ANTI MICROBIAL
EFFECTS *Anacardiumoccidentale* (CASHEW) AND *Vernonia amygdalina* (BITT ER LEAF) AQUEOUS AND ETHANOLIC EXTRACTS ON ORAL BACTERIA

BY

EGIYI MUNACHIMSO VERA
GOU/U22/MCB/523

A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, FACULTY OF NATURAL SCIENCE AND ENVIRONMENTAL STUDIES, GODFREY OKOYE UNIVERSITY, UGWUOMU NIKE, ENUGU. ENUGU STATE.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE BACHELOR OF SCIENCE (B.Sc), DEGREE IN MICROBIOLOGY

SUPERVISOR: UKWUEZE, N. M.

JULY, 2026

ABSTRACT

Oral bacterial infections remain a major public health concern due to their association with dental caries, periodontal diseases, oral mucosal infections, and systemic health complications. The increasing emergence of antibiotic-resistant bacteria has created the need for effective, affordable, and safer antimicrobial agents from medicinal plants. *Anacardium occidentale* (cashew) and *Vernonia amygdalina* (bitter leaf) are widely used in traditional medicine for treating microbial infections; however, information on the antibacterial activities of their stem extracts against oral bacterial pathogens remains limited. This study evaluated the antimicrobial activities of aqueous and ethanolic stem extracts of *Anacardium occidentale* and *Vernonia amygdalina* against selected oral bacterial isolates and determined their Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Fresh stems were collected, authenticated, air-dried, pulverized, and extracted using distilled water and ethanol. Oral bacterial isolates were identified using colony morphology, Gram staining, and standard biochemical tests. Antibacterial activity was assessed using the agar well diffusion method at concentrations of 100, 50, 25, and 12.5 mg/mL. MIC was determined by broth dilution, while MBC was established by subculturing MIC broths showing no visible growth onto Mueller-Hinton agar. Amoxicillin and dimethyl sulfoxide (DMSO) served as the positive and negative controls, respectively. The isolates identified were *Bacillus spp., Staphylococcus spp.,* and *Streptococcus spp*. Both plant extracts demonstrated antibacterial activity against the test organisms. *Vernonia amygdalina* showed the highest activity, with maximum inhibition zones of 40 mm (aqueous) and 41 mm (ethanolic), while *Anacardium occidentale* produced inhibition zones of 37 mm (aqueous) and 29 mm (ethanolic). Antibacterial activity generally increased with extract concentration. The aqueous stem extract of *Anacardium occidentale* exhibited both MIC and MBC values of 25 mg/mL against the F4 (Bacillus spp.) isolate. These findings provide scientific evidence supporting the traditional use of both plants in managing oral bacterial infections and highlight their potential as sources of natural antimicrobial agents.

Keywords: Anacardium occidentale, Vernonia amygdalina, oral bacteria, antimicrobial activity, MIC, MBC, medicinal plants.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Our mouths are teeming with hundreds of bacterial species that constantly navigate the fragile line between oral health and oral disease; in fact, oral cavity remains one of the richest microbial ecosystems in the human body. The microbes inhabit dense oral biofilms in tooth crevices, gingival margin, and oral mucosa and the shift to dominance of oral pathogenseven in a healthy host, the ecological balance can be shifted toward pathogens (Baker et al., 2024). The majority of human diseases are caused by oral bacterial pathogens such as caries, gingivitis, periodontitis, halitosis and odontogenic infections.

Oral infections if left untreated can also aggravate other disorders, for example, respiratory diseases, oral complications in diabetes, and cardiovascular diseases (Morrison et al., 2023; Baker et al., 2024).

Oral microorganisms of most clinical relevance are Lactobacillusspecies that contribute to the pathogenesis of the disease lesion;S.aureus,an opportunistic oral pathogen that colonises the oral cavity and may aggravates oral infections and systemic infections;andS.mutans,a major etiologic factor in dental caries owing to their highly acidogenic and aciduric nature (Gao et al., 2023). Historically, management of oral microbial pathogens involve use of mechanical plaque removal and application of chemical agents such as antibiotic therapy and antiseptic Mouthrinses. However, injudicious and routine application of antibiotics/antiseptics have caused reduced potency of agents and development of resistance to antibiotics/antiseptics along with side effects such as irritation of the oral mucosa, discolouration of the teeth, taste changes and dysbiosis of oral microflora (World Health Organization, 2023). Microbial resistance (AMR) remains a significant threat to public health globally, particularly in developing countries where availability of alternative treatment modalities might be limited.

There are reports that Streptococci and Staphylococci isolates recovered from infected oral infections are becoming increasingly resistant, suggesting oral pathogens are not exempted (World Health Organization, 2023).

The increased incidence of drug-resistant oral microflora necessitate continued investigation in search for novel, cheap and safer agents. Among various potential natural antimicrobial agents, medicinal plants are emerging as a valuable source of these bioactive compounds. Medicinal plants have been used in treatment of various diseases from many centuries by the people ofAfrica,Asia andLatinAmerica.

Plants possess bioactive phytochemical compounds like flavonoids, tannins, alkaloids, saponins, terpenoids, and phenolic acids that exhibits broad range of antimicrobial activity (Okello et al., 2020). Plant extracts as opposed to antibiotics and antiseptic, exert effects on more than one target site thereby reducing development of resistance to such agents, thus sparking great interest among researcher to evaluate using a properly designed experiment on vitro the antimicrobial potential of those commonly use in folklore medicine. Anacardium occidentale Anacardium occidentale L., commonly known as cashew, belonging to the family Anacardiaceae is a tropical evergreen tree widely cultivated in Nigeria and other West African countries.

Anacardium occidentale has long been used traditionally as source of remedy against diseases such as diarrhea,skin infection, sore throat and toothaches with its leaves, stem bark, roots and nut used for various purpose (Akinpelu, 2001).

Phytochemical analysis revealed presence of anacardic acids, tannins, flavonoids and phenolic compounds having antibacterial properties (Agedahet al., 2010). It was demonstrated that cashew stem bark and leaf extracts exhibit antibacterial activity against Gram-positive bacteria such as StaphylococcusaureusandStaphylococcus.species as well as Gram-negative organisms likeEscherichia coli andStaphylococcusspecies (Akinpelu, 2001). These findings suggested the possibility of utilising cashew stem bark as natural antimicrobial agents against common oral bacterial infections. In the same vein, another most useful medicinal plant inAfricais Vernonia amygdalina commonly known asbitter leaf from thefamily Asteraceae; it is widely consumed as vegetable and applied traditionally as treatment for infection, diabetes, gastrointestinal complaints and malaria.

Traditional used as chewing stick in management of toothache and for general oral hygiene is of importance to our work (Alabiet al., 2011). Phytochemical screening has reveal the presence of numerous bioactive secondary metabolites that confer antibacterial and anti-inflammatory properties to Vernonia amygdalina such as sesquiterpene lactones, alkaloids, tannins, saponins and flavonoids (Ugboguet al., 2021).

Multiple study has proved Vernonia amygdalina exhibit antibacterial effect on several Oral bacteria likeStaphylococcusaureusandStaphylococcusand other non-oral bacteria.

The choice of extraction solvent and extraction methods is instrumental to determining the potency of the plant extract to inhibit microorganisms. Aqueous extraction is generally safe, non-polluting, and akin to how the plants have been traditionally prepared. Some lipid-soluble active components may not, however, be completely extracted with water.

Water may not also extract all bioactives.

Other lipophilic constituents such as flavonoids and phenolics have been documented to be extractable by alcoholic solvent such as ethanol with subsequent enhancement of the antimicrobial properties of extracts (Azwanida, 2020). To know the optimum extract medium, and patterns of change of antimicrobial activities as solvent vary between water and ethanol it is necessary to conduct a comparative study. Although both plants (Anacardium occidentale and Vernonia amygdalina) have been explored, few reports on their stem extracts are available, and there is insufficient knowledge on their comparative activities on oral bacteria. Previous researchers tend to investigate their leaf or bark extracts with insufficient information concerning variation of solvent impact or patterned susceptibility exhibited by the tested bacterial.

The antimicrobial activity of the two plants under uniform experimental conditions is relatively unreported.

1.2 Statement of the problem

Oral bacterial infections are among the most frequent human disease that cause the highest proportion of oral ailments such as dental caries, periodontitis, and gingivitis in all the world. Pathogens inhabit the oral cavity and can result in infection, inflammation, discomfort, tooth loss, and in more severe cases, can cause complications to the whole body systems. The extensive and persistent use of prescribed antimicrobial antibiotics for their treatment has lead to development of drug resistance by various oral bacterial pathogens, thus minimizing the effectiveness of available agents in combating the Infections.

Therefore, there is an urgent and paramount need for alternative sources of effective antimicrobials.

The use of medicinal plants is considered a potent alternative as it contains a large number of secondary metabolites possessing antimicrobial characteristics. The plant, Anacardium occidentale (cashew) and the plant, Vernonia amygdalina (bitter leaf) have a long history of traditional use in the management of infections including various diseases in which oral infections form a component of concern. The scientific literature on antibacterial activities of leaves, barks and roots of these plants on pathogenic bacteria has been documented, but minimal or lack of documentation on the stems of these two plants with respect to their activities against oral bacterial is evident. Furthermore, less attention has been paid on the evaluation of comparative antibacterial activity ofaqueousandethanolicstem extractsofthesestwo plantsandtotheirinhibitionofbacterialgrowth (bactericidalactivity), or only inhibiting bacteria growth (bacteriostatic).

Hence this present study was carried out to determine the antimicrobial activities of Aqueous and Ethanolic Stem ExtractsofAnacardium occidentaleandVernoniaamygdalinaon Selected Oral Bacterial Isolates and their MIC (Minimum inhibitory concentration) andMBC (Minimum bactericidal concentration) values were determined.

1.3 Aim of the study

OBJECTIVES 1.3

Aim of the study This research study was conducted to determine the antimicrobial activities of the aqueous and ethanolic stem extract of Anacardium occidentale and Vernonia amygdalina against some selected oral bacteria isolate.

1.4 Specific Objectives

The objectives of this research are: To extract the aqueous and ethanolic stem extracts of Anacardium occidentale and Vernonia amygdalina. To isolate and characterize the Oral bacteria pathogens from the oral swab sample using standard microbiology and biochemical methods.

To determine the antibacterial activities of the aqueous and ethanolic stem extracts of Anacardium occidentale and Vernonia amygdalina against isolated Oral bacteria by the method of agar well diffusion.

To compare the antibacterial activities of the aqueous and ethanolic stem extracts against the test bacterial isolates. To determine the Minimum Inhibitory Concentration (MIC) of the active stem extract against sensitive oral bacterial isolate. To determine the Minimum Bactericidal Concentration (MBC) of the active stem extract against sensitive oral bacterial isolates. To compare the antibacterial activities of the plant extract against amoxicillin (the positive control).

CHAPTER TWO

LITERATURE REVIEW

2.1 Importance of oral health in overall human health

It comes as no surprise to learn that poor oral health has a negative impact on your overall health and well-being, and it has recently begun to be treated as a part of systemic health rather than as an isolated condition limited to the oral cavity. The World Health Organization defines oral health as: ‘a state of being free from chronic mouth and facial pain, oral and throat cancer, oral infection and sores, periodontal disease, tooth decay, tooth loss and other diseases that impair an individual’s ability to bite, chew, smile, talk, and psychosocial well-being’ (World Health Organization, 2022). Clearly, oral health isn’t only a matter of being free from disease, but also one that concerns functional, psychological, and social aspects of your health.

The human oral cavity is the entry point of the human body and performs numerous essential physiological functions including chewing, digestion, speech, taste, and immunity.

It is an active, complex environment containing over 700 different species of bacteria, viruses, fungi, and protozoa (Peng et al., 2022; Santacroce et al., 2023). The microbes form organized colonies called biofilms on the surfaces of teeth, tongue, in the gaps between the gingiva, and in the mucosal tissues. While there are many friendly microbes within the oral microbiota that keep it in homeostasis, the balance of microbes can be thrown off, leading to dysbiosis and subsequently, the development of oral health issues. Because of the relationship between the health of the oral cavity and systemic health, poor oral health has been cited as being the culprit behind numerous systemic conditions.

Cardiovascular disease (atherosclerosis, myocardial infarction and stroke) is closely related to periodontitis, with both conditions linked by inflammation throughout the body and the transit of oral pathogens into the bloodstream (Etta et al., 2023; Arbildo-Vega et al., 2024).

Similarly, diabetes mellitus is known to correlate with chronic oral infections because the inflammatory mediators produced by periodontal tissue can alter the mechanisms for insulin signaling (Punic et al., 2023). This mutual relationship means that diabetes can actually trigger periodontitis, and periodontitis, in turn, can cause diabetes to progress more rapidly. Recent findings have even linked oral health to poor outcomes in pregnancy.

Women with untreated periodontitis while pregnant are at a greater risk of pre-eclampsia, premature delivery, and low birth weight (Bida et al., 2025). Oral infections can be spread systemically via pathogens and inflammatory cytokines that have an effect on the placenta and the developing fetus. Poor oral health can affect the elderly through a reduction in nutrient uptake, a risk of aspiration pneumonia, and a decrease in quality of life, particularly among individuals whose immune systems have weakened or who are struggling to access dental services (Rajasekaran et al., 2024).

Oral infections also pose a considerable burden,both socially and psychologically.

Loss of teeth, foul breath (halitosis), and obvious tooth decay can have a significant impact on a person’s confidence, relationships, and prospects of getting work. People who suffer from oral infections can experience social ostracism, feelings of shame, and reduced work efficiency, thereby causing a toll on mental health and financial stability (Rajasekaran et al., 2024). In the global sphere, oral disease is one of the most common chronic conditions among humankind.

Worldwide,3.5 billion people suffer from cavities, and nearly 10% of the population suffers from advanced periodontitis(World Health Organization, 2022).

Many oral diseases can be prevented easily but cause major difficulties for healthcare systems-particularly those in developing and middle-income countries that have limited access to dental treatment and where preventative care is often neglected.

The treatment of poor oral health is another enormous economic problem. Restorative dentistry, tooth removal, prothetics and continuous maintenance treatments represent a large segment of overall health spending. Oral care expenses worldwide have been estimated to be in excess of$500 billion per year, making it one of the most expensive healthcare concerns (FDI World Dental Federation, 2021). For resource-scarce regions, these expenses can be directly borne by the patients, leading to delayed diagnoses and exacerbated illness. A function in the oral cavity of immune defence of the host additionally strengthens their significance at a systemic level.

In order to counter the passage of microorganisms into the body, a substantial defence line, which include an arsenal of salivary enzymes, antimicrobial peptides, immunoglobulins, and cellular immune defenses, is in operation at oral mucosal barriers (Mizrahi et al., 2023; Sedghi et al., 2024). Lysozyme, lactoferrin, histatins, and secretory immunoglobulin A in the saliva all have defensive roles to prevent adhesion of microbes and to control the entry of bacterial toxins and the inflammatory cascade. Any impairment in these defence systems because of persistent poor oral hygiene and infections might lead to the increased likelihood of opportunist infections

Over the past decade, there has been increased interest in the so-called oral-systemic health axis. Oral cavity has been considered as an endogenous reservoir of pathogenic microorganisms and inflammatory mediators that can be disseminated by hematogenous routes into various distant organs. Several oral bacteria (i.e., Streptococcus mutans, Porphyromonasgingivalis, and Fusobacterium nucleatum) have been found in atherosclerotic lesions, brains, and colonic tumors, and it has been suggested that these bacteria are potentially contributing to various systemic diseases (Buiet al., 2022; Hajishengallis&Chavakis, 2021; Zhang et al., 2023).

Consequently, maintaining oral hygiene is no longer a dental concern alone, but is important to prevent other health problems too.

Recently, the issue of oral health as part of public health and a priority has become part of a global discussion, with WHO Assembly resolutions passed in 2021 that highlight the role of oral health in universal health coverage and in primary healthcare policies. World Health Organization (2022). Health at a Glance 2022: OECD indicators. Paris, OECD Publishing.

In developing countries, oral health challenges are also worsened due to inadequate infrastructure, limited dental professionals, expensive treatments, and prevalent use of traditional medicines.

In most communities in Africa and Asia, medicinal plants remain the only available option for many people to cope with health problems such as toothache, gum bleeding, mouth ulcers and infection, for which these plants have been widely used. Many individuals, especially in the African and Asian regions use herbal remedies as primary medicine to treat various ailments including oral disorders, and there has been a growing emphasis on exploring the use of traditional medicines in treating various conditions including oral ones Bamideleet al. (2023).

The high prevalence of oral disease around the world, coupled with increasing scientific understanding of the connection between oral health and systemic health as well as the adverse effects of antimicrobial agents, has prompted investigation into safer, less expensive, and culturally acceptable ways of preventing and controlling oral bacterial infections. Plants with proven antimicrobial activity, such as Anacardium occidentale and Vernonia amygdalina that are commonly used in traditional African and Asian practices, can offer an important resource for natural approaches in this direction.

2.2 Epidemiology of Oral Infections

The oral infections pose a threat of a considerable public health interest because their highest prevalence and chronicity and significance for human life quality. Epidemiology can be explained as a scientific discipline concerned with the distribution, incidence, causes, and prevention of disease in population groups, in relation to oral health, epidemiology can offer the necessary evidence based on the patterns, underlying causes and outcomes of diseases of the oral cavity. Oral infection is the major type of human infections and they can occur in both all races, people from all countries, social classes, sexes, and ages. Nevertheless the advancements that occurred in the filed of preventive dentistry and public health promotion efforts,oral infections continue to be highly prevalent worldwide and predominantly in the third world countries which have inadequate dental access and few preventive methods.

2.2.1DEFINITION OF ORAL INFECTIONS

Oral infection is disease of oral region that are brought by invasion and growth of pathogenic microorganisms such as viruses, bacteria, and fungi. The affected oral sites include hard tissues and soft tissues of the oral cavity which range from teeth, periodontal ligament, tongue, Gingiva, alveolar bone and oral mucosa. Some forms of infection in oral region areLocalized (dental caries and gingivitis) and others escalate into systemic infections that can turn severe without treatment. World Health Organization (2022) offers an extensive report about oral diseases which include dental caries, periodontal diseases, oral candidiasis, pulpitis, periapical abscesses, oral viral lesions and Necrotizing Periodontal Diseases. Theoral cavity is suitable for supporting microbes to live due to adequate moist environment, constant and proper temperatures and enough availability of nutrition and ecological niches. These conditions encourage proliferation of communities of microbes that change between the healthy commensal and pathogenic stages when oral hygiene is compromised and when host immunity is weakened. Oral dysbiosis (unbalanced microbiota in terms of structure and activity) is a root cause for transitioning between states of oral health and disease (Houet al., 2022; Sedghiet al., 2024).

2.2.2 Global Prevalence and Burden of Oral Infections

Oral infections represent one of the largest group of diseases in the world. The Global Burden of Disease Study identified that the most common diseases in the world are the untreated caries of the permanent teeth (more than 2.5 billion people) and severe periodontal disease (1 billion in total) (Global Burden of Disease Oral Disorders Collaborators, 2022; World Health Organization, 2022). These data suggest the seriousness of oral infections as an ever-existing health problem. The prevalence of oral infections can vary significantly depending on populations in different geographical regions. In the developing countries, dental caries and periodontal disease are reduced in young people, due to their better oral health awareness, fluoride use and dental service; however, these are still major problem in elderly people and the poor populations. On the other hand, oral diseases are disproportionately common in middle and lower income countries, due to the restricted access to the oral care services, incomplete preventive care, inadequate nutrition and lack of oral health education (Benzianet al., 2023). In Africa and the other developing regions, oral infections are usually underreported and untreated. According to epidemiologic studies, the rate of dental caries and periodontal disease in school children and adults are very high and most of patients access the services when their conditions is critical. Use of traditional medicine and self-medication because of the cultural background leads to the delay of treatment and the severity of the disease (Adeniyiet al., 2022).

2.2.3 Etiology and Risk Factors for Oral Infections Oral infections have a multifactorial etiology and they involve the complex interplay of various micro-organisms, host factors, and environmental factors. Various oral organisms cause these infections, but bacteria are primarily involved. Oral cavity is rich in the diversity of microorganisms and in presence of ecological disturbance pathogenic organisms may arise. Caries are caused by acidogenic and aciduric bacteria such as Streptococcus mutans and Lactobacillus species, which digest the fermentable carbohydrates to organic acids, lower the oral pH and consequently demineralize tooth enamel (Lemoset al., 2022; Baker et al., 2023). These decay, also enabled by persistent sugars and lack of regular oral cleaning. The pathogens involved in the development of periodontal diseases include Porphyromonasgingivalis, Tannerella forsythia, and Treponema denticola, and they generate complex biofilms within periodontal pockets causing chronic inflammatory reactions that destroy supporting tooth structure (Hajishengallis&Chavakis, 2021; Sedghiet al., 2024). The host immune system plays role in inflammation process by secreting cytokines, proteolytic enzymes, and reactive oxygen species, and contributing to tissue damage. Fungus related oral infections such as oral candidiasis is mainly due to Candida albicans in patients with weakened immune system, denture users or in those under antibacterial treatment. Common virus infections in the oral cavity are oral herpessimplex virus, human papillomavirus lesions, Epstein-Bar virus and related infections, appearing as ulcers, warts, or mucosal lesions (Villa et al., 2023; Patilet al., 2022).

2.2.4 Types of Oral Infections

Oral infections can be generally classified into dental, periodontal, mucosal and odontogenic infection. Dental infections affect primarily the hard tissues of the tooth; these include dental caries, pulpitis and periapical abscesses. These conditions are usually initiated when bacteria invade the dental pulp subsequent to damage to the enamel and dentin.

Periodontal infections affect the supporting tissues of the teeth and consist of gingivitis and periodontitis.

Gingivitis involves inflammation of the gingiva with no loss of attachment whereas periodontitis involves destruction of periodontal ligament and alveolar bone with consequent tooth migration and loss. Mucosal infections affect soft tissues in the oral cavity, examples are oral candidiasis, aphthous and viral oral lesions. Infections can signify the status of the host immune system and predict underlying illness. Dental infections that occur in the teeth can progress into adjacent anatomical spaces leading to conditions like cellulitis, osteomyelitis and potentially life-threatening infections such as Ludwig angina; these conditions represent situations where oral infection may progress to a medical emergency if untreated (Jevon et al., 2023).

2.2.5 Pathology and Disease Progression

The underlying pathophysiology of oral infections revolves around the concepts of microbial colonization and biofilm formation, along with host immune responses. A biofilm is a community of bacteria that are attached to a surface and enclosed in an extracellular polymeric matrix which protects the biofilm from adverse environmental conditions and antibacterial treatments. Within a biofilm, Bacteria communicate via quorum sensing systems which facilitate gene expression and enhance virulence (Sedghiet al., 2024).

Plaque microbes produce acid which lead to recurrent cycles of demineralization and remineralization.

When the rate of demineralization exceeds the rate of remineralization the tooth structure is irrevocably lost. Chronic bacterial challenge also triggers a chronic inflammatory response in periodontal disease that lead to breakdown of the connective tissue and loss of bone mediated by osteoclasts (Hajishengallis&Chavakis, 2021). The host immune response to infection plays a double role; it protects against microbial challenge but it can cause disease as well when the immune response over reacts or is unbridled. This is especially the case in periodontitis where the extent of damage of the tissue can largely be explained by the host's own inflammation, and not the bacteria alone.

Oral infection may become chronic, serving as a reservoir for pathogen and inflammatory mediator dispersal throughout the body, potentially contributing to other health problems; routine activities such as eating and brushing may induce bacteremia, seeding other parts of the body and contributing to cardiovascular diseases, rheumatoid arthritis, and pregnancy outcomes (Buiet al., 2022).

2.2.6 Epidemiological Implications for Public Health

The oral infections are of considerable concern to the community at large due to their high prevalence and long term nature. Oral disease share common risk factors such as poor diet, use of alcohol, and tobacco, and socio-economic factors with non-communicable diseases. Combined approach that tackle the risk factors of life-style and the social determinants of health is warranted (Watt et al., 2023). The populations such as the elderly, the young, the rural community and disadvantaged group are disproportionately affected. In many low and middle-income countries the dentist to the population ratio is too low, and dental preventive services are not adequately accessible. As a consequence, oral infections occur at higher rates, remaining untreated, resulting in pain, suffering and inability to live a normal life.

Oral infections result in enormous expenditure from healthcare systems in both direct and indirect forms. Direct costs result from medical interventions in the form of care delivery and the use of resources. Indirect costs are represented by productivity losses from school absenteeism and working time; long term disability also contributes to indirect costs. Such burdens make affordable interventions (preventive and therapeutic) necessary especially in low income areas. These epidemiological figures strongly suggest the need for alternative prevention and treatment strategies. Existing treatment regimens like antimicrobial drugs, although generally effective, contribute to increasing antibiotic resistance, drug interactions and are costly. Medicinal plants represent an affordable and locally acceptable alternatives for treating oral infections in cultures where traditional treatments are predominantly utilized. The very high frequency of oral infections and the limitations of current management strategies represent a good reason to develop scientific support for plant-based anti-infective agents. It would be appropriate to use the epidemiological knowledge about oral infection to select appropriate targets for research and develop control strategies. In order to address these global public health priorities, research assessing the antimicrobial effects of Anacardium occidentale and Vernonia amygdalina against oral pathogen will bridge this research gap.

2.3 Prevention and Control of Oral Infections

Oral infections and oral disease prevention and control Oral diseases cause high morbidity and morbidity in the community and are considered a chronic health problem with potential systemic effects; therefore their prevention and control are essential part of community dentistry, as well as clinical dentistry. The goal of prevention interventions is to reduce incidence of oral infections by modifying risk factors and improving oral hygiene practices and early diagnosis and treatment. Control intervention strategies focus on limiting the progression of existing disease, complications and recurrences by using specific interventions and sustained long-term oral hygiene maintenance.

Basic pillars for preventing oral infections consist of individual oral care, professional oral hygiene and population based approach in the field of public health.

Individual oral hygiene can be seen as the cornerstone in prevention: it consists of regularly brushing the teeth with fluoridated toothpaste, removing interdental debris by using dental floss or interdental brushes, and rinse the mouth. By this, they eliminate dental plaque, which is primary etiology for dental caries and periodontal diseases. The caries preventative action of fluoride consists in increasing enamel mineralization, lowering microbial metabolism thus diminishing the production of acids in the biofilms on tooth surface (Twetman, 2022). The dental profession, through routine clinical examinations, professional cleanings, application of preventive agents like fluoride varnishes, and pit and fissure sealants, contribute to prevention of oral diseases.

Furthermore, dental professionals are well placed to identify the earliest signs of oral disease and educate patients about optimal practices.

Early intervention is of utmost importance in cases of reversible demineralization of enamel, which with timely treatment can avoid progression into irreparable tissue damage, especially in the most serious stage (World Health Organization, 2022). Within public health sphere preventive strategies targeting oral infections rely on health promotion, disease monitoring, and policymaking, with focus on the improvement of access to dental services, healthier diets, and promotion of dental hygiene in schools. These kinds of interventions are particularly needed in developing countries with high burden of oral infections and lacking of availability of preventive services.

The World Health Organization advocates that Oral health needs to be integrated in general health policies and promotion strategy due to existing common risk factors for oral infections and noncommunicable diseases such as cardiovascular diseases or diabetes (World Health Organization, 2022). It has long been recognized that oral health is intimately linked to our diet, especially in case of dental caries. Dietary factors are one of the main causes of oral disease.

High intake of fermentable carbohydrates and particularly of sugar enhances the activity of cariogenic microorganisms that demineralise tooth enamel, and it may consequently lead to a dental cavity.

Eating a balanced diet rich in vegetables, fruits and whole grains, while avoiding diets rich in sugar, plays a crucial role in preventing cavities and promoting overall oral health. It is recommended that total sugar intake should account for less than 10% of total daily energy intake, with additional benefits in consuming even less than 5% of total energy as sugar (World Health Organization, 2022). Oral infection control relies on therapeutic intervention that are aimed at eliminating pathogenic microorganisms, reducing inflammation, and repairing any damage done to the tissue. Controlling periodontal disease is achieved by a comprehensive regimen of procedures such as Scaling and Root Planing (SRP), which involves the removal of bacterial plaque and calculus from teeth, enabling host immune system to restore oral health.

However, if the disease is advanced, more complex periodontal surgical procedures may be required to remove infection and restore tissues to a more normal state (Sanz et al., 2022).

Pharmacological methods of treatment are extremely important in the control of oral infections. When the application of non-pharmacological treatment methods are not sufficient, oral infections are managed with antimicrobial medications to minimize bacteria or to cure the infection. These include topic agents such as chlorhexidine, mouth rinses containing essential oils, and in serious infections, oral antibiotics are given in order to eradicate the bacterial.

The best antiseptic in dentistry and the 'gold standard' of treatment for periodontitis, in addition to the antimicrobial properties of mouth rinses containing herbs, Chlorhexidine works as an oral antiseptic because it stays attached to oral tissues and can have long-term antimicrobial effects; however, oral application of chlorhexidine leads to tooth discolouration, altered taste perception, irritation to mucosa with long-term use (Brookes et al., 2020).

Systemic antibiotic agents are prescribed for the management of spreading or severe infections of oral cavity which include odotogenic abscesses, necrotic periodontal lesions, oral infections in immunosuppressed patients, etc. In these cases, dentists commonly prescribe drugs like amoxycillin, clindamycin, metronidazole etc. The constant use of such antibiotics has developed resistant bacteria, which cause many complications and hinder treatments throughout the world (Cope et al., 2022). In response to widespread and worrying concern about antibiotic resistance, clinicians are now exploring alternative methods to treat and prevent oral infections as a way to avoid over reliance on antibiotics.

This is done through the use of oral prebiotics, anti-microbial peptides and host-modulation treatments.

These treatments provide good bacterial strains that enhance oral environment with their ability to limit oral inflammation, control the number of oral bacteria and promote dental health by inhibiting the formation of oral biofilm. Studies on several types of oral probiotics such as Lactobacillus reuteri indicate they have the capacity to lower the occurrence of oral diseases by reducing the production of plaque and preventing inflammation of the gum (Akramet al., 2023). The method called host modulation therapy controls oral infection by stimulating and weakening the body's natural defense mechanisms against pathogens causing oral diseases.

Sub-antimicrobial-dose doxycycline, commonly used to manage periodontitis, inhibits destructive matrix metalloproteinases, thus preventing degradation of tissue (Martandeet al., 2022). Many research works in recent years has emphasized on use of natural compounds derived from plants for the prevention and treatment of oral infections because of their affordability, accessibility, reduced adverse effects, and cultural acceptability. Traditional medicine systems across the world (Africa, Asia, South America) have been using plant based natural products (as paste, infusion, decoction, etc) since before the advancement of conventional medicine.

These natural compounds contain compounds that act against oral infections.

Some of these compounds are flavinoids, tannings, alkaloids, saponins, and essential oils. It appears the mode of actions of these products is that they disrupt the cell membrane of bacteria, blocks the formation of enzyme, inhibits the action of bacteria to produce DNA and also prevent forming oral biofilm (Altemimiet al., 2023). These components derived from plants act in various mechanisms unlike traditional drugs that act in only specific function which lead to formation of resistence in body. Many scientific studies and researches have shown effective action of natural plants extract against different microbials that cause oral disease, like Azadirachta indica (neem), Salvadorapersica (miswak) and Ocimumgratissimum (scent leaf) in the commercialization of toothpastes, mouth wash etc (Soleimaniet al., 2023).

Although oral infections can effectively be treated or controlled with natural herbal medicine, they may cause side effects depending on dosage, the plant species used, and mode of usage (AgThese factors include variation of the phytochemical compounds due to differences in plant species, location of harvesting, procedures employed in the plants harvesting, procedures employed in the plants extraction and poor standardization. Clinical trials are also few to allow for generalized prescription. The toxicity and safety of the most of medicinal plant are not well elucidated which call for rigorous scientific study. Generally, management and prevention of oral diseases is a multifactorial issue which necessitates integration of lifestyle interventions, oral hygiene practices, oral preventive interventions, and dental interventions and treatment modalities. While traditional agents still play a part, it is critical to consider alternate means, particularly given the shortcomings of the traditional interventions. Growing interest in natural plant-based antimicrobials mirrors the growing need for culturally relevant and sustainable approaches to healthcare. Thus, the investigation of plants asAnacardium occidentaleand Vernonia amygdalinaas a source of novel, safe, and effective oral health agents in oral infection is opportune.

2.3.1 Chemical Formulations and Drugs Used in the Control of Oral Infections.

The use of chemical formulations and drugs remains the key strategy in oral infections clinical management. They serve to eradicate, mitigate or prevent replication of bacteria, reduce inflammation, control pain, and halt spread of the infection to adjacent tissues or systemic site. Prescription of drugs or formulations is directed by the type and severity of the oral infection, the predominant micro-organism(s), the condition of the patient, and any existing comorbidities. Oral rinse, gels, spray, and toothpaste were among other methods of application used with their mode of action of killing or preventing adhesion and colonization by plaque-forming bacteria and killing microbes on their way to causing disease. While mechanical methods such as scaling and brushing have proven beneficial, the utilization of chemical agents in clinical settings is essential to prevent complications and treat established infection.

A majority of patients suffering from oral infection utilize the topical antiseptics. These agents can be formulated as rinse, mouth sprays, tooth gels and toothpaste and are mainly responsible for killing oral microbes or for the prevention of plaque formation and deposition onto oral structures. Some agents are specifically designed to have high binding affinity with dental structure and tissues in the oral cavity (also termed as substantivity). This characteristic gives long-lasting therapeutic effect even after washing of the oral cavity (Brookes et al., 2020).

Among such antiseptics, the chlorhexidine gluconate is one of the widely reported topical antiseptic with wide spectrum of antibacterial, antiviral, and anti-fungal activity (Brookes et al., 2020).

yareet al., 2022).

When the dentist recommends that his or her patient use an antimicrobial preparation such as an antiseptic mouthwash or an antibacterial paste and an effective antibacterial agent is needed the use of chlorhexidine often comes to the fore as it has a broad range of applications. Most frequently it is employed for patients experiencing periodontitis (periodontal disease) because of its excellent effect on periodontitis as well as in patients who have been treated for post-operative infections, not to mention, as an alternative in treatment of gingivitis (Woodward et al. 1994). It is clinically demonstrated that chlorhexidine has a great impact on plaque and gingival inflammation with mechanical control of plaque. However, there are several side effects with the long-term use of this antibacterial mouthwash, for instance, it may stain teeth, alters sense of taste, may contribute to calculus formation and may be irritating to the mucosa and therefore, restricts long-term application thus need other therapeutic agents with less side effects (James et al. 2023).

Other well-used antibacterial chemical preparations in dental care are those based on essential oils. This kind of mouthwash normally containing the combined therapeutic potential of the active compounds like thymol, eucalyptol, menthol, and methyl salicylate. They carry antimicrobial activity, and show anti-inflammatory and anti-microbial characteristics (Tufekci et al. 2022). Essential oils-based mouth rinses decrease the prevalence of plaque and gingival gingivitis by targeting bacterial cell walls and interfering with enzymes. They exhibit less side effects and can be applied in long-term daily oral care, unlike chlorhexidine mouthwashes (Tufekci et al. 2022).

Fluoride-based materials used in oral care play a key role in prevention and control of dental caries. The remineralization of enamel is stimulated by fluoride. It is anti-demineralization agent, decrease activity of bacterial metabolism and in the same way it shows anti-microbial activity against most common micro-organisms, such as S. Mutans, F. Nucleatum, S. Sanguis. There are many ways to apply Fluorides in oral practice; the main applications are in the toothpaste, mouth rinse, gels, and varnishes. Fluoride-based materials have already been proven their preventive role on caries; thus the caries rate has declined in several countries where fluoridation programs were implemented (Twetman, 2022). Despite its wide acceptance and proven benefits,excess fluoride intake, especially in children, may lead to development of dental fluorosis, hence a need for controlled intake (Twetman, 2022).

Systemic antibiotics (oral or injected) is normally given to patients who develop an acute oral infections with sever signs or symptoms and a condition that involve spreading or the infection affects the system in general (e.g., acute odontogenic abscess, necrotizing periodontal diseases, acute infections involve the system in general) (Cope et al. 2022). Various antibiotics like beta-lactams (Amoxicillin), lincosamides (Clindamycin) macrolides (Erythromycin, Azithromycin) and nitroimidazoles (Metronidazole) are generally recommended in dentistry. Amoxicillin, the broadest of this class of agents, is considered the first choice in manyodontogenic infectioncases because of its broad coverage of dental microbes, predictable clinical response, acceptable toxicity and ease of administration (Cope et al. 2022).

Metronidazole is highly effective against anaerobic bacteria which are predominant in the pathogenesis of periodontal and periapical infections, and are often supplemented by a broad spectrum antibacterial like amoxicillin, resulting in synergistic effect and achieving the highest response rates. Clindamycin has a very good bone and soft tissue penetration thus making it an excellent choice for deep seated oral infections and typically prescribed to patients who have penicillin allergies. These antibiotics, despite being effective, are also associated with numerous systemic side effects and can disturb the normal microbial population of the oral cavity, leading to development of antibiotic resistance (Teo et al. 2023).

Development of resistance in microbes toward conventional antibiotics has been the greatest problem and has seriously affected dental infections treatment protocols. Rising cases of antibiotic-resistant strains such as Streptococcus spp, Staphylococcus spp, and anaerobic organisms have been identified in dental practice. Issues including antibiotic abuse, failure to follow dosage regimen, and self-medication among the patients, have contribute greatly to the emergence of resistant strains which hinder the conventional antibiotic efficacy. Henceforth there is an urge to discover a new alternative therapy and increase the emphasis on antimicrobial stewardship practices (Miller et al. 2022).

Antifungal agents play a significant part in the treatment of fungal infection of oral region specifically oral candidiasis. The following antifungaldrugs have found to be clinically useful nystatin, clotrimazole and fluconazole. Nystatin, which used topically in forms of suspension andlozenges, works on interfering with the fungal cell membrane, while fluconazole, a systemic agent for serious or recurrent infections and especially for patients with immune-compromising conditions, may present some interactions with other medications and resistance when used long term,requiring regular follow ups (Villa et al. 2023).

Antiviral medication is usually prescribed for patients having oral viral diseases like herpes simplex virus and human papillomavirus (HPV). The more popular and clinically indicated antivirals includeacyclovir, valacyclovir and famciclovir. They interfere with viral replication processes by directly acting on viral DNA. Their use can reduce the severity and the duration of viral infections outbreaks particularly among the immunocompromised patient. The antiviral treatment, however, does not eradicate underlying viral latent reservoir (Patil et al. 2022).

In addition, auxiliary treatments may include the use of analgesics and anti-inflammatory drugs to relieve pain and inflammation due to the occurrence of oral infections. Normally, nonsteroidal anti-inflammatory drugs are used for symptom management. Some of the commonly used medications include ibuprofen, paracetamol, etc.

Corticosteroids may be used, with due caution, when the patient has severe inflammation to suppress extreme immune responses. These drugs do not carry any antimicrobial activities, but can provide relief to patients and promote their recovery by inhibiting tissue inflammatory effects (Hargreaves & Abbott, 2022).

In the same vein, use of antibacterial, antifungal, antiviral and auxiliary therapy also plays a critical role in the treatment and management of oral infection disease. However, these challenges from undesired outcomes, antimicrobial resistance and unavailability of sustainable oral healthcare drugs necessitated research into other and complementary means of treating the disease. Chemical substances and medicinal products generally have limitations in the control of oral infections even when most widely utilized and effective in this treatment modality. Limitations including severe side effects, adverse reactions/interactions, high costs, poor access, and resistance of micro-organisms compromise the long-term effectiveness of such treatment agents.

The limitation and cost constraints of many essential oral healthcare medications, particularly in the developing world where access to basic oral medical products is a major challenge and where access and availability of essential medications are affected by costs and lack of appropriate infrastructure, support the application of alternative approaches and supplements in the management of oral infection.

Alternative oral infection management strategies are needed due to the failure and other limitations of traditional medications. Plant-derived products have appeared as a suitable candidate because they contain novel compounds with broader spectrum of biological activities including, broad antimicrobial range, unlike synthetic compounds, which often targeted a specific microbial biochemical pathway and they also had lower risk of emerging resistance. Moreover, plant products have shown poly-functional and diverse biological activities and they offer the possibility of being used as adjunct or alternatives to standard pharmaceuticals in treating oral infections particularly when available in resource-constrained environment and readily accessible and socially acceptable by the communities for oral healthcare interventions.

Overall, the efficacy of these drug formulations in the oral infection control cannot be downplayed as topical antiseptics, system antibiotic, antiviral, anti-fungal and ancillary therapies has vital part to play in management oforal infectiondisease. Nevertheless, some shortcomings related to side effects, microbial resistance, poor availability and lack of sustainable source point out the necessity of alternative therapies, as an adjunct or supplement in controlling oral infections. This creates an avenue for Scientific Investigation in the Plant-based antimicrobial Agents that will either provide replacement or complementation of commercial drugs in future.

A review of medicinal plants, such as A. Occidentale and V. Amygdalina, to this cause is relevant.

2.3.2 Herbal Remedies in the Management of Oral Infections Herbal medicines for oral infection are a subject of long-term application and its traditional utilization is corroborated by modern research. Phytochemicals in herbal medicines generally show multiple mechanisms of action against micro-organisms unlike the synthetics which normally act by single mechanism pathway of action which are: disorganisation of microbial membrane cell, disruption of cellular process, inhibiting enzyme system, interference with the gene expression, attenuation of virulance and interference with biofilm formation. There is generally less or even no adverse effects in the use of herbal agents in treatment or management of infection than the long-term utilization of synthetic chemical substances such as chlorhexidine that have side effects including dental stain, taste disorders and oral mucosal lesions (Jameset al., 2023). The Oral diseases which are frequently polymicrobial infections such asDental caries, gingivitis and Periodontitis arecaused byS. Mutans, Lactobacillus spp, P. Gingivalis, andA. Actinomycetemcomitans. Biofilm production, as an organized microbial structure embedded in extracellular polymeric material (EPM), leads to significantly increased tolerance towards antimicrobial agents (Sedghiet al., 2024).

Herbal antimicrobials provide an attractive alternative since many of their compounds have shown efficacy against biofilm forming micro-organisms.

One of the most researched plants used in treating oral infection is Azadirachta indica A. Juss, commonly called as neem. It has been used in traditional South Asian oral hygiene practice for centuries.

In a random clinical trial that measured effects of neem mouthwash versus chlorhexidine in gingivitis patients, it was concluded that neem was effective in decreasing the gingival inflammation and plaque scores, as comparable to chlorhexidine, and with no major adverse events over the 4-week period (Gupta et al., 2022).

Studies have demonstrated the presence of compounds in neem extracts, such as nimbidin, azadirachtin, and quercetin, that display anti-inflammatory and broad-spectrum antibacterial activities against S. Mutans and other cariogenic and periodontopathogenic bacteria (Bharathiet al., 2023; Mistry et al., 2022).

Salvadorapersica L. Is known as miswak or toothbrush tree and is recognized as a natural oral hygiene agent by the World Health Organization (WHO) for its plaque removal and antimicrobial properties (World Health Organization, 2022). Its antimicrobial potential comes from chemical constituents like potassium salts, benzyl isothiocyanate, and fluoride which helps to reduce bacteria adhesion and their enzymatic action (Soleimaniet al., 2023). The findings from a systematic review have confirmed significant reduction in plaque accumulation and gingival bleeding with the use of miswak compared to untreated individuals and comparable effectiveness as conventional tooth brushing (Halawany, 2022). Miswak has also demonstrated its ability to inhibit both caries-causing S. Mutans and periodontal disease-associated Porphyromonasgingivalis (Alghamdiet al., 2022).

Ocimum sanctum L., more commonly referred to as holy basil, is yet another promising medicinal plant with demonstrated oral antimicrobial action. Several studies suggest that extracts derived from O. Sanctum leaves possess antigrowth activity against various oral pathogenic microorganisms, including S. Mutans and Lactobacillus acidophilus (Khan et al., 2022). It is well documented that eugenol and other essential oils in holy basil exert their antibacterial effects by interfering with the cell membranes and virulent factors of microbes. An Indian clinical study showed that using holy basil leaf extract as a mouth rinse resulted in noticeable reductions in both dental plaque and gingival inflammation, making it a viable adjunct to mechanical oral cleaning (Rao et al., 2023).

In the realm of oral health, the plant Camellia sinensis (L.) Kuntze, commonly known as green tea, is another noteworthy candidate. Particularly, its active compound, epigallocatechin-3-gallate (EGCG) from the group of catechins, is proven to be effective in inhibiting the growth, acid production, and biofilm formation of S. Mutans (Reygaert, 2022).

A double-blind clinical study using green tea extract as a mouthwash found that it significantly reduced both gingival bleeding and plaque when compared to a placebo (Nassaret al., 2022).

As antioxidants and anti-inflammatories, catechins may play a role in protecting periodontal tissues from damage (Shen et al., 2023).

Turmeric, also identified as Curcuma longa L., is a well-established antimicrobial and anti-inflammatory agent traditionally used in South Asia. The active component, curcumin, has been observed to inhibit the growth of S. Mutans and other periodontal pathogens in laboratory settings (Sharma et al., 2022). In a randomized clinical trial, applying a curcumin-based gel to periodontal pockets resulted in improved outcomes, with lower levels of gingival inflammation and probing depth compared to chlorhexidine gel, and better tolerance (Moghadamet al., 2023). Curcumin's antioxidant capabilities also offer potential benefits in mitigating oxidative stress associated with persistent periodontal inflammation (Hewlings&Kalman, 2022).

The plant Glycyrrhizaglabra L., or licorice root, is known to produce compounds like glycyrrhizin and glabridin that exhibit antibacterial activity against bacteria involved in dental caries. Laboratory studies have demonstrated that licorice extracts can inhibit S. Mutans and Lactobacillus species, while also decreasing acid production and their adhesion to enamel (Amin et al., 2023). Licorice extract has also been utilized in confectionery to lower S. Mutans levels in children's saliva, suggesting potential for caries prevention (Hu et al., 2022).

Garlic (Allium sativum L.) contains an organosulfur compound, allicin, which has a broad spectrum of antimicrobial activity and has been shown to be inhibitory against oral streptococci and Candida species in vitro (El-Saber Batihaet al., 2023). Although the strong taste of raw garlic makes it less palatable as a direct oral therapy, numerous studies show that its extracts can be incorporated into various oral healthcare products, such as mouth rinses or lozenges.

African medicinal plants hold significant promise as well. It has been found that the African plant Garcinia kola Heckel (bitter kola) exhibits antimicrobial activity against oral pathogens like S. Mutans. Its seed extracts were found to inhibit bacterial adhesion and acid generation in vitro, indicating its suitability for oral hygiene formulations (Okunlolaet al., 2022).

Furthermore, the leaf extracts of another African plant, Psidiumguajava L. (guava), contain flavonoids and phenolic acids responsible for their antibacterial effects against S. Mutans, P. Gingivalis, and Fusobacterium nucleatum (Akinmoladunet al., 2023).

Guava leaf extract mouth rinses have been clinically demonstrated to reduce the levels of plaque and gingival inflammation (Priya et al., 2022).

The anacardium occidentale L (cashew) and vernonia amygdalina del (bitter leaf), which are the subject of this research, have also been the subject of research into their antimicrobial potential, but studies directly relating them to the pathogens in question are few in comparison to those mentioned above. However, phytochemistry studies have demonstrated that both species contain compounds with a broad spectrum of antimicrobial activity, which forms a scientific basis for this research. Anacardium occidentale extracts (leaves, bark, stems) have been demonstrated to inhibit various different species of bacteria such as S. Aureus, E. Coli, and Salmonella spp (Oladeji et al., 2022; Eze et al., 2023) and are all attributed to the presence of phenol compounds, tannin,flavonoids, anacardic acid which have been known to interfere with the microbial membrane, prevent enzymatic system.

Although limited study tested cashew stem extract on oral pathogens, leaf and bark have also exhibited antimicrobial activity on oral bacteria as such that the research that hypothesis may also active on them is well supported.

In a number of studies, bitter leaf has also exhibited antimicrobial activity on both gram-positive and gram-negative bacteria including S. Aureus, E. Coli, Pseudomonas aeruginosa andKlebsiella spp(Adesanoye et al., 2022; Nwafore et al., 2023). This effect is suspected to be due to the compounds isolated sesquiterpenoids lactones, Flavonoids, saponins in bitter leaf. Furthermore, Bitter leaf extracts have been found to inhibit Candida albicans and block formation of dental bacterial biofilms, suggesting the applicability in the prevention oforal candidiasis and diseases caused by cariogenic microorganisms(Ojo et al., 2022), but strictly limited studies evaluating oral specific oral pathogen so far is one of the factors why thecurrent study was designed.
Although all above studies suggest great potential in most of them medicinal plants there are

limitations of several nature, such as variability of phytochemical constituent depending on the geographic origin, part of the plant selected, method of harvesting, time of harvesting, extraction method and process of standardization of these method.

Lack of clinical evaluation is a main barrier to apply these natural agents in oral therapy as clinical trials are necessary to ascertain the efficacy, safety, adequate formulation and dosing as well as toxicity.

To conclude, use of herbal products in oral care offers cost-effective approach in preventing and controlling oral infections in populations with limited access to dental services. Most studies in medicinal plants, including neem, miswak, basil, green tea, turmeric, licorice, garlic, guava, and bitter kola, have shown antimicrobial effects on the microorganisms responsible for oral disease. Due to the high level of compounds found in Anacardium occidentale and Vernonia amygdalina, their investigation as natural oral antimicrobials are scientifically supported.

Natural based treatments supplemented with chemical treatment under specific standardization and with proper clinical validation can be the basis of new direction in sustainable and culturally competent oral healthcare.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area and material

This study was carried out in the laboratory of Godfrey Okoye University, a research center which was suitably outfitted with facilities essential for microbiological work and an examination of anti-microbial agents. Equipment in the lab included a range of incubators, autoclaves, laminar airflow cabinets, hot air ovens, microscopes and an analytical weighing balance-all needed in the processing of plants extracts, the nurturing of bacterial isolates and the assay of anti-microbial actions. The study was undertaken using strict adherence to sterile lab surroundings to ensure the results obtained were valid, dependable and accurate and to exclude any sort of contaminants. Aseptic approaches were followed at all stages during the study, from cleansing glassware and growth media to using only sterile instruments and managing bacterial samples carefully.

3.2 Obtaining and identifying plant material

We obtained fresh Anacardium occidentale (cashew) and Vernonia amygdalina (bitter leaf) stem cuttings from nearby botanical vegetation where they exhibited sound, healthy growth. These fresh plant materials were scrubbed using sterile distilled water before being laid out for air-drying for seven days on a sterile surface in the lab. Once the stems were adequately dry, they were ready to be ground into powder.

3.3 Producing the plant extracts

We ground both Anacardium occidentale and Vernonia amygdalina stem samples separately into a fine powder and subjected these samples to both aqueous and ethanolic extraction processes using maceration methodology, making only small modifications to standard pharmacognistic processing.

3.3.1 Aqueous extraction

For aqueous extraction, we measured 50g of powdered stem samples from each plant, placed each into sterile 1-L conical flasks and poured 500ml of sterile distilled water into each sample. Each flask was vigorously mixed and tightly covered with sterile aluminum foil. Each sample was allowed to macerate at ambient temperatures (between 25c - 30c) for 48 hours with shaking performed at regular 6-hour intervals to effectively extract water-soluble phytochemicals.

Upon conclusion of the extraction, the mixture in each flask was passed through muslin cloth, and the filtrate was subsequently concentrated using a water bath heated to 70c until the product was in a semi-solid state.

The concentrated extracts were decanted into labeled sterile airtight amber bottles and set aside until they were required for anti-microbial susceptibility testing.

3.3.2 Ethanolic extraction

The extraction with ethanol was conducted in exactly the same way as the aqueous extraction. Fifty grams of the finely ground powder of each stem was weighed out separately and added to sterile conical flasks, to each of which 500ml of 95% ethanol had been added. The flasks were securely sealed with aluminum foil and set aside to macerate for 48 hours, shaking occasionally to encourage penetration of the solvent and more efficient extraction.

At the end of the extraction period, each mixture was filtered in muslin cloth and then the filtrates were concentrated in a controlled water bath at 50Oc until a semi solid crude was obtained. The concentrated extracts were then transferred into sterile airtight amber bottles, labeled appropriately and stored until required for antimicrobial susceptibility testing (Kumar et al., 2023; Peiris et al., 2023).

3.4 Test microorganisms

We isolated obtained clinical oral bacteria from oral swab samples collected from park-lane hospital's dental clinic patients with ethical clearance. We then cultured the bacterial isolates on appropriate microbiological media and incubated for 18-24hrs to obtain pure culture. We also sub cultured to confirm the purity of the isolates before they were used for anti microbial susceptibility testing.

We performed gram staining and relevant biochemical tests to authenticate the isolates following established laboratory protocols.

Following identification, the confirmed isolates were maintained on nutrient agar slants and stored until required for experimental analysis.

3.5 Standardization ofinoculum

Fresh bacterial colonies from 18-24 hours pure culture was aseptically transferred to sterile normal saline by using sterile inoculating loop We adjusted the turbidity of each bacterial suspension to compare the 0.5 McFarland standard for bacterial density by observation visually compare with bacterial suspension with 0.5 McFarland McFarland standard under a good light We also prepared new batch for new bacterial suspension by adjusting to the density that appeared less turbid, with the additions of fresh colonies. Add sterile saline for density that appeared higher than the standard and the bacterial cultures should be adequately and uniformly dispersed in the standardized inoculum by mixing prior to the inoculation process We adjusted the bacterial suspension density to compare to 0.5 McFarland standard under a good lighting. Add saline for adjustment of density for denser sample and additions of new colonies when the sample less density till reached to 0.5 McFarland McFarland.

The standardized inoculum was mixed thoroughly to Ensure uniform cell suspension of the bacterial cells prior to its utilization 3.6 Anti microbial susceptibility testing 3.6.1 Agar well diffusion method The Mueller Hinton agar were prepared according to manufacturers instruction then sterilized by autoclaving at 1210c for 20 minutes.

After that, we cooled it up to 45-500c and dispense it into sterile Petri dish and allow to solidified on surface level prior use. Each sterile Petri dish containing Muller Hinton agar media was uniformly inoculated by spreading the standardized bacterial suspension to compare the 0.5 McFarland McFarlandstandard using sterile cotton swab The inoculated Petri dishes were allowed to stand for an hour to make proper absorption of the inoculum into the agar surface. Using sterile cork borers of diameter 8mm uniform holes (wells) were created on the inoculated agar media by removing agar plug using sterile forceps. The formed wells were each filled aseptically with 100 µl of prepared aqueous and ethanolic extracts of plant at different concentration as stated in (3.4) with adequate care was taken to ensure that not overflow the extracted solution when Dispensed into the wells.

The inoculated petri dishes were then left to stand on the bench at room temperature for 30 minute for proper diffusion of extract into agar medium before incubation of plate on the incubation machine for 24 hours at 370C under an inverted position 3.6.2 Controls Both positive and negative control have been incorporated into each experimental batch to provide verification for the antibacterial susceptibility assay Positive control contains amoxicillin (30 µg)/ciprofloxacin to ensure that the organisms were still sensitive to a known antibiotic and Negative control contain 5% DMSO as described in (3.5.2) to verify that only the extract had inhibited microbial growth 3.7Determination of Antimicrobial Activity Antimicrobial activity of both aqueous and ethanolic stem extract of Anacardium occidentale and Vernonia amygdalina were evaluated and characterized by measurement of diameter of clear zones (zone of inhibition) around wells on Mueller-Hinton agar plate After incubation for 24 hrs at 370 C, the zones of inhibition present in each plates are measured.

The diameters of clear zones including the wells were measured with a Transparent metric ruler to the nearest mm in diameter in three separate lines and the mean zone diameters of the inhibition are recorded, average of the measurements were recorded Where inhibition zones are irregular two measurements along perpendicular axis, including the diameter of wells was recorded and the mean value determined. 3.8Minimum Inhibitory Concentration (MIC) Two-fold serial dilutions of each of the crude extract were prepared in sterile Mueller-Hinton broth and used to generate series of different extract concentrations The standardized bacterial inoculum that equivalent to 0.5 McFarland standard (1.5 x 108 CFU/mL) was added to tubes (or microplate wells) that containing different concentration of the extract Equal volumes were used in both positive (broth and bacteria without extract), negative (broth only) and the different extract concentration tubes. The tubes (or microplates) were incubated aerobically at 370C for 24 h After incubation, bacterial growth was evaluated by visually observing turbidity in the tubes or wells and lowest concentration at which the complete inhibition of bacterial growth occurs was identified as the minimum inhibitory concentration (MIC)

CHAPTER FOUR

RESULTS

4.1 Extraction of the stem plants

The extraction were carried out to identify the aqueous of Anacardium occidentale (cashew), ethanolic, the aqueous and ethanolic of Vernonia amygdalina (bitter leaf) in the stem. The results gave crude pastes for both extracts.

Table 4.1: Results for the extraction of stem plants

Extracted Plant Species/ sample code	Solvent	Physical Appearance
Anacardium occidentale	Ethanol	Thick dark-brown residue
Anacardium occidentale	Water	Light-brown semi-solid extract
Vernonia amygdalina	Ethanol	Dark greenish-brown viscous extract
Vernonia amygdalina	Water	Greenish-brown semi-solid extract

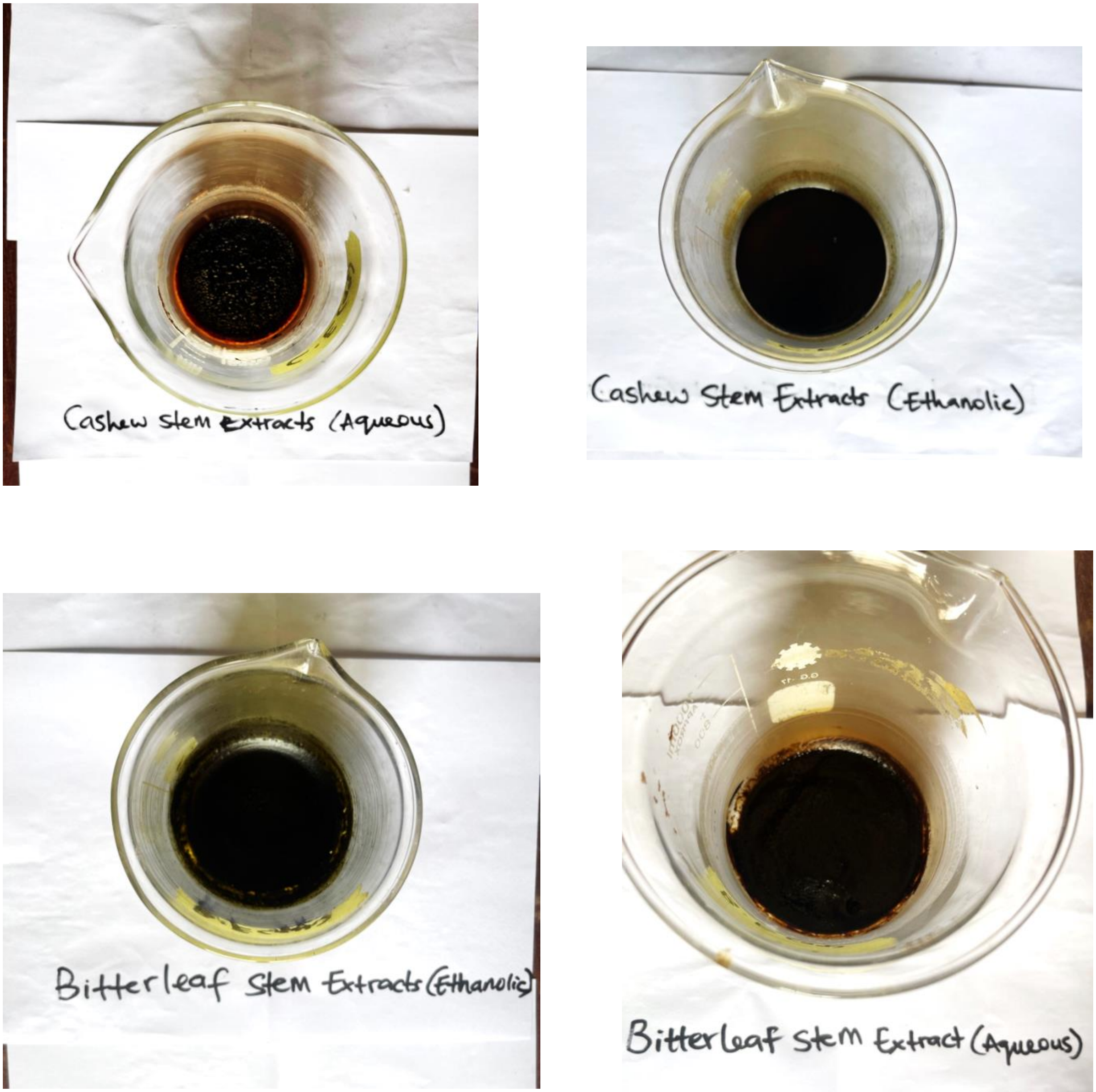

Figure 4.1: Results for the extraction of stem plants

4.2 Characterization and biochemical isolation of the oral bacteria isolated.

Following isolation, the bacterial colonies were characterized based on their colony morphology, gram staining characteristics, catalase reactions and oxidase reactions.

Table 4.2: Characterization and biochemical isolates of the oral bacteria isolated.

Isolate	Colony morphology	Gram reaction	Catalase	Oxidase	Most probable organism
F4(BA)	Dull white, irregular, dry, raised	Gram +	-	+	*Bacillus spp.*
F2(MSA)	Pink/ red, irregular, dry, raised	Gram +	+	+	*Staphylococcus spp.*
F5(MSA)	Pink/red, irregular, dry, raised	Gram +	+	+	*Staphylococcus spp.*
F5 (BA)	Dull white, irregular, dry, raised	Gram +	++		*Bacillus spp.*
F6(BA)	Dull white, irregular, dry, raised	Gram +	++	+	*Bacillus spp.*
M1(BA)	Dull white, irregular, dry, raised	Gram +	+	+	*Bacillus spp.*
M2(BA)	Dull white, irregular, dry, raised	Gram +	++	+	*Bacillus spp.*
F6(MA)	Transperant, spherical	Gram +	+	+	*Streptococcus spp.*
M3(BA)	White, spherical	Gram +	+	+	*Streptococcus spp.*
M4(BA)	White, spherical	Gram +	++	+	*Streptococcus spp.*

Keywords: + (positive) – (negative)

F is sample code and media used

4.3 Zones of inhibition

The aqueous stem extracts of *Anacardium occidentale* (bitter leaf) and *Vernonia amygdalina* (cashew) showed the anti-microbial activity of the plant extract if the oral bacterial isolation is presented below.

Aqueous *Vernonia amygdalina* is higher than aqueous *Anacardium occidentale* which showed that *Vernonia amygdalina* extracts (40mm) has significantly stronger anti-bacterial activity than the aqueous stem extracts of *Anacardium occidentale* (37mm).

Table 4.3: Zones of inhibition for aqueous stem extracts

Plant extracts	Concentration in mg/ml											
	Bacillus spp.				***Staphylococcus spp.***				***Streptococcus spp.***			
	100mg/ml	**50mg/ml**	**25mg/ml**	**12.5mg/ml**	**100mg/ml**	**50mg/ml**	**25mg/ml**	**12.5mg/ml**	**100mg/ml**	**50mg/ml**	**25mg/ml**	**12.5mg/ml**
***A. occidentale* (aqueous)**	22	21	21	21	23	22	28	25	36	27	27	26
	35	24	33	29	37	33	31	25	23	20	19	22
	28	29	37	28	-	-	-	-	24	24	22	23
	21	23	19	19	-	-	-	-	-	-	-	-
	21	17	23	18	-	-	-	-	-	-	-	-
***V. amygdalina* (aqueous)**	28	19	23	24	20	17	14	22	23	36	32	20
	29	16	26	27	31	34	38	40	21	28	22	30
	34	35	31	37	-	-	-	-	39	30	32	17
	25	31	31	36	-	-	-	-	-	-	-	-
	25	13	18	20	-	-	-	-	-	-	-	-
Negative control(DMSO)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Postive control(Amoxcillin)	52	35	28	20	52	35	28	20	52	35	28	20

Keywords: - (NIL)

4.4 Zones of inhibition for Ethanolic stem extract

The ethanolic stem extracts of *Anacardium occidentale* (bitter leaf) and *Vernonia amygdalina* (cashew) showed the anti-microbial activity of the plant extract if the oral bacterial isolation is presented below.

Ethanolic *Vernonia amygdalina* is higher than ethanolic *Anacardium occidentale* which showed that ethanolic *Vernonia amygdalina* extracts (41mm) has significantly stronger anti-bacterial activity than the ethanolic stem extracts of *Anacardium occidentale*(29mm).

Table 4.4: Zones of inhibition for ethanolic stem extracts

Plant extracts	**Concentration in mg/ml**											
	Bacillus spp.				***Staphylococcus spp.***				***Streptococcus spp.***			
	100mg/ml	**50mg/ml**	**25mg/ml**	**5mg/ml**	**100mg/ml**	**50mg/ml**	**25mg/ml**	**5mg/ml**	**100mg/ml**	**50mg/ml**	**25mg/ml**	**5mg/ml**
***A. occidentale*(ethanol)**	22	19	20	18	28	27	21	27	28	27	20	16
	29	0	25	21	23	19	24	19	27	26	27	15
	23	23	29	19	-	-	-	-	24	23	19	15
	0	0	27	29	-	-	-	-	-	-	-	-
	22	24	0	22	-	-	-	-	-	-	-	-
***V. amygdalina*(ethanol)**	33	40	35	40	33	37	35	41	34	35	32	37
	32	28	28	29	31	28	27	25	35	33	36	29
	35	39	30	36	-	-	-	-	38	36	37	34
	25	30	31	29	-	-	-	-	-	-	-	-
	38	35	39	38	-	-	-	-	-	-	-	-
Negative control(DMSO)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Positive control(Amoxicillin)	52	35	28	20	52	35	28	20	52	35	28	20

Keywords: (-) NIL

4.5 Minimum inhibitory concentration of the stem plant extracts

The minimum inhibitory concentration results revealed that aqueous extracts of *Anacardium occidentale* (cashew) required lower concentration to inhibit bacterial growth which indicates higher potency of aqueous as an extraction solvent. The minimum inhibitory concentration (MIC) lowest concentration was showed on the test isolates of oral bacteria which signify lowest concentration and it was on F4(BA) of *Bacillus spp*. that showed minimum bactericidal concentration (MBC)

Table 4.5: Minimum inhibitory concentration (MIC)

Sample isolate	Plant extracts	Solvents	Mlc(mg/ml)
F4(BA)	***A. occidentale***	Aqueous	25mg/ml
F5(MA)	***A. occidentale***	Ethanol	25mg/ml

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

Since the rising issue of the prevalence of oral bacterial infections and the threat of growing resistance to antimicrobial drugs, an interest has been expressed in identifying alternatives antimicrobial from medicinal plants. In the present study, antimicrobial activity of the aqueous and ethanolic stem extracts of Anacardium occidentale (cashew) and Vernonia amygdalina (bitter leaf) against selected oral bacteria isolates was evaluated. This study showed that these two medicinal plants have appreciable antimicrobial activities against the oral bacteria, Bacillus spp., Staphylococcus spp., and Streptococcus spp. This has scientifically confirmed their usage as antimicrobials for the treatment of infections as widely accepted and practice.

Bacterial Isolates from this research have been identified based on colony morphology, Gram-stain characteristics, catalase, and oxidase test reaction to be Bacillus spp., Staphylococcus spp. And Streptococcus spp. These are known among those bacteria associated in the human mouth and are capable to cause opportunistic infections of the mouth when the oral normalflora has been disturb. For Streptococcus spp. Such as Streptococcus mutans; the well known culprits in dental plaque, dental caries, is as a result of its capacity to digest the diet carbohydrates to organic acid to demineralize the tooth enamels (Sedghi et al., 2024). Likewise, Staphylococcus spp. Can result to infections of the oral mucous membrane, denture-associated stomatitis, post surgical infections of the wounds, even though the Bacillus spp. Are generally regarded not to be oral bacteria but can be found in oral cavities and under appropriate conditions become opportunists (Brookes et al., 2020). Identification of the organisms confirmed that they are of clinic significance in the assessment of antimicrobials meant for oral hygiene.

Crude extracts obtained from Anacardium occidentale and Vernonia amygdalina stem using both water and ethanol solvents showed that effectiveextraction of biological components can occur using both methods and that they yielded a difference in consistency and color, thus suggests a difference in their phytochemical profiles. Ethanol is widely recognized as one of the most effective solvent for extracting plant constituents since it dissolves many plant bioactive components like alkaloids, phenolics, terpenoids and flavonoids. Water extracts mostly contain those with highly polarity, such as polysaccharides and most phenolic compounds, and hence this has made this method often perceived as less potent for plant based drug development as many compounds of medicinal plants have limited solubility in water. Therefore, an explanation for variations between aqueous and ethanolicextract antibacterial activities in the study has been provided (Kumar et al., 2023; Peiris et al., 2023).

Antibacterial effect of Anacardium occidentale and Vernonia amygdalina stem extracts on the target bacterial organisms was demonstrated by all the tests. The tested plant extracts are capable to inhibit all the target oral bacteria. This study also corresponds with studies by Oladeji et al. (2022) and Nwafor et al. (2023) that indicated a significant antibacterial activity in Anacardium occidentale and Vernonia amygdalina plant. This activity might have been due to presence of some antimicrobial compounds such as flavonoids, phenolics, tannins, saponins, alkaloids, and sesquiterpene lactones that kill bacteria via destruction of cell wall, disruption of membrane permeability, inhibition of enzyme activity, nucleic acids biosynthesis prevention and prevention of formation of bacterial biofilms (Agyare et al., 2022; Altemimi et al., 2023).

From table 1 and figure 1, both water extracted samples of the tested plants exhibited noticeable antibacterial activities against the bacterial isolates examined. Nevertheless, Vernonia amygdalina showed superior activities than that of Anacardium occidentale, the highest inhibition zone shown by Vernonia amygdalina aqueousextract being 40 mm and an inhibition zone of 37 mm shown by the aqueous extract of Anacardium occidentale. These findings suggested that water soluble compounds with good antibacterial potential exist in the extracts of the two medicinalplants and that Vernonia amygdalina has higher antibacterial compounds in water soluble forms than Anacardium occidentale. Thisis in consonantwith studies that report on the activity of vernodalin in Vernonia amygdalina as a compound containing phenolics, alkaloids, saponins and flavonoid and that it has showed excellent effects on some other bacteria in the studies of Adesanoye et al. (2022) and Ojo et al. (2022).

The ethanolic extracts also showed an interesting activity on all tested oral bacteria. The maximum inhibitionzone recorded was with vernodalin (41 mm), followed by Vernonia amygdalina stem ethanolic extract with inhibition zone of 41 mm, the ethanolic extract of Anacardium occidentale extract have a 29 mm zone of inhibition as compared to others extracts. This is indication of greater potential in ethanolic extracts of the two plants against all the bacterial Isolates used and again Vernonia amygdalin had demonstrated higher potency against them than Anacardium occidentale. This is likely due to greater quantity of flavonoids, alkaloids and phenolic contents in the plant species of vernacular Vernoniaamygdalina,vernodalin andvernolin A and E which is know to possess strong antibacterial activity on a wide range of bacterial strains (Nwafor et al., 2023). According to El-Saber Batiha et al. (2023) the presence of phenolics in plants enhances antibacterial action because phenolics damage cellular membranes and inhibits enzymatic processes in the body of the organism.

Even though the zones of inhibition exhibited by Anacardium occidentale were less when compared to that of Vernonia amygdalina, but this plant has still shown effective activity against all bacteria. This finding is in line with research conducted by Oladeji et al. (2022) and Eze et al. (2023) that indicated Anacardium occidentaleextract contains anacardic acid, flavonoids, and tanninswhich inhibits various harmful bacteria, by means of mechanisms like damage to enzyme function, increase in membrane permeability, and reduction of protein synthesis which inhibit bacterial multiplication and survival. Although Anacardium occidentale show less activity compared to Vernonia amygdalina it has shown considerable potential in managing oral infections.

The result of the present study shows that the antibacterial activities of both plant extracts were concentration-dependent. The high extract concentrations (100 and 50 mg/mL) exerted greater inhibitory activity as seen by their corresponding larger zones of inhibition compared to those observed with lower extract concentrations (25 and 12.5 mg/mL). These findings suggest that an increase in extract concentration increases the concentration of the active phytochemicals that interact with bacteria, which enhances bacterial inhibition. These results are comparable with those reported in previous studies, such as that of Sharma et al. (2022) and Bharathi et al. (2023) on plant extract antimicrobial effects on various bacterial strains.

In order to compare the degree of bacterial inhibition by A. Occidentale and V. Amygdalina. Extracts a method described by Olayemi et al. (2005) was employed, the result of which shown in Tables.2 The antibacterial activities displayed by V. Amygdalina and A. Occidentale are possibly attributed to the varying array of bioactive constituents present in the plants. For example, previous researchers had reported presence of sesquiterpene lactones (vernodalin and vernolides), and various flavonoids, which exhibited a broad range of activities and possibly act against diverse bacteria oral bacteria in V. Amygdalina (Adesanoye et al., 2022; Nwafor et al., 2023) These compounds may exhibit activity through damaging the cell wall, altering membrane permeability, impairing protein and nucleic acid synthesis and inhibition of bacteria biofilm formation (Altemimi et al., 2023).

Since bacterial biofilm formed by oral pathogenic bacteria often increases the risk of colonization and persistence within the oral cavity, biofilm formation is known to play a crucial role in oral pathogenicity (Sedghi et al., 2024).

Likewise the observed antibacterial activity of A. Occidentale against oral isolates may be attributed to the richness of anacardic acids, tannins, flavonoids and other phenolics which are previously demonstrated to have bactericidal and bacteriostatic activity against diverse range of organisms through the inhibition of crucial enzymes, altering the permeability of bacterial cell membrane and affecting the cell's metabolism (Oladeji et al., 2022; Eze et al., 2023). Although the inhibition zone by A. Occidentale are generally small as compared to that of V. Amygdalina the activity is consistent in all oral isolates tested indicating its efficacy as a medicinal plant and a source of potential antibacterial compound.

Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentrations (MICs) of the plant extracts against tested bacteria strains shown in Table 3 confirmed the antibacterial activities noted in the agar diffusion method. MIC is the minimum concentration of an antimicrobial agent that prevents the visible growth of a microorganism after a given incubation period. In this study, it was observed that the extracts suppressed bacterial growth at fairly low concentrations, showing substantial potency against oral pathogens.

SmallMIC values indicate a more potent antimicrobial agent because less amounts of it will be needed to inhibit bacterial proliferation (Kumar et al., 2023).

Thus the results from the MIC tests suggest that both plants contains highly active phytochemical agents that are effective in suppressingoral bacteria infection. The most active MIC of 1.56 mg/ml was recorded against Streptococcus sanguinis and Enterococcus faecalis by A. Occidentale extract while Staphylococcus aureus was most sensitive to V. Amygdalinaextracts withMIC of 1.56mg/ml and all plant extracts were also effective against P. Micra. Table 3MIC of ethanolic extracts of A. Occidentale and V. Amygdalinaagainst Oral bacterial isolates (mg/ml).

Positive control (Amoxicillin) produced largest inhibition zone among all treatment used on the bacterial isolates (Table 2). Amoxicillin a purified broad-spectrum antibiotic with specific mechanism of inhibiting the synthesis of the bacterial cell wall (Cope et al., 2022) gave positive result as it was expected. Althoughplant extracts gave less inhibition zones than amoxcillin, the effectiveness of plants againstoral bacteria in the present study indicated that medicinal plants could be used as adjunct to current antibiotic therapy. Unlike classical antibiotics that normally operate through one specific pathway, traditional medicines possess numerous bioactive phytochemical compounds which attack pathogens in multiple sites at the same time thereby circumventing or reducing chances of developing antimicrobialresistance(Altemimi et al., 2023; World Health Organization, 2023).

Even with the hopeful results, there are a few setbacks to this research. The antimicrobial testing took place within a laboratory (in vitro) which might not be completely reflective of the environment within the human mouth. It would be more of a representation of the environment of the oral cavity due to factors like saliva composition, host immunity, pH of the mouth, and the interaction between normal oral microorganisms, all of which would affect the overall efficacy of these plant extracts.

Moreover, the plant extracts weren't individually separated nor was a specific phytochemical component identified responsible for the bacterial-inhibiting effect.

Future research should involve the identification of these compounds, tests assessing safety andtoxicity, as well as investigations into the mode of action and the Minimum Bactericidal Concentration (MBC) of the plant extracts, in order to determine safety and effectiveness. If the plants prove to be both safe and effective for the purposes of a dental treatment plan, the extracts should then be formulated into an orally-administered solution, gel, paste or mouth rinse that can be used.

To sum up, the results of this research suggest that Anacardium occidentale and Vernonia amygdalina can be successfully utilized as antibacterial agents in the treatment of certain oral microorganisms. Vernonia amygdalina's greater antibacterial potency than Anacardium occidentale has led this study to assume that it is a potentially more potent resource of natural antimicrobial compounds and agents, which may be applied in oral hygiene. These results lend some scientific credence to the oral applications traditionally attributed to these medicinal plants and reinforce the notion of herbal treatments for dental care.

Another observation from this particular study is that the antibacterial effect of the extracts was concentration dependent. The extracts' concentrations at 100 mg/mL and 50 mg/mL generated larger zones of inhibition than concentrations at 25 mg/mL and 12.5 mg/mL. This is as a result of increased numbers of bioactive compounds present at the high concentrations, and these increased quantities are able to more readily interact with the bacteria to inhibit their growth.

Similar observations to the findings of this study have previously been observed by Sharma et al. (2022) and Bharathi et al. (2023) who have established that antibacterial effects of medicinal plant extracts generally increased in their efficacy with the increasing concentrations of the extracts.

The increased effectiveness of Vernonia amygdalina over Anacardium occidentale may be attributed to variation in the compounds that they contain. It has previously been reported that the major compounds found in Vernonia amygdalina extracts, namely alkaloids, saponins, flavonoids, tannins, sesquiterpene lactones and phenolic compounds possess various antimicrobial activity [9,10]. These various compounds kill bacterial pathogens by damaging cell walls, enhancing membrane permeability, prohibiting protein synthesis, affecting the ability to duplicate dna and by hindering the creation of bacterial colonies. The ability of the plant extracts to inhibit bacterial colonies is a significant finding, as oral pathogens utilize colonies for adhesion as well as disease progress and the creation of colonies has also shown to contribute to the pathogenicity of certain bacteria [11].

The bacteriostatic and bactericidal activities recorded for the Anacardium occidentale extract were contributed to by its main constituent compounds, namely anacardic acid, tannin, flavonoids and phenolics. [12,13]. As the growth zone of Anacardium occidentale's activity was slightly lower than the zones of inhibition obtained using Vernonia amygdalina, the inhibition recorded using the former was consistent among all three bacteria and the plant also proved to be potentially effective in targetingoral infections.

The data from the measurement of the minimum inhibitory concentration (MIC) are further evidence of the antibacterial effects observed during the diffusion method. The MIC can be described as the minimum quantity of an antibiotic agent required to stop the progress of bacteria as evident by the naked eye. Plant extracts that showed a lowMICvalueare thought to be of high effectivenessas it has been establishedthat such a small quantity of extract is sufficient to prohibit bacteria growth [14]. In this research, the minimum inhibitory concentrations calculated for the extracts indicated the effective action of compounds contained within the extracts against a selection of oral bacteria.

The zones of inhibition generated with the positive control (amoxicillin) against all of the tested bacteria were significantly larger than those created by either extract, which was to be expected due to amoxicillin's broad spectrum activity and mechanism of action (inhibiting the cell wall of bacteria) [15]. In addition, plant extracts are likely to have a variety of mechanisms of actions due to the number of phytochemicalspresent within the extracts which act on bacteria through several targets simultaneously. This multipronged approach may reduce the likelihood of developingresistance in microorganisms and this has garnered a substantial amount of scientific interest in recent years [16,17]. The lack of an inhibition zone when the negative control (5% DMSO) was used demonstrates that the solvent does not possess any bacterial-inhibiting properties and, therefore, the measured inhibition caused by the extracts is solely as a result of active constituents in the plants. The use of positive and negative controls makes this research valid.

The results of this study can be useful for oral health in areas of developing countries where medical and dental facilities are scarce. Diseases affecting the mouth remain the most prevalent non-communicable diseases worldwide and affect as many as billions of people, causing an immense amount of pain, loss of teeth, and significant medical expenses [17]. In an effort tocombat antibiotic resistance, oral and medical practitioners need to consider more options to manage infections as an alternative to the conventional antibiotic treatments. Medicinal plant compounds such as those found within Anacardium occidentale and Vernonia amygdalina are widely used in the areas of many countries to combat the progression oforal infections. Theseplants couldbe incorporated intooral toothpastes, gels, rinses and mouthwashes due totheir abundance and affordability.

Even with the hopeful results, there are a few setbacks to this research. The antimicrobial testing took place within a laboratory (in vitro) which might not be completely reflective of the environment within the human mouth. It would be more of a representation of the environment of the oral cavity due to factors like saliva composition, host immunity, pH of the mouth, and the interaction between normal oral microorganisms, all of which would affect the overall efficacy of these plant extracts. Moreover, the plant extracts weren't individually separated nor was a specific phytochemical component identified responsible for the bacterial-inhibiting effect. Future research should involve the identification of these compounds, tests assessing safety andtoxicity, as well as investigations into the mode of action and the Minimum Bactericidal Concentration (MBC) of the plant extracts, in order to determine safety and effectiveness. If the plants prove to be both safe and effective for the purposes of a dental treatment plan, the extracts should then be formulated into an orally-administered solution, gel, paste or mouth rinse that can be used.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) also confirmed the antibacterial action of the stem extracts against the oral bacteria. The F4 (Bacillus spp.) treated with the aqueous stem extract of Anacardium occidentale was the only isolate that had a significant MIC value at 25 mg/mL, in which no turbidity was observed and the broth was completely clear (no bacterial growth). However, all the remaining bacterial isolates showed turbidity at all concentrations tested which showed that the concentrations used for the test were not sufficient to inhibit growth and that these isolates were less susceptible to plant extracts.

The Minimum Bactericidal Concentration (MBC) was determined by taking aliquots from the MIC tube of the F4 (Bacillus spp.) treated with aqueous stem extract of Anacardium occidentale (25 mg/mL) and then streaking them on sterile Mueller-Hinton agar. There was no growth of bacteria after 24 hr of incubation which indicates that the bacteria was killed not inhibited by the extract. Therefore, the MIC and MBC of the aqueous stem extract of Anacardium occidentale on F4 (Bacillus spp.) was found to be 25 mg/mL. Having the same MIC and MBC indicates that aqueous stem extract of Anacardium occidentale showed a bactericidal effect against the sensitive isolate.

Lack of MIC values for the other isolates did not mean that the extracts were inactive, as clear zones were found against the other isolates in the agar well diffusion assay; rather, it means that higher concentrations may be needed in the broth to cause inhibition (Kumar et al., 2023; Oladeji et al., 2022). Higher MICs observed against the other bacterial isolates might be due to differences in bacterial cell wall structure, intrinsic resistance, or other resistance mechanisms of bacterial species, variations in inoculum size, or even different sensitivities to the specific compounds (phytochemicals) present in plant extracts.

Both MIC and MBC were obtained for F4(Bacillus spp.) isolate, indicating the potential to exhibit both bacteriostatic and bactericidal effect on the susceptible isolate by aqueous stem extract of Anacardium occidentale and suggests its possible application as a natural oral antimicrobial agent. However, it may be worth evaluating the effectiveness of aqueous stem extract of Anacardium occidentale on the other bacterial isolates with higher concentrations and further analysis should focus on purification of the active phytochemical(s) and subsequent in vivo testing.

5.2 Conclusion

The study evaluated the antimicrobial properties of the aqueous and ethanol stem extracts of Anacardium occidentale (cashew) and Vernonia amygdalina (bitter leaf) against selected oral bacteria such as Baccilus spp, Staphylococcus spp., and Streptococcus spp. The results revealed that the aqueous and ethanol stem extracts of Anacardium occidentale and Vernonia amygdalina have significant antibacterial activity against all the selected oral pathogenic bacteria, Vernonia amygdalina has superior antibacterial activity against the isolates while the aqueous stem extract of Anacardium occidentale exhibit inhibitory and bactericidal effects on the F4 (Bacillus spp.) at 25 mg/mL. These findings reinforce the medicinal properties of these plants which can be applied in oral antibacterial management.

Recommendation

To explore the isolation and characterization of the compounds contributing to the antibacterial activity. However, further works need to be conducted with regarding to determine the toxicity study, pharmacology study, preparation of the herbal oral care products and conducted in vivo study.

REFERENCES

Adeniyi, A. A., Sofola, O. O., and Kalliecharan, R. V. (2022). Oral health care utilization and traditional practices among populations in sub-Saharan Africa: A public health review. *BMC Oral Health*, 22, 487.

Adesanoye, O. A., Akinpelu, D. A., and Aiyegoro, O. A. (2022). Antimicrobial and phytochemical properties of Vernonia amygdalina: A review. Journal of Herbal Medicine, 32, 100531.

Adesanoye, O. A., Farombi, E. O., and Olatunji, L. A. (2022). Antimicrobial and phytochemical properties of *Vernonia amygdalina* extracts against selected pathogenic microorganisms. *BMC Complementary Medicine and Therapies*, 22, 184,

Advanced extraction techniques for herbal drugs: A comprehensive review. *Research Journal of Pharmacognosy and Phytochemistry, 17*(3), 229–240.

Agyare, C., Appiah, T., Boakye, Y. D., and Apenteng, J. A. (2022). African medicinal plants: Bioactive compounds, antimicrobial activities and therapeutic applications. Academic Press.

Agyare, C., Boakye, Y. D., Bekoe, E. O., Hensel, A., Dapaah, S. O., and Appiah, T. (2022). Review of African medicinal plants with antimicrobial activities against oral pathogens. *Journal of Herbal Medicine*, 32, 100540.

Akinmoladun, A. C., Ibukun, E. O., and Dan-Ologe, I. A. (2023). Antibacterial activities of *Psidium guajava* leaf extracts against oral pathogens. *Journal of Herbal Medicine*, 39, 100672.

Akram, Z., Safii, S. H., Vaithilingam, R. D., Baharuddin, N. A., Javed, F., and Vohra, F. (2023). Probiotics in the management of periodontal disease: A systematic review and meta-analysis. *Clinical Oral Investigations*, 27(2), 485–498.

Alghamdi, F., Al-Zahrani, M. S., and Alqahtani, A. S. (2022). Antimicrobial efficacy of *Salvadorapersica* against periodontal pathogens: A systematic review. *Clinical and Experimental Dental Research*, 8(5), 1245–1254.

Altemimi, A. B., Alhelfi, N., Ali, S. A., Pasqualone, A., andFidan, H. (2023). Phytochemicals: Extraction, isolation, and role against microbial pathogens. *Molecules*, 28(3), 1158.

Altemimi, A. B., Al-Hilphy, A. R. S., Watson, D. G., andIbrahim, S. A. (2023). Phytochemicals: Extraction, isolation and identification of bioactive compounds from medicinal plants. Plants, 12(4), 856.

Amin, M., Rakhshandeh, H., and Niknam, S. A. (2023). Antibacterial effects of *Glycyrrhiza glabra* on cariogenic microorganisms: A review. *Frontiers in Oral Health*, 4, 1182247.

Arbildo-Vega, H. I., *et al*. (2024). Periodontal disease and cardiovascular disease: Umbrella review. *BMC Oral Health*, 24, Article 4907.

Baker, J. L., Mark Welch, J. L., Kauffman, K. M., McLean, J. S., and He, X. (2024). The oral microbiome: Diversity, biogeography and human health. *Nature Reviews Microbiology, 22*(2), 89–104.

Baker, J. L., Faustoferri, R. C., and Quivey, R. G. (2023). Acid tolerance response and virulence of *Streptococcus mutans*: A review. *Frontiers in Oral Health*, 4, 1162748.

Bamidele, O. M., Adebayo, E. T., and Adeyemi, O. O. (2023). Ethnomedicinal approaches to oral healthcare management in developing countries: A review of medicinal plants used in Africa and Asia. *Journal of Herbal Medicine*, 38, 100640.

Benzian, H., Guarnizo-Herreño, C. C., Kearns, C., Muriithi, M. K., and Watt, R. G. (2023). Untreated oral diseases and inequalities in low- and middle-income countries. *The Lancet Global Health*, 11(7), e1040–e1041.

Bharathi, D., Ramesh, A., and Venkatesan, A. (2023). Antimicrobial activity of neem extracts against oral pathogens associated with dental caries and periodontal disease. *Journal of Oral Biology and CraniofacialResearch*, 13(4), 412–418.

Bharathi, R., Prakash, M., and Kumar, S. (2023). Evaluation of antibacterial activity of medicinal plant extracts against pathogenic bacteria. Journal of Applied Biology and Biotechnology, 11(3), 95–103.

Bida, F. C., *et al*. (2025). The systemic link between oral health and cardiovascular disease. *Journal of Clinical Medicine*, 14(1), 1–15.

Brookes, Z. L. S., Bescos, R., Belfield, L. A., Ali, K., andRoberts, A. (2020). Current uses of chlorhexidine for management of oral disease: A narrative review. *Journal of Dentistry*, 103, 103497.

Brookes, Z. L. S., Bescos, R., Belfield, L. A., Ali, K., andRoberts, A. (2020). Effects of chlorhexidine mouthwash on the oral microbiome. *Scientific Reports*, 10, 5254.

Bui, F. Q., Almeida-da-Silva, C. L. C., Huynh, B., Trinh, A., Liu, J., Woodward, J., Asadi, H., and Ojcius, D. M. (2022). Association between periodontal pathogens and systemic disease. *Biomedical Journal*, 45(1), 27–35.

Clinical and Laboratory Standards Institute. (2023). *Performance standards for antimicrobial susceptibility testing* (33rd ed.). CLSI.

Cope, A. L., Francis, N. A., Wood, F., & Chestnutt, I. G. (2022). Antibiotic prescribing in dentistry and antimicrobial resistance. *British Dental Journal,* 232(1), 25–30.

Cope, A. L., Francis, N. A., Wood, F., and Chestnutt, I. G. (2022). Antibiotic prescribing in dentistry and antimicrobial resistance. *British Dental Journal*, 232(1), 9–14.

El-Saber Batiha, G., Alqahtani, A., Ojo, O. A., Shaheen, H. M., Wasef, L., Elzeiny, M., Ismail, M., Shalaby, M., Murata, T., Zaragoza-Bastida, A., and Rivero-Perez, N. (2023). Bioactive compounds and pharmacological actions of garlic (*Allium sativum*). *Foods*, 12(2), 345.

Etta, I., *et al*. (2023). A systematic review on the impact of periodontal disease and cardiovascular diseases. *International Journal of Dentistry*, 2023, 1–10.

Eze, E. A., Okeke, C. U., and Nwankwo, I. U. (2023). Antibacterial properties of Anacardium occidentale stem extracts against selected pathogenic bacteria. *African Journal of Microbiology Research,* 17(2), 58–67.

Eze, P. M., Abonyi, D. O., and Abba, C. C. (2023). Antibacterial and phytochemical evaluation of *Anacardium occidentale*stem bark extracts. *South African Journal of Botany*, 156, 79–86.

Gao, Z., Chen, X., Wang, C., Song, J., Xu, J., Liu, X., Qian, Y., and Suo, H. (2023). New strategies and mechanisms for targeting *Streptococcus mutans* biofilm formation to prevent dental caries: A review. *Microbiological Research, 278*, 127526.

Global Burden of Disease Oral Disorders Collaborators. (2022). Global, regional, and national burden of oral disorders and associated risk factors, 1990–2019: A systematic analysis for the Global Burden of Disease Study 2019. *The Lancet Public Health*, 7(1), e54–e67.

Gupta, D., Bhaskar, D. J., Gupta, R. K., Karim, B., Jain, A., Dalai, D. R., and Grewal, Y. S. (2022). Comparative evaluation of neem mouthwash and chlorhexidine on gingival health: A randomized clinical trial. *International Journal of Dentistry*, 2022, 1–8.

Hajishengallis, G., and Chavakis, T. (2021). Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. *Nature Reviews Immunology*, 21(7), 426–440.

Halawany, H. S. (2022). A review on miswak (*Salvadorapersica*) and its effect on various aspects of oral health. *Saudi Dental Journal*, 34(1), 1–10.

Hargreaves, K. M., and Abbott, P. V. (2022). Drugs for pain management in dentistry. *Australian Dental Journal*, 67(S1), S89–S98.

Hewlings, S. J., and Kalman, D. S. (2022). Curcumin: A review of its effects on human health. *Foods*, 11(15), 2167.

Hou, K., Wu, Z. X., Chen, X. Y., Wang, J. Q., Zhang, D., Xiao, C., Zhu, D., Koya, J. B., Wei, L., Li, J., Chen, Z. S., andChen, G. (2022). Microbiota in health and diseases. *Signal Transduction and Targeted Therapy*, 7(1), 135.

Hu, C. H., He, J., Eckert, R., Wu, X. Y., Li, L. N., Tian, Y., Lux, R., Shuffer, J. A., Gelman, F., Mentes, J., and Shi, W. (2022). Potential cariostatic effects of licorice extracts in children. *Caries Research*, 56(2), 176–184.

Ide, M., Karimova, M., and Setterfield, J. (2022). Oral health, antimicrobials and care for patients with chronic oral diseases: A review of knowledge and treatment strategies. *Frontiers in Oral Health,* 3, 866695.

Jain, N., Dutt, U., Radenkov, I., and Jain, S. (2024). WHO's global oral health status report 2022: Actions, discussion and implementation. *Oral Diseases,* 30(1), 73–79.

James, P., Worthington, H. V., Parnell, C., Harding, M., Lamont, T., Cheung, A., Whelton, H., and Riley, P. (2023). Chlorhexidine mouthrinse as an adjunctive treatment for gingival health. *Cochrane Database of Systematic Reviews*, 4, CD008676.

Jevon, P., Abdelrahman, A., and Pigadas, N. (2023). Management of odontogenic infections and sepsis: An update. *British Dental Journal*, 234(5), 311–317.

Khan, M. M., Ahmad, I., and Wahab, S. (2022). Antimicrobial effects of *Ocimum sanctum* against oral pathogens: An in vitro study. *BMC Oral Health*, 22, 219.

Kumar, A., Kumar, M., Jose, A., Tomer, V., Öz, E., Proestos, C., Zeng, M., Elobeid, T., K., S., and Öz, F. (2023). Major phytochemicals: Recent advances in health benefits and extraction methods. *Molecules,* 28(2), 887.

Kumar, S., Pandey, A. K., and Singh, P. (2023). Medicinal plants as sources of antimicrobial agents: A review. *Journal of Herbal Medicine,* 38, 100642.

Lemos, J. A., Palmer, S. R., Zeng, L., Wen, Z. T., Kajfasz, J. K., Freires, I. A., Abranches, J., and Brady, L. J. (2022). The biology of *Streptococcus mutans*. *Microbiology Spectrum*, 10(1), e00796-21.

Martande, S. S., Kumari, M., Pradeep, A. R., Singh, S. P., Suke, D. K., and Guruprasad, C. N. (2022). Host modulation therapy in periodontal disease management. *Journal of International Society of Preventive and Community Dentistry*, 12(1), 1–9.

Miller, W. R., Munita, J. M., and Arias, C. A. (2022). Mechanisms of antibiotic resistance in oral pathogens. *Clinical Microbiology Reviews*, 35(2), e00113-21.

Mistry, K. S., Sanghvi, Z., Parmar, G., and Shah, S. (2022). Neem and its role in oral health care: A review. *Journal of Traditional and Complementary Medicine*, 12(3), 201–209.

Mizraji, G., Heyman, O., Van Dyke, T. E., and Wilensky, A. (2023). The role of the oral mucosa immune system in maintaining oral health and systemic protection. *Frontiers in Immunology*, 14, 1186042.

Moghadam, E. T., Yazdanian, M., Tahmasebi, E., Tebyanian, H., Ranjbar, R., Seifalian, A., and Yazdanian, A. (2023). Curcumin-containing formulations in periodontal therapy: A clinical review. *Frontiers in Dentistry*, 20, 18.

Morrison, A. G., Sarkar, S., Umar, S., Lee, S. T. M., andThomas, S. M. (2023). The contribution of the human oral microbiome to oral disease: A review. *Microorganisms, 11*(2), 318.

Nassar, M., Hiraishi, N., Islam, M. S., Otsuki, M., and Tagami, J. (2022). Clinical effectiveness of green tea mouthwash on plaque and gingivitis control. *Clinical Oral Investigations*, 26(5), 3921–3929.

Nwafor, I. C., Okoli, C. O., and Onyeto, C. A. (2023). Antibacterial activities of *Vernonia amygdalina* leaf extracts against multidrug resistant bacteria. *Scientific African*, 20, e01642.

Ojo, O. A., Ojo, A. B., Ajiboye, B. O., and Oyinloye, B. E. (2022). Biofilm inhibitory and antifungal activities of *Vernonia amygdalina* extracts. *Journal of Applied Microbiology*, 133(4), 2104–2115.

Okello, D., Jun Yeob Lee, and Kang, Y.-M. (2020). *Ethnopharmacological Potential of Aspiliaafricana for the Treatment of Inflammatory Diseases*. *2020*, 1–11.

Okunlola, A., Afolabi, O. C., and Oyelami, O. A. (2022). Antimicrobial properties of *Garcinia kola* against oral pathogens associated with dental caries. *African Journal of Clinical and Experimental Microbiology*, 23(2), 159–167.

Oladeji, O. S., Adelowo, F. E., Ayodele, D. T., and Odelade, K. A. (2022). Phytochemistry and pharmacological activities of Anacardium occidentale: A review. *Heliyon,* 8(8), e10223.

Oladeji, O. S., Adelowo, F. E., Ayodele, D. T., and Odelade, K. A. (2022). Phytochemistry and antimicrobial properties of *Anacardium occidentale*: A review. *Heliyon*, 8(10), e11012.

Patil, S., Rao, R. S., Majumdar, B., and Anil, S. (2022). Viral infections and oral health manifestations: Current updates and perspectives. *Journal of Oral Biology and Craniofacial Research*, 12(5), 640–648.

Păunică, I., *et al*. (2023). The bidirectional relationship between periodontal disease and diabetes mellitus. *Diagnostics*, 13(4), 681.

Peiris, D. S. H. S., Fernando, D. T. K., Senadeera, N., and Ranaweera, C. (2023). Phytochemical screening for medicinal plants: Guide for extraction methods. *Asian Plant Research Journal, 11*, 13–34.

Peiris, L. D. C., Silva, P., & Perera, M. (2023). Influence of extraction solvents on phytochemical yield and antimicrobial activity of medicinal plants. Plants, 12(9), 1824.

Peng, X., Cheng, L., You, Y., Tang, C., Ren, B., Li, Y., Xu, X., and Zhou, X. (2022). Oral microbiota in human systematic diseases. *International Journal of Oral Science*, 14(1), 14.

Priya, B. M., Selvaraj, R., and Nivetha, R. (2022). Clinical evaluation of guava leaf mouthrinse on plaque-induced gingivitis. *Journal of Indian Society of Periodontology*, 26(4), 348–354.

Rajasekaran, J. J., Krishnamurthy, H. K., Bosco, J., Jayaraman, V., Krishna, K., Wang, T., and Bei, K. (2024). Oral microbiome: A review of its impact on oral and systemic health. *Microorganisms*, 12(9), 1797.

Rao, P. K., Ramesh, A., and Nagarajan, S. (2023). Efficacy of *Ocimum sanctum* mouthwash in plaque and gingivitis control: A randomized controlled trial. *Journal of Herbal Medicine*, 37, 100621.

Reygaert, W. C. (2022). Green tea catechins: Their use in treating and preventing infectious diseases. *BioMed Research International*, 2022, 9105261.

S., and Öz, F. (2023). Major phytochemicals: Recent advances in health benefits and extraction methods. *Molecules, 28*(2), 887.

Santacroce, L., Bottalico, L., Charitos, I. A., and Ballini, A. (2023). Oral microbiota in human health and disease: A perspective. *Pathogens*, 12(11), 1321.

Sanz, M., Herrera, D., Kebschull, M., Chapple, I., Jepsen, S., Beglundh, T., Sculean, A., Tonetti, M. S., and EFP Workshop Participants. (2022). Treatment of stage I–III periodontitis. *Journal of Clinical Periodontology*, 49(S24), 4–60.

Sawant, P., Ingle, N., Belavale, A., Shinde, A., Gunjal, A. A., and Kakade, R. T. (2025). Advanced extraction techniques for herbal drugs: A comprehensive review. *Research Journal of Pharmacognosy and Phytochemistry,* 17(3), 229–240.

Sedghi, L., DiMassa, V., Harrington, A., Lynch, S. V., and Kapila, Y. L. (2022). The oral microbiota: Community composition, influencing factors, pathogenesis and interventions. *Frontiers in Microbiology,* 13, 895537.

Sedghi, L., DiMassa, V., Harrington, A., Lynch, S. V., andKapila, Y. L. (2024). The oral microbiome and host interactions in oral and systemic health. *International Journal of Molecular Sciences*, 25(3), 1458.

Sharma, P., Dietz, A., and Kalra, S. (2022). Curcumin and oral health: Biological properties and therapeutic implications. *Molecules*, 27(19), 6405.

Sharma, P., Verma, R., & Singh, A. (2022). Concentration-dependent antibacterial activity of medicinal plant extracts against clinical bacterial isolates. *Journal of Applied Pharmaceutical Science,* 12(5), 112–120.

Shen, P., Zhao, J., and Walker, G. D. (2023). Green tea catechins and periodontal health: Current evidence and therapeutic potential. *Nutrients*, 15(6), 1421.

Soleimani, M., Bahramian, H., and Gholami, F. (2023). Traditional chewing sticks and oral health: Antimicrobial perspectives of miswak. *BMC Oral Health*, 23, 411.

Teoh, L., Stewart, K., Marino, R. J., and McCullough, M. J. (2023). Antibiotic resistance and relevance to oral infections. *Australian Dental Journal*, 68(2), 107–118.

Tufekci, E., Dixon, J. S., Gunsolley, J. C., and Lindauer, S. J. (2022). Effectiveness of essential oil mouthrinses in plaque and gingivitis control. *American Journal of Orthodontics and Dentofacial Orthopedics*, 161(4), 558–566.

Twetman, S. (2022). Prevention of dental caries as a non-communicable disease. *European Journal of Oral Sciences*, 130(3), e12806.

Ugbogu, E. A., Emmanuel, O., Dike, E. D., Agi, G. O., Ugbogu, O. C., Ibe, C., and Iweala, E. J. (2021). The Phytochemistry, Ethnobotanical, and Pharmacological Potentials of the Medicinal Plant-*Vernonia amygdalina* L. (bitter Leaf). *Clinical Complementary Medicine and Pharmacology*, *1*(1), 100006.

Villa, A., Abati, S., and Sonis, S. T. (2023). Oral candidiasis: Epidemiology, clinical manifestations, and management strategies. *Journal of Fungi*, 9(4), 421.

Watt, R. G., Daly, B., Allison, P., Macpherson, L. M. D., Venturelli, R., Listl, S., Weyant, R. J., Mathur, M. R., Guarnizo-Herreño, C. C., Celeste, R. K., Peres, M. A., Kearns, C., and Benzian, H. (2023). Ending the neglect of global oral health: Time for radical action. *The Lancet*, 401(10380), 1189–1200.

World Health Organization. (2022). Global oral health status report: Towards universal health coverage for oral health by 2030. World Health Organization.

World Health Organization. (2022). *Global oral health status report: Towards universal health coverage for oral health by 2030*. World Health Organization.

World Health Organization. (2023). *Antimicrobial resistance: Global report and surveillance update*. World Health Organization.

Zhang, S., Li, C., Liu, J., Geng, F., Shi, X., Li, Q., and Meng, X. (2023). *Fusobacterium nucleatum* and its association with colorectal cancer: Mechanisms and clinical implications. *Frontiers in Oncology*, 13, 1171986.