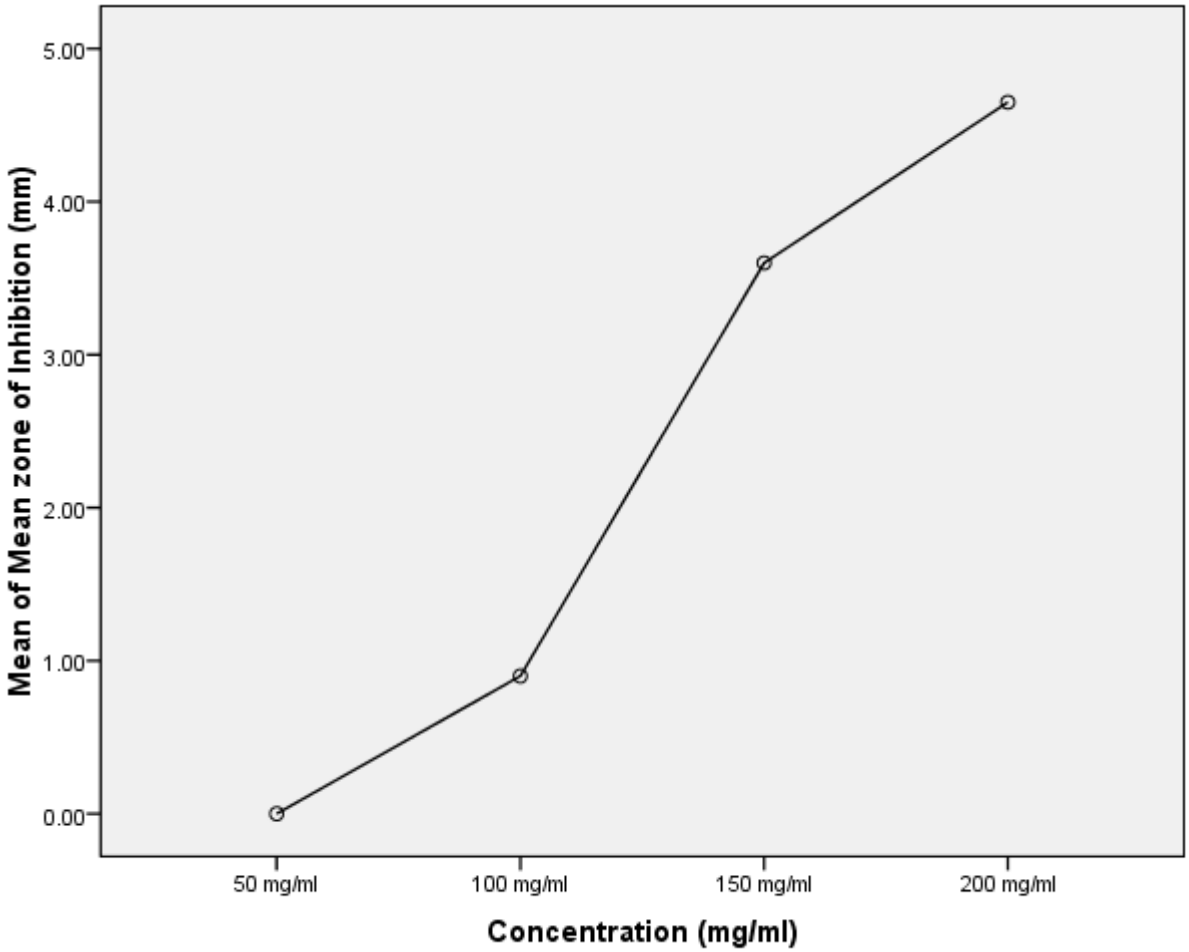

Figure 8: A Mean Plot of Zone of Inhibition against the Concentration of Zanthozylum *leprieurii*

Appendix 4: Consent Form

Participant ID Number:

Participant Name:

Study Title: Antibacterial activities of three medicinal plants on organisms associated with dental plaque.

Dear Participant,

Your permission is being sought to participate in a study which is described below. Before you decide whether or not to participate, you can talk to anyone you feel comfortable with. If certain aspects are not clear to you, you are at liberty to seek further clarification and I will take time to explain better. If there are other questions or issues bothering your mind, do not hesitate to ask me for answers. Your participation in this study is entirely voluntary. The information you will provide and the outcome of the analysis of your samples provided will not be used in any way that would go against your interest. Your participation and test results will be coded and therefore will remain confidential instead of your names. Therefore, if you decide not to consent or you consent and later decide to withdraw, there shall be no consequences attached to it and your decision shall be accepted in good faith.

The study in few words

Dental plaque is the predominant cause of tooth decay. Although the affliction is not life threatening, it causes nagging pain and thus possesses physical as well as psychological discomfort. The economic burden of the disease is also very high. Poor oral health affects the general population and it is often related to systemic diseases such as endocarditis, atherosclerosis, stroke, bacterial pneumonia and diabetes mellitus. Bacteria existing in the plaque or biofilm play an important role in the development of both dental caries and periodontal diseases. I am conducting a research into the

antibacterial activities of three medicinal plants namely *Sida acuta*, *Zanthoxylum leprieurii* and *Anthocleista nobilis* on *Streptococcus mutans* and *Streptococcus sanguis*.

Procedure

We will take dental plaque samples from your teeth. The dental plaque sample will be examined for isolation of *Streptococcus mutans* and *Streptococcus sanguis.*

Risks

There is no identifiable risk that this study may pose to you since no invasive procedure is employed.

Benefit

There may be no immediate personal benefit to you. On the other hand, results these test will be communicated to your clinician and copies kept in your folder.

Alternatives

The alternative to participating in this study is not participating.

Voluntary participation and confidentiality

Once again, your participation in this study is voluntary and you are free to withdraw from the study at any time. Any information you give us will be used for this study and will remain confidential and numbers and not your names will be used on samples.

Contact

Any questions concerning this study may be addressed to Mr. Clement E. Nyadroh (0245279207) of the Department of Microbiology, University of Ghana Medical School.

Participant: I understand all the above and hereby agree to participate or allow my ward to participate in this study.

_____	_____	_____
Name of participant	Signature/Thumbprint	Date
_____	_____	_____
Name of witness	Signature/Thumbprint	Date
_____	_____	_____
Name of investigator	Signature/Thumbprint	Date

Appendix 5: The Pictures of Selected Medicinal Plants

(http://www.rain-tree.com/htm)

Figure 8: *Sida acuta Burm*

(http://www.rain-tree.com/htm)

Figure 9: *Zanthozylum leprieurii*

(http://www.rain-tree.com/htm)

Figure 10: *Anthocleista nobilis*

Appendix 6: Ethical Clearance

UNIVERSITY OF GHANA MEDICAL SCHOOL
COLLEGE OF HEALTH SCIENCES
ACADEMIC AFFAIRS OFFICE

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My Ref. No: MS-AA/C.2/Vol.18[A]

P O Box 4236
Accra
Ghana

27th February, 2014

Your Ref. No.

Mr. Clement Eleseshie Nyadroh
Dept. of Microbiology
UGMS

ETHICAL CLEARANCE

Protocol Identification Number: MS-Et/M.4 – P 3.3 /2013-2014

The Ethical and Protocol Review Committee of the University of Ghana Medical School on 25th February, 2014 unanimously approved your research proposal.

TITLE OF PROTOCOL: **"Antibacterial Activities of three Medicinal Plants on Organisms Associated with Dental Plaque"**

PRINCIPAL INVESTIGATOR: **Mr. Clement Eleseshie Nyadroh**

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol Review Committee at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the Ethical and Protocol Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till December, 2014.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed:.....
PROFESSOR JENNIFER WELBECK
(CHAIRPERSON, ETHICAL AND PROTOCOL REVIEW COMMITTEE)

cc: Ag. Dean
Head of Department
Research Office