ANTIMICROBIAL AND PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACTS OF GUAVA AND NEEM YOUNG STEMS USED AS LOCAL CHEW STICKS ON COMMON DENTAL CARIES ORGANISMS*; LACTOBACILLUS SP. AND STREPTOCOCCUS SP.*

BY

ENEJE LOVINA IFUNANYA

GOU/U22/BTG/175

BIOTECHNOLOGY B.SC. PROGRAMME
DEPARTMENT OF BIOLOGICAL SCIENCES
FACULTY OF NATURAL SCIENCES AND ENVIRONMENTAL STUDIES
GODFREY OKOYE UNIVERSITY
UGWU-OMU NIKE.

JULY, 2026.

TITLE PAGE

ANTIMICROBIAL AND PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACTS OF GUAVA AND NEEM YOUNG STEMS USED AS LOCAL CHEW STICKS ON COMMON DENTAL CARIES ORGANISMS*; LACTOBACILLUS SP. AND STREPTOCOCCUS SP.*

BY

ENEJE LOVINA IFUNANYA
GOU/U22/BTG/175

PROGRAMME BIOTECHNOLOGY
DEPARTMENT OF BIOLOGICAL SCIENCES
FACULTY OF NATURAL SCIENCE AND ENVIRONMENTAL STUDIES,

A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCE, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE (B.Sc) IN BIOTECHNOLOGY

SUPERVISED BY PROF. C. O. ONYIA.

JULY, 2026

APPROVAL PAGE

This project entitled "**ANTIMICROBIAL AND PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACTS OF GUAVA AND NEEM YOUNG STEMS USED AS LOCAL CHEW STICKS ON COMMON DENTAL CARIES ORGANISMS (*Lactobacillus spp.* AND *Streptococcus spp.*)**" by Eneje Lovina Ifunanya (GOU/U22/BTG/175) has been examined and approved as meeting the requirements for the award of the Bachelor of Science (B.Sc.) Degree in Biotechnology, Department of Biological Sciences, Faculty of Natural Sciences and Environmental Studies, Godfrey Okoye University.

	……..	…….
Prof. C. O. Onyia	**Signature**	**Date**
Supervisor		
	……..	…….
External Examiner	**Signature**	**Date**
	……..	…….
Dr. Frances N. Olisaka	**Signature**	**Date**
Head of Department		
	……..	…….
Prof. Chidi Uchegbu	**Signature**	**Date**
Dean, Faculty of Natural Sciences and Environmental Studies		

CERTIFICATION

This is to certify that this research project entitled "**ANTIMICROBIAL AND PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACTS OF GUAVA AND NEEM YOUNG STEMS USED AS LOCAL CHEW STICKS ON COMMON DENTAL CARIES ORGANISMS (*Lactobacillus spp.* AND *Streptococcus spp.*)"** was carried out by me, Eneje Lovina Ifunanya (GOU/U22/BTG/175) in the Department of Biological Sciences, Faculty of Natural Sciences and Environmental Studies, Godfrey Okoye University, Enugu, in partial fulfillment of the requirements for the award of the degree of Bachelor of Science (B.Sc.) in Biotechnology.

_____ _____

Eneje Lovina Ifunanya **Date**

GOU/U22/BTG/175

DEDICATION

This project is dedicated first and foremost to God Almighty, whose grace, wisdom, strength, and unfailing love have sustained me throughout my academic journey.

It is also dedicated to my beloved brother Mr Samson.C. Eneje and family for their immeasurable love, prayers, sacrifices, encouragement, and unwavering support, which made this achievement possible.

ACKNOWLEDGEMENTS

I return all glory, honour, and thanksgiving to Almighty God for His abundant grace, mercy, protection, and faithfulness throughout my years of study. Without His guidance and strength, this work would not have been possible. My profound gratitude goes to my supervisor, Prof. C. O. Onyia and Mr Solomon Uchechukwu for their invaluable guidance, patience, constructive criticisms, encouragement, and professional advice throughout the course of this research. Their dedication greatly contributed to the successful completion of this work. I sincerely appreciate the Head of the Department of Biological Sciences, Dr Frances .N. Olisaka and Prof. Ezeonu, Mr. Mike, Prof. Marire, Dr. Okafor, Dr. Charles for their knowledge, mentorship, and contributions to my academic development. I am deeply grateful to the laboratory technologists and staff for their assistance during the laboratory aspect of this research. My heartfelt appreciation goes to my family for their unconditional love, prayers, financial support, encouragement, and sacrifices throughout my educational journey. I also appreciate my friends and classmates for their support, encouragement, and the memorable experiences we shared throughout our undergraduate years. Finally, I acknowledge everyone who contributed directly or indirectly to the successful completion of this research. May God richly bless you all.

ABSTRACT

Dental caries (tooth decay) is a prevalent oral health problem caused mainly by bacteria such as *Streptococcu*s spp. and *Lactobacillus* spp. Growing resistance to conventional antibiotics has increased interest in plant-based alternatives. This study examined the antibacterial activity of ethanol extracts from the twigs of Guava and Neem against bacteria associated with dental caries. French guava and neem twigs were collected, dried, and extracted using ethanol. Bacteria were isolated from oral samples and identified through standard microbiology, biochemical, and molecular methods. Phytochemical screening was performed to identify the bioactive compounds present in the extracts. Antibacterial activity was tested using the agar well diffusion method, and the minimum inhibitory concentration (MIC), the lowest concentration needed to prevent bacterial growth, was determined by broth dilution. Phytochemical screening revealed alkaloids, flavonoids, glycosides, terpenoids, and steroids, in both extracts, with tannins and saponins found exclusively in neem extract. Both extracts showed antibacterial activity against the test organisms. Neem extract performed better overall, producing inhibitory zone of 22mm against *Streptococcus* spp., and 21mm against *Lactobacillus* spp., compared to 19mm and 18mm for guava extract. MIC values ranged from 6.25 to 25 mg/ml across all isolates. These results suggest that ethanol extracts of neem and guava twigs have notable antibacterial properties against dental caries-causing bacteria, with neem showing stronger activity. This supports their potential as natural, affordable alternatives for dental caries prevention and management.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The most common dental disease that people experience around the world is dental caries. According to WHO data, cases of untreated dental caries impacts about 2.5 billion adults and 573 million children worldwide (Kassebaum *et al*., 2017), creating a significant burden on healthcare systems and society at large. The prevention and treatment of caries is of importance to this study. Dental plaque functions as the main factor which starts the caries process and controls its development with time. Environmental factors and host-related influences and interactions among microorganisms cause continuous changes in the oral microbiota. The ever-evolving bacterial communities of the human body play an essential role in sustaining general human well-being. The delicate balance exists between two extremes, which scientists call dysbiosis. This condition has links to several human diseases, including inflammatory bowel disease and type 1 diabetes (Gilbert *et al*., 2018). People need to maintain a balanced microbial environment in their mouths because it protects their dental health. The ecological plaque hypothesis explains that dental caries develops when microbial communities lose their stable ecological condition during periods of ecological upheaval.

The number of acid-producing bacteria and acid-resistant cariogenic bacteria in dental plaque shows an upward trend which results in the creation of multiple organic acids through the process of glycolysis. The acids present in the environment decrease its pH levels which results in the demineralization of tooth enamel (Pitts *et al*., 2021). The successful management of dental caries requires maintaining a healthy balance of oral microorganisms while controlling the spread of cavity-causing bacteria. The traditional approaches dentists use to treat cavities through

manual and mechanical techniques create pain and fear for their patients especially when treating young patients who refuse to cooperate. The research demonstrates how bacteria cause secondary cavities and treatment failures which creates a demand for methods that use minimal treatment approaches with antimicrobial capabilities. The dental practice employs new treatment methods to eliminate the bacterial infection that causes dental caries and secondary dental caries which will help dentists accomplish their professional goals. The Er:YAG and Nd:YAG laser technologies have gained increasing popularity for their use in this particular application (Pagano *et al*., 2020; Valenti *et al*., 2021). The dental field uses technological solutions together with antimicrobial agents to effectively manage the spread of cavity-causing bacteria. The research has examined and implemented various antibacterial substances which include cationic agents and metal-based disinfectants and natural products which contain plant-based antibacterial extracts. The substances demonstrate effective antibacterial properties but they also create certain difficulties. The substance affects beneficial bacteria which leads to the destruction of the natural oral microbial ecosystem (Fishbein *et al*., 2023; Lathakumari *et al*., 2024). The ongoing application of these substances creates conditions that enable the development of bacteria which possess resistance against antibiotics (Ahmed *et al*., 2024).

The oral borne bacteria *Streptococcus mutans* became an important medical research topic starting from the 1950s. By 1966 researchers had discovered through both clinical and animal research that *Streptococcus mutans* serves as a major factor which causes dental caries. The human oral cavity serves as its main dwelling area which exists primarily inside dental plaque that develops as a biofilm on dental surfaces.

The cariogenic potential of *Streptococcus mutans* is attributed to three main characteristics:

1. Its ability to permanently colonize hard surfaces by synthesizing a substantial amount of extracellular glucan polymers from sucrose.
2. Its capacity to transport and metabolize various carbohydrates into organic acids which produces high acid levels.
3. Its resilience in hostile environments especially in low pH conditions. *Streptococcus mutans* uses its ability to develop polysaccharide-based biofilms together with its capability to reduce environmental pH for creating conditions that enable the growth of acidogenic and aciduric bacteria which results in dental caries progression. The presence of *Streptococcus mutans* shows different patterns throughout the oral cavity because researchers found that the tongue's dorsum area contains the highest concentration of *Streptococcus mutans* bacteria (Lemos *et al*. 2019).

Lactobacillus species exist as non-pathogenic organisms but specific strains of these bacteria have been found in dental cavities which are associated with caries development. The bacteria exist in children who lack caries but they function as major contributors to caries development because they do not initiate the dental disease. The *Lactobacillus* species generate lactic acid through their metabolic process which functions by converting glucose into energy for their activities. The second most common cariogenic bacteria in the mouth belong to this particular group of bacteria. The *Lactobacillus* species which include *Lactobacillus gasseri*, *Lactobacillus fermentum* and *Lactobacillus casei* represent the most common bacteria that cause dental caries. The ba bncteria possess two main cariogenic traits which enable them to stick to surfaces and produce harmful acids. The S-layer protein present in their cell wall serves as the primary element that generates hydrophobic characteristics on Lactobacillus cell surfaces. The research

conducted by Ahirwar *et al*. (2019) demonstrates that *Lactobacillus* species produce Exopolysaccharides which function as essential components in biofilm development.

1.2 Statement of the problem

In recent times, there have been the advent of many oral care formulations from different mouthwashes with antibacterial and even antifungal properties, to toothpastes with activated charcoal and the likes to even special mechanized toothbrushes with ultra-cleaning capabilities. All these inventions are geared towards having healthier teeth and gum, which is the vital essence of oral hygiene. However, cases of tooth decay, dental plaque and caries still linger and may lead to more complicated issues if left untreated and unchecked. With that being said, new alternatives are being sought after which could potentially eliminate these threatening health risks and issues in regards to oral health and wellness. The use of plant based remedy in this regard is a technique of known ancient applications which is even better adapted with recent technological advances in science. These plant-based products could be more precise and tailored to delivering specific therapeutic effects to patients facing dental issues and they have proven to be safer, cheaper, available and renewable in nature, and could potentially replace majority of the synthetically processed products for use in maintaining good oral hygiene. The use of both the Neem and Guava plant as twigs, as a form of cleaning agent for the teeth has been practiced for decades within the rural communities in the African continent. This means formulations could be derived and converted into more suitable forms for better use in oral hygiene maintenance and treatment of dental caries and the likes.

This study's significance is centered on the need to have safer and more readily available alternatives to maintaining oral hygiene, especially in rural communities who potentially face

lack of proper medical facilities. It could potentially lead to the development of remedies to issues like dental caries and tooth decay, making this alternative easily accessible.

1.3 Aim and Objectives

Aim

The aim of this study is to determine the antimicrobial activity of ethanolic extracts of Guava (*Psidium guajava)* and Neem or Dogonyaro (*Azadirachta indica*) twigs or sticks against Dental Caries-causing bacteria (*Lactobacillus* spp. and *Streptococcus* spp.).

The Objective of the study were to:

1. Analyze the bioactive compounds of the Guava and Neem tree crude extract qualitatively.
2. Determine the Antimicrobial potential of the plant extracts against common selected oral pathogens.
3. Compare the antimicrobial potential of the two plant extracts (Guava and Neem Stem).
4. Isolate and molecularly identify the test organisms.

CHAPTER TWO

LITERATURE REVIEW

2.1. Overview of Diseases Associated with Oral Health

Throughout their entire life span, people across all countries experience oral diseases, which rank as one of the most common health issues that people face. The World Health Organization states that approximately 50 percent of people worldwide experience oral diseases (Homayouni *et al*., 2023). Dental diseases, particularly dental caries, represent significant public health challenges which rank among the most common medical conditions that affect people worldwide (Chen *et al*., 2020). Dental caries, which affects both children and adults, ranks among the top health problems in developed countries. (Pitts *et al*., 2021). The World Health Organization estimates that 2.4 billion people worldwide suffer from permanent tooth decay, while 532 million children experience primary tooth decay. The recent epidemiological study discovered that the global permanent teeth caries cases showed a 46.1 percent increase from 1990 to 2019 (Grabek-Lejko *et al*., 2023).

The oral cavity contains more than 700 microbial species but only a small number of these species lead to dental caries according to Mosaddad *et al*. (2019). The main cause of caries development as per Wolfoviz-Zilberman *et al*., (2021) is the *Streptococcus mutans* bacteria which function as a primary cause of the disease. *S. mutans* exists as a Gram-positive bacterium which produces acids and can survive in acidic conditions and naturally exists in human mouths (Ben-Zaken *et al*., 2021). The organism's capability to create dental cavities depends on its metabolic functions and its ability to develop colonies in dental biofilms according to Grabek-Lejko & Hyrchel, (2023). *S. mutans* digests carbohydrates to produce organic acids which enable them to survive in environments with low pH levels. The bacteria create extracellular polymeric

substances (EPSs) which consist of glucans and fructans that help them grow while they also employ these substances to adhere to teeth. This process leads to biofilm development according to Grabek-Lejko & Hyrchel, (2023). Glucans specifically promote the growth of cariogenic Streptococci which results in the biofilm becoming more dangerous to health (Zhu *et al*., 2023). The development of caries primarily depends on dental biofilms. The biofilms will establish various physiological features which create complex systems that result in both tooth demineralization and systemic inflammation when their management fails. The scientific community acknowledges that people must use mechanical techniques which include tooth brushing and flossing to prevent dental biofilms (Takenaka *et al*., 2019).

The use of antimicrobial agents together with toothpastes and mouthwashes creates an enhanced partnership which improves both mechanical plaque control and daily oral hygiene routines. The agents stop plaque formation and biofilm development in areas where effective cleaning proves challenging (Chatzopoulos *et al*., 2022). The dental treatment employs a combination of vitamins and probiotics and antibiotics which include amoxicillin and erythromycin and penicillin to stop microbial growth (Isola, 2020; Yang & Kang, 2020; Chatzopoulos *et al*., 2022; Heliawati *et al*., 2022; Milia *et al*., 2023). The commercial oral hygiene products provide benefits to users yet they contain specific restrictions (Isola, 2020). The majority of products use synthetic chemicals which create non-biocompatible reactions when they touch oral tissues (Heliawati *et al*., 2022). The excessive use of fluoride creates toxicity problems and the substance serves as a health risk for children under six because it can cause enamel discoloration and structural damage (Isola, 2020). The current status of chlorhexidine (CHX) establishes it as the primary standard for antiseptic testing. The extended application of this product results in multiple detrimental health outcomes. The health problems which arise from this condition involve yellow-brown tooth

staining, altered taste perception, irritation of oral mucosa, desquamative lesions, ulceration, increased calculus buildup, and cytotoxic effects on human fibroblasts and osteoblasts (Kumar *et al*., 2021; Chatzopoulos *et al*., 2022; Milia *et al*., 2023). Triclosan demonstrates restricted ability to bind within the oral cavity which results in decreased effectiveness against bacteria. The administration of antibiotics results in nausea, vomiting, and diarrhea as common side effects (Yang & Kng, 2020). The ongoing use of antimicrobial agents (Milia *et al*., 2023) has created a major problem which has existed for 100 years because it enables cariogenic germs to develop resistance (Oluwasina *et al*., 2019; Yang *et al*., 2020; Kumar *et al*., 2021). The investigation of natural products as new antimicrobial agents offers an effective method to stop and treat oral microbial infections (Zhu *et al*., 2023). This review presents a collection of essential dental caries medicinal plants which show antibacterial effects and real-world applications in dental treatment. Natural products present a strong medicinal option which competes with synthetic drugs because medicinal herbs possess diverse bioactive compounds that can lead to the creation of new antimicrobial therapies (Milutinovici *et al*., 2019).

2.1.1. Overview of the Cariogenic Microbiota (Caries Causing Microbes)

The characteristics of cariogenic microbiota need to be studied because dysbiosis of microorganisms functions as a primary driver for caries development. *Streptococcus mutans* exists as a primary cariogenic microorganism which researchers have studied since its discovery as the first identified pathogen who causes dental caries. The research about cariogenic microbiota has revealed that *Streptococcus mutans* exists as one of multiple factors which cause dental caries. Dental caries development depends on the interaction between different bacterial species which work together to advance the disease. *Streptococcus mutans* constitutes only a small fraction of the bacterial population which ranges between 0.02% and 0.73%, while 15% of

people with cavities lack *Streptococcus mutans* (Banas & Jake, 2018). The development of effective caries-prevention methods should concentrate on the complete range of cariogenic microorganisms instead of targeting one specific organism. The cariogenic microbiota shows significant microbial diversity loss across different species. People with caries show reduced diversity of their microbiota when compared to people without caries. The research by Kim *et al*. (2018) and Hurley *et al*. (2019) shows that dental caries advanced through different stages lead to decreased microbial diversity. The study by Zhu *et al*. (2018) found that children lost oral microbial diversity as their caries progressed. Active lesions show less microbial community abundance than static caries but both types of lesions maintain similar beta diversity according to Mitwalli *et al*., (2019) and Mei *et al*., (2020). The studies of Jiang *et al*., (2016); Jiang *et al*., (2018); Xu *et al*. (2018); Kalpana *et al*., (2020); and Pang *et al*., (2021) demonstrate that caries species show distinct abundance patterns throughout the process of caries development. The study by Mei *et al*., (2020) found that caries related bacteria display different abundance patterns because active lesions show higher levels of these bacteria compared to arrested lesions. The study by da Costa Rosa *et al*., (2021) found that identified caries show balanced *Streptococcus* and *Veillonella* distribution together with other bacterial types unlike active lesions which display dominance of these two species. The fungal flora patterns in dental caries plaques follow the same pattern as *Candida* shows increased presence which establishes itself as a key factor shaping the microbial makeup according to Havsed *et al*., (2021).

Research should focus on studying microbial interactions because they determine whether caries will develop through their various impacts on microbial community complexity. Microorganisms can interact with one another through two distinct methods which include synergy and antagonism. Microorganisms in the oral cavity display synergy through their combined function

which breaks down salivary glycoproteins. The complete breakdown of mucin from saliva depends on multiple enzymes which no microorganism has to achieve this task (Zhang *et al*., 2024). The food chain demonstrates multiple cases where one species metabolite serves as primary energy source for its partner species (Gralka *et al*., 2020). The production of bacteriocins together with hydrogen peroxide creates antagonistic effects which impact community aggregation patterns according to Huang *et al*., (2018). The most researched microbial interaction currently exists between *Streptococcus mutans* and other bacteria that cause dental cavities. The enzyme glucosyltransferase (Gtfs) serves as an external enzyme which *Proteus* releases to convert sucrose into extracellular glucan which forms the main part of EPS. Bacteria stick better to tooth surfaces because extracellular glucan helps them to connect with other bacteria which leads to cariogenic biofilm development (Bowen *et al*., 2018). The dental caries microbiome system causes dental caries because it creates an ecological imbalance which leads to dental caries development. The research community now studies microecological regulation together with precise antibacterial research instead of focusing on total bacteria elimination.

2.2. The Botanical Description of the Guava (*Psidium guajava*) plant

The common name for *Psidium guajava* is guava and it grows as a small tree or shrub which reaches maximum heights between 2 and 7 meters. The guava plant displays evergreen leaves which have a leathery texture and grow in opposite pairs along its branches as shown in Figure 2.1. The plants display their short petioles together with various irregular shapes which include oval shapes and other non-standard patterns. The guava blossoms display their large size through their stem support and their appearance which creates a strong visual effect while producing a pleasant scent. Guava plants develop branchlets which exhibit four different angular positions and their fruits show various shape possibilities which include pyriform and oblate and rounded

and ellipsoidal and oval and cylindrical forms. Guava trees establish their roots in shallow soil depths while their fruits stay fresh for only three to five days when stored at room temperature. The fruit maintains a brief shelf life because it displays high respiration rates combined with ongoing metabolic processes (Toma & Lucian, 2019; Hussain *et al*., 2021). The plant provides multiple segments which include leaves and fruits to deliver various medicinal properties that range from antimicrobial effectiveness to their possible anti-cancer properties. The medicinal properties of guava include its ability to treat gastrointestinal infections which people have used as a traditional remedy for diarrhea. The guava leaf extract shows antinociceptive activity which helps to reduce pain. (Daswani *et al*., 2017; Naseer *et al*., 2018).

It's classified below based on its taxonomy;

Table 2.1. The Taxonomical Classification of the Guava Plant

Taxonomical Rank	Classification
Kingdom	Plantae
Phylum	Magnoliophyta (Flowering plants)
Class	Magnoliopsida (Dicotyledons)
Subclass	Rosidae
Order	Myrtales
Family	Myrtaceae
Genus	*Psidium*
Species	*Psidium guajava*

Source: Hussain *et al*., 2021.

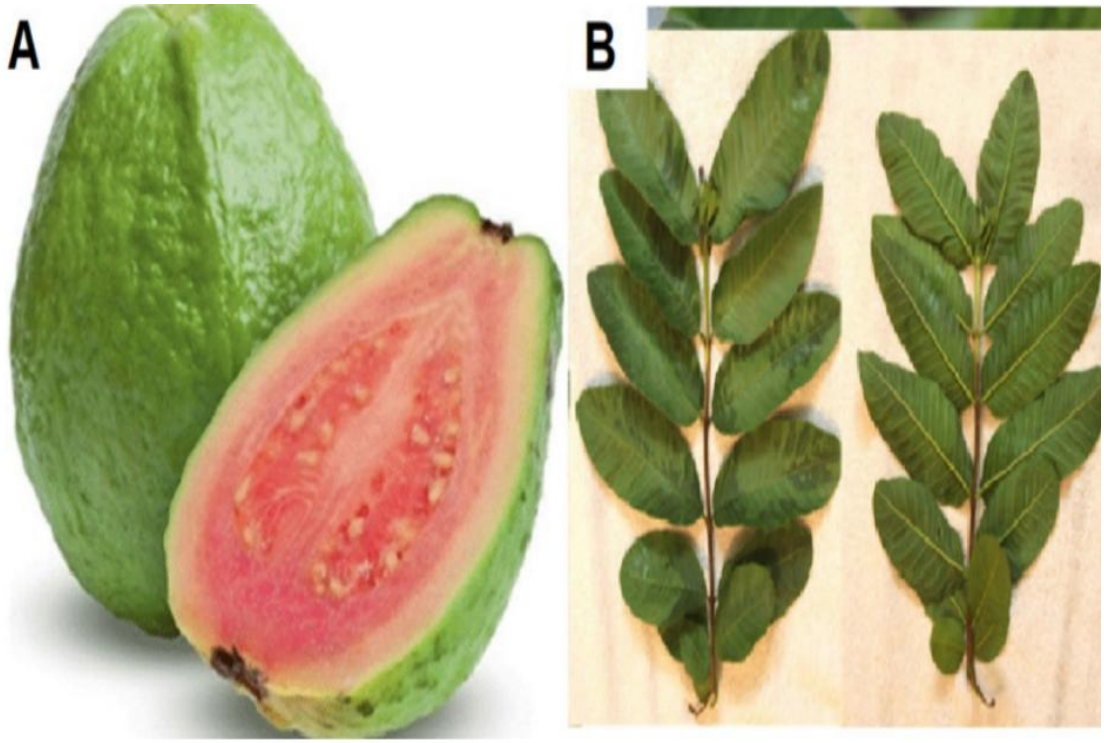

Figure 2.1 The Fruit (A) and Leaves (B) of the Guava Plant

Source: Roma and Lucian, 2019 and Hussain *et al*., 2021.

2.3. The Phytochemistry of the Guava Plant

The various parts of the Guava Plant like it's leaves, twigs, fruits and more have different bioactive compounds like phenolic compounds, carbohydrates, glycosides, alkaloids, saponins, sterols, fatty acids, essential oils, terpenoids and much more. Guava provides important vitamins essential for body function as well as vital minerals and therapeutic electrolytes. . The high content of these minerals makes them appropriate for human consumption. Proteins serve as essential macromolecules which form the basic framework of biological functions throughout living systems. Guava leaves, which contain approximately 10% protein content exhibit this protein percentage on a dry weight basis (Kumari *et al*., 2020). Guava contains high amounts of antioxidant pigments which include carotenoids and polyphenols. Multiple carotenoids which included lycopene, â-carotene, zeaxanthin and diepoxy-â-carotene was identified and 16 carotenoids was extracted from red guava flesh which identified lycopene as the main pigment. The fruit contains between 0.04 to 4.04 mg of the substance per 100 grams which decreases when the pulp color changes from dark pink to white (Leiton-Ramírez *et al*., 2020; Kumari *et al*., 2020; Abdullahi, 2021; Otálora *et al*., 2022).

2.4. Some Pharmacological Activity of the Guava Plant

The Guava Plant has numerous benefits which are as a result of its pharmacological activities, and they include the following:

1. Antioxidant activity

Lipid autoxidation begins when lipophilic radicals create their initial radical which then drives the autoxidation process forward. The biological system uses various oxidase enzymes to produce hydrogen peroxide through their enzymatic functions. Hydrogen peroxide acts as a signaling molecule because it generates hydroxyl free radicals which manage the production and

activation of inflammatory mediators. The pathways produce signaling molecules which activate major pathways that result in tissue destruction and multiple diseases like diabetes. Guava contains a significant amount of essential antioxidants which provide it with radio-protective capabilities. This contains multiple vitamins together with numerous minerals. Guava contains phenolic compounds which include flavonoids and essential antioxidants which include lycopene and flavonoids. The components eliminate cancer cells while they prevent the formation of wrinkles on skin surfaces. Quercetin serves as the strongest antioxidant present in guava leaves, which can provide control over muscle spasms. Guava extracts, when tested with both aqueous and organic solvents, show an extensive antioxidant capacity which can effectively stop oxidation processes (Naseer *et al*., 2018).

2. Antibacterial action

The *P. guajava* leaves possess some antibacterial properties due to the presence of flavonoids. Researchers conducted extensive studies on guava to determine its antibacterial properties which showed that its flavonoid components functioned as effective antibacterial agents. The dedicated liquid form of *P. guajava* leaves demonstrates impressive bacterial killing ability against multiple tested bacterial strains. The antibacterial activity of guava leaf extracts which contain flavonoids has been documented in scientific studies. The antibacterial activity of guava leaf extract occurs because its flavonoid glycosides contain (Morin-3-Oalpha-Llyxopyranoside) and (morin-3-OalphaLarabinopyranoside) (Ahamad & Ansari, 2022). The studies established that those flavonoids display antibacterial properties through their effects. to exhibit antibacterial activity. The study demonstrated that the flavonoid compound guaijaverin which belongs to guava plants works against plaque development (Kakad *et al*., 2022).

3. Hepatoprotective activity

Guava leaf extract demonstrates its ability to combat free radicals through its antioxidant properties which result from its high phenolic content. The compound demonstrates an ability to protect the liver from damage that occurs due to free radicals which affect diabetic rats that receive alloxan treatment. The extract from guava leaves provides two health advantages because it lowers blood sugar levels and protects the liver from damage (Luo *et al*. 2019; Parker *et al*. 2022).

2.5. The Botanical Description of the Neem (*Azadirachta indica*) Plant

The *Azadirachta indica* tree belong to the *Meliaceae* family and grows at a fast pace during all stages of its existence. Neem which originates from the Indian subcontinent can now grow in tropical and subtropical regions worldwide as a result of its various medicinal and environmental applications. The neem tree grows between 15 and 20 meters in height and develops a trunk with 2.5 meter circumference while its branches create a dense canopy of dark green foliage. This broad-leaved evergreen tree needs more than two hundred years to complete its full life cycle. The leaves of the plant develop into a pinnate structure which contains oblong leaflets that create a distinctive powerful fragrance. The tree produces yellowish fruit which contains oily seeds and produces tiny white flowers. The tree features rough grayish-brown bark while its seeds and leaves function as the main medicinal elements that people use for treatment purposes (Handa *et al*., 2018; Koul *et al*., 2018).

Figure 2.2. The Leaves and Stem Bark of the Neem Tree.

Source: Khare and Kamble, 2025.

The taxonomical classification of the neem tree is recorded in the table below

Table 2.2. The Taxonomical Classification of the Neem Tree

Taxonomical Rank	Classification
Kingdom	Plantae
Sub-Kingdom	Tracheobionta (Vascular plants)
Super-Division	Spermatophyta (Seed plants)
Division	Magnoliophyta (Flowering plants)
Class	Magnoliopsida (Dicotyledons)
Order	Sapindales
Family	Meliaceae (Mahogany family)
Genus	*Azadirachta* A. Juss *(Azadirachta)*
Species	*Azadirachta indica* A. Juss (Neem)

Source: (Uzzaman, 2020; Haji *et al*., 2023).

2.6. The Phytochemistry of the Neem Plant

Researchers identified azadirachtin as the primary compound which neem trees produce in their most concentrated form (Chaudhary *et al*., 2017; Pasquoto-Stigliani *et al*., 2017; Kilani-Morakchi *et al*., 2021). *A*. *indica* provides all its components which include stem, bark, roots, leaves, gum, seeds, fruits, flowers and other parts for use as home remedies to treat human health problems. According to Gupta *et al*. (2017) neem twigs function as dental hygiene chewing sticks which millions of people worldwide use. Scientists who study modern medicine and infectious diseases investigate neem tree properties because this tree shows potential as an antimicrobial solution which medical professionals use in oncology, dentistry, dermatology and endocrinology. The neem plant's phytochemical compounds can be recovered through extraction methods that utilize different solvents to isolate the plant's active ingredients yet researchers in laboratories depend on water, ethanol, methanol, chloroform and ether extracts to examine the biological active compounds of neem. Research studies most commonly use methanol and ethanol extracts to conduct their antimicrobial assessments. The neem oil extracts which researchers tested demonstrate their complete biological function because they contain various secondary plant metabolites that include isoprenoids and non-isoprenoids (Saleem *et al*. 2018). The neem tree contains more than 300 distinct compounds yet researchers found that azadirachtin, gedunin and nimbolide serve as the main phytochemicals which deliver its essential biological functions and medicinal properties. These compounds exhibit bioactive properties that perform distinct functions according to the findings of (Braga *et al*. 2020; Nagini *et al*. 2021).

Table 2.3. The Major Phytochemical Compounds of Neem Plant and their known reported Activities

Phytochemicals	Plant parts	Type of Compound	Reported Antimicrobial Activity	Mechanism of Action
Nimbolide	Leaves, Flowers	Limonoid	Antibacterial, Antifungal, Antiparasitic	Induces oxidative stress, inhibits microbial DNA/RNA.
Azadirachtin	Seed, Leaves	Limonoid (Triterpenoid)	Antibacterial, Antifungal, Antiviral	Disrupts microbial metabolism and cell replication.
Nimbin	Bark, Seed oil	Triterpenoid	Antibacterial, Antifungal	Disrupts microbial membrane integrity.
Gedunin	Seeds	Limonoid	Antimalarial, Antibacterial	Protein denaturation, mitochondrial disruption.
Nimbidin	Seed, Kernel oil	Triterpenoid	Antibacterial, Antifungal	Inhibits bacterial cell wall synthesis, reduces inflammation.

Source: Khare and Kamble, 2025

2.7. Some Pharmacological Activity of the Neem Plant

1. Anticancer activity

The process of carcinogenesis involves multiple factors which create a complex pathway that starts from precancerous lesions and ends with neoplasia. The normal process of cell growth becomes disrupted by carcinogenesis which causes normal cells to transform into cancer cells (Moga *et al*., 2018). The neem leaves extract showed anticancer properties in aqueous and ethanolic forms which proved effective against cancer cells in laboratory tests using breast and lung and cervical tissue that had developed neoplasia (Ahmad *et al*., 2017).

2. Anthelmintic activity

Parasitic helminth diseases maintain their status as major production threats for livestock operations which particularly affect small ruminant herds in tropical and subtropical regions. The developing world experiences its highest productivity losses from parasite infections which lead to both immediate and future economic declines. Most of the economic losses from nematode parasite infections occur through hidden symptoms which internal parasitism studies show to have major financial effects (Tibebu *et al*., 2017).

2.8. The Brief History and Development of Medicinal Plants in Dentistry

2.8.1. Brief history of phytotherahy in dentistry

Throughout history people have used natural herbs as medicinal treatments but modern development methods create obstacles that prevent people from recognizing these strong natural resources. Research has shown that herbal medicines which contain medicinal herbs and herbal preparations and phytotherapeutic compounds provide vital health advantages to humans according to Delimont & Carlson, (2020). People widely use these phytotherapeutic products because natural substances demonstrate high safety levels and users can tolerate them well.

Researchers have found that medicinal plants show both general and specific biological functions through their antimicrobial properties and antioxidant effects and anti-inflammatory capabilities and antifungal activities and antiviral functions and analgesic effects which help maintain oral health (Isola, 2020; Milutinovici *et al*., 2021). Human beings from ancient times used plant materials to create various products which include chewing sticks and latex and tooth powders and mouth rinses for dental health maintenance and disease prevention and oral health promotion (Isola, 2020).

2.8.2. Review on the Development of Medical Formulations from Plants

Phytodentistry has emerged as a new treatment method that uses plant and herb medicine to treat various dental problems which provides a safe and affordable alternative to synthetic medications (Moghadam *et al*., 2020). The practice of using plants to both prevent and treat dental diseases has existed for many centuries. Plant extracts show effectiveness because they bind to particular chemical receptors that exist within the human body (Shaalan & El-Rashidy, 2023) The oral cavity sustains its oral health through a delicate balance which exists between its two types of microorganisms: beneficial microorganisms and harmful microorganisms. When pathogenic microorganisms take control of the oral cavity after its microbial equilibrium gets disrupted, the result leads to the emergence of oral diseases (Sedigh-Rahimabadi *et al*., 2017). The development of common oral diseases occurs when a person's mouth bacteria decrease and dental biofilms start to develop (Yang & Jang, 2020). As recorded by Eltay *et al*., (2021), the formed biofilms have different microbial species such as *Lactobacillus acidophilus, Enterococcus faecalis, Streptococcus mutans, Candida albicans, Candida glabrata, Fusobacterium nucleatum, Veillonella dispar* and so much more. The scientific community has established dental caries as a dysbiosis which develops through intricate interactions between

dental structures microbial biofilms and sugars found in food (Eltay *et al*., 2021). The main factor that leads to dental caries development occurs when *S. mutans* bacteria infect the teeth. The primary way to stop dental caries development depends on controlling the bacterium's biological activities.

The research study conducted by Mandava *et al*. in (2019) evaluated how different medicinal plants would protect against S. mutans glucosyltransferases (GTFs) through their anticaries properties. The researchers conducted experiments with six natural sources which included four plant species and two essential oils: *Azadirachta indica, Psidium guajava, Pongmia pinata, Terminalia chebula* and clove essential oil from *Syzygium aromaticum* and peppermint essential oil from *M. piperita*. The researchers tested the ability of hydroalcoholic plant extracts and essential oils to stop the GTFs which they isolated from *S. mutans*. The tested samples showed strong GTF inhibition results. The polyherbal mouthwash demonstrated 95.936% GTF inhibition while the chlorhexidine control produced 54% GTF inhibition which proves its potential as a future dental caries control product according to Mandava *et al*., (2019). Valadas *et al*. (2019) conducted tests to determine which antimicrobial agents showed the strongest results against *S. mutans* in children when they applied dental varnish made from *Copaifera langsdorffii* copaiba oil-resin. The study showed that dental varnish made from copaiba oil-resin produced strong antibacterial effects against *S. mutans*. The formulation functions as an effective strategy which prevents dental diseases in children who belong to the age group between 3 and 5 years.

The results showed that using *T. polium* mouthwash at regular intervals helps people decrease their chances of developing dental caries. Milutinovici *et al*., (2019) conducted a comprehensive review of commonly used medicinal plants in dentistry. The authors discovered that natural extracts has more healing power when their plant chemical components create combined effects.

Traditional dental treatments face competition from medicinal plants and their active compounds which offer a valuable treatment option according to Milutinovici *et al*. (2019).

2.9. The use of Different Medicinal Plant Extracts in Dental Caries Treatment

The disease known as dental caries serves as the most common dental condition that affects people throughout the world, which creates significant health issues and financial burdens for numerous nations. The untreated condition can progress to various complications that include tooth loss and gingivitis and periodontitis and oral cancer. The initial stages of dental caries show high preventable potential, but untreated cases lead to advanced disease development. Dental caries results because infectious biofilms develop on oral surfaces and establish permanent attachment to these surfaces. The process leads to biofilm formation because cariogenic bacteria use their metabolic byproducts together with saliva and dietary components to create biofilms on tooth surfaces. The biofilm matrices protect microorganisms through their physical and chemical attributes which enable them to survive against both host immune attacks and environmental threats, thus creating a significant barrier that prevents people from achieving their best oral health (Bowen *et al*. 2018). The typical composition of pathogenic biofilms contains Streptococcus species which include *S. mutans, S. salivarius, S. sanguinis, Porphyromonas, L. acidophilus* and *C. albicans* yeast. Dental diseases have been treated with medicinal plants for hundreds of years because they serve essential functions in both disease prevention and disease treatment. The first human people started to chew plant leaves and fruit and root materials before they developed the skill to create herbal infusions and mouth rinses. Modern dental practices use plant materials to create extracts which dental products use to develop active ingredients that fight dental caries (Milia *et al*., 2023).

Researchers use an established method to test plant anticariogenic effects by testing their ability to kill S. mutans bacteria in laboratory conditions (Mandava *et al*., 2019). People need to practice proper oral hygiene because it helps their entire body while nature supplies numerous natural treatments that contain compounds which fight bacteria and provide antioxidant benefits and anti-inflammatory effects and protection against dental diseases. *C. canephora, C. sativum* and *V. vinifera* contain phenolic acids which work as antimicrobials against both types of bacteria, while these compounds exhibit antibacterial effects by destroying *S. mutans* oral bacteria through their capacity to break down bacterial cell walls and block the enzymes which enable biofilm creation. They also eliminate *C. albicans* cells while showing effectiveness against *S. mutans* biofilms which create dental cavities (Yadav *et al*., 2017). Periodontal disease prevention uses *C. sinensis* (black tea) because its epicatechin-3-gallate polyphenolic compounds function as antioxidants and anti-inflammatory agents and antibacterial substances (Mazur *et al*., 2021).

The anthocyanins, flavonols, and proanthocyanidins in *V. macrocarpon* compounds block bacterial colonization of *P. gingivalis* and *F. nucleatum* while they stop *P. gingivalis* from sticking to different proteins which reduces periodontal disease development risk. The essential oil from *L. sidoides* shows strong antibacterial effects against cariogenic bacteria while it effectively controls supragingival biofilm development (Nowaczyk *et al*., 2021; Pellerin *et al*., 2021; De Assis *et al*., 2022). The neem plant, Embelia, *J. regia, P. guajava* and *C. sinensis*, which people consider excellent for oral health, serve as their main polyphenol sources which include tannins and flavonoids that make them important for oral care (Shetty *et al*., 2020). The phytochemical composition of *A. sativum* contains alliin which transforms into allicin, an antimicrobial compound that effectively fights against oral bacteria (Sasi *et al*., 2021). Dental applications make use of phytochemical compounds which require ethanolic or aqueous plant

extracts as the primary extraction method for their development of mouthwashes and toothpaste products. The plant extracts used in this study came from multiple plant sources which include leaves, roots and seeds. Research groups adopt different extraction methods because standardized procedures for extract preparation do not yet exist. The clinical research studies which assess phytochemical effectiveness use multiple testing methods which include bacterial counts plaque reduction and antioxidant activity assays to measure biological effects. The majority of these studies exhibit short study periods which last from two weeks to two months. Dental studies require extended study times because plant extracts need additional time to demonstrate their ability to prevent or reverse dental caries. The results of many clinical studies present antimicrobial findings that lack information about the effectiveness of their treatments on other medical conditions according to Suleiman, (2020).

2.10. The Effectiveness and Safety Profile of Plant-Based Therapies in Dental Treatments

2.10.1. The Efficacy Analysis of Natural Plant-based Therapies

As of recent times, many researchers have given much attention to the use of Plant-based natural products in preventing and treating denatal caries as seen in (Figure 2.3). Polyphenols serve as essential bioactive compounds which demonstrate their strong effectiveness to scientists. Natural products offer lower preventive power because their efficacy falls short when compared to standard treatment methods which use antibiotics and fluoride. Researchers continue to face challenges when they attempt to achieve complete understanding of natural compound action mechanisms. Ancuceanu *et al*., in their (2019) study examined more than 56 clinical trials which investigated how herbal products prevent dental caries. The studies primarily investigated how the products killed bacteria through their effects on *S. mutans* and their limited impact on other bacterial and fungal microorganisms. Some studies assessed different factors which included

dental plaque presence and pH measurement and salivary flow and ionic level assessment and human salivary amylase function. People mostly used mouthwash as their primary method of taking medication while they used dentifrices and other delivery systems as their secondary option. Some studies conducted research on only pediatric patients and most of the used interventions lasted for less than seven days with about one-third of the studies using one-time treatment. The studies utilised chlorhexidine as a positive control in one-third of the research, while about 17.86% of the study had no control group. The four out of five studies showed methodological weaknesses together with potential biases which made results interpretation more challenging. The research showed *C. sinensis* and *T. chebula* and *Glycyrrhiza uralensis* as the most studied species. The research findings showed that 85.71% of the trials achieved positive results based on Ancuceanu *et al*. (2019) research, yet the overall study quality remained deficient. A subsequent 2021 review assessed 47 clinical studies which tested plant extracts and pharmaceutical products that contained standardized plant compounds to treat dental caries. The study measured both microbiological and clinical results. Ancuceanu found that only 15 of the 47 studies included environmental data which showed most studies documented decreases in both *S. mutans* levels and total bacterial counts. Ancuceanu *et al*. (2019) documented that multiple studies showed clinical results which decreased both plaque and gingival indices. The natural treatments produced few side effects for patients yet they reported experiencing dental stains plus a bitter taste and an unpleasant mouth odor and a mild burning sensation (Furquim dos Santos Cardoso *et al*.,2021).

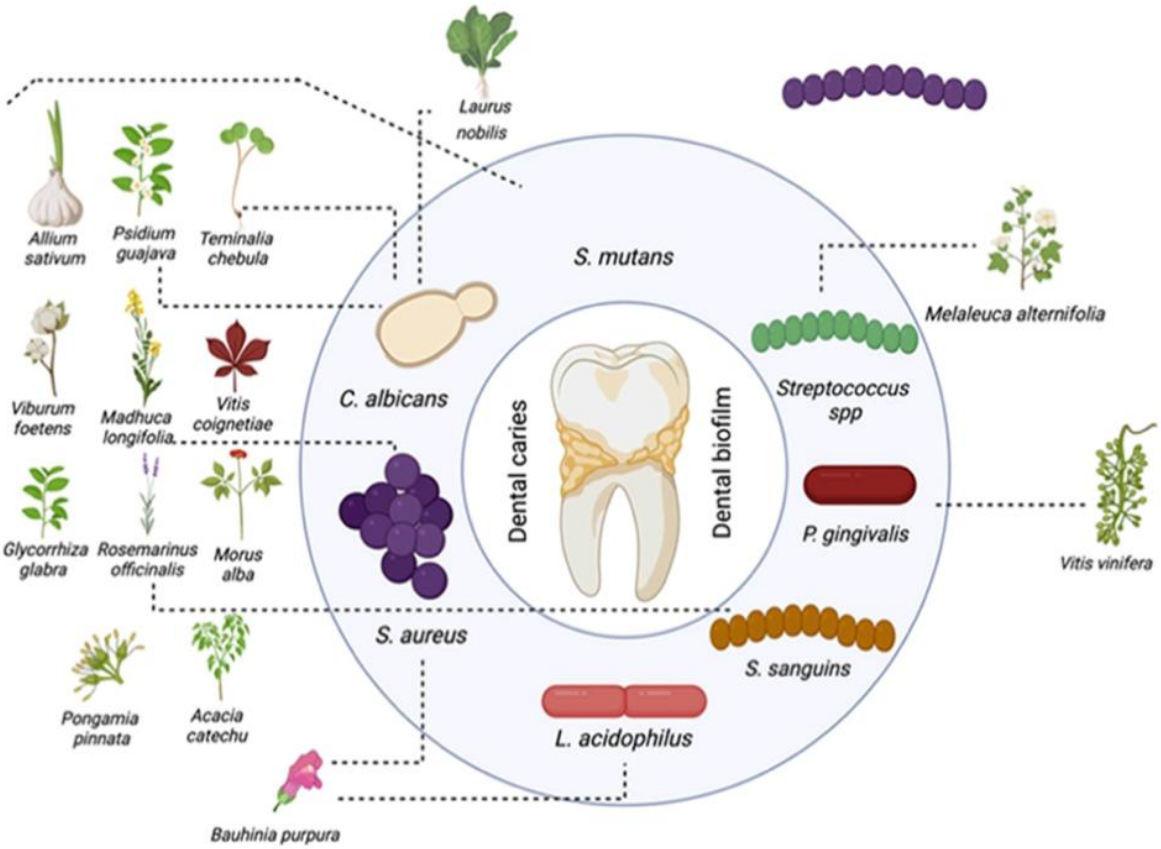

Figure 2.3. Natural Plant-based Products for Caries Treatment

Source: Gloria-Garza *et al*., 2025.

2.10.2. Comparison of Natural Plant-based Therapies with Conventional Techniques

The standard method for treating dental caries is divides into three primary treatment methods which include repair techniques, treatment materials and patient education efforts. Clinical dentistry currently uses established repair techniques which include both remineralization methods and restorative treatment methods. The process begins with root canal disinfection which continues until the team completes sealing work that destroys all pathways for bacterial entry. The procedure requires dental professionals to extract dead pulp tissue while using antimicrobial irrigants to decrease bacterial presence in root canals through sequential or combined application. Sodium hypochlorite (NaOCl) serves as the primary endodontic irrigation solution because it provides complete antimicrobial coverage while dissolving dead tissue. The solution presents several major problems which include producing offensive smells and tastes together with harmful effects which damage nearby biological structures. Chlorhexidine (CHX) functions as a cationic bisbiguanide which dentists use as their final endodontic irrigant solution. The substance demonstrates its ability to kill bacteria while preventing bacterial attachment to dentin through its 2% concentration which offers complete bacterial protection. The bisbiguanide compound Alexidine (ALX) which has structural similarities to CHX, that enables it form hydrophobic interactions with lipid membranes to produce electrostatic affinity to bacteria cell surfaces and functions as an antiseptic agent in mouthwashes. The bactericidal effects of ALX operate more quickly than CHX because ALX enables greater bacterial membrane penetration. ALX reacts with NaOCl to generate an insoluble para-chloroaniline (PCA) precipitate while CHX does not create this reaction. Biofilms maintain strong resistance against disruption despite the effectiveness of these agents. Antimicrobial treatments require two years of continuous

application before they begin to change biofilm structure and decrease cavities risk. People who have low biofilm levels but face other danger elements will not experience major advantages from antimicrobial treatments. The development of an effective treatment strategy for each patient requires personalized solutions (Kermeoglu *et al*., 2018). The antimicrobial agent CHX has undergone more research studies than other available options according to its effectiveness against cariogenic bacteria.

2.11. New Technological Approach: The Use of Plant-Based Antibacterial Nanoparticles for Treatment of Dental Caries

1. Nano particles

Nanomaterials exist as materials which occupy a size range between 1 nanometer and 100 nanometers. The morphological characteristics together with the particle size distribution of nanoparticles (NPs) function as essential elements which determine their innovative biomedical usage in oral hygiene products (Ahmed *et al*. 2023). Silver nanoparticles (AgNPs) have emerged as the most studied metal nanoparticles because they function as effective antimicrobial agents which protect prosthetic materials and adhesives and implants from microbial contamination and enable caries treatment and biofilm eradication and bone tissue formation (Noronha *et al*. 2017). The production process for AgNPs now enables the creation of particles which maintain controlled dimensions and shapes together with uniform size distribution and special active properties which can achieve specific requirements through molecular capping agents, like protein macromolecules and small hydrophobic and hydrophilic chemical groups. AgNPs function as dental material components while they serve multiple purposes in dental treatment methods which use them throughout the oral cavity (Noronha *et al*. 2017). Bacteria flora of the oral cavity form biofilms which are necessary for their growth, their ability to defend against the

immune system and their resistance to antibiotics. Scientists need to study biofilm structure to develop effective NPs. The NPs' characteristics determine their effectiveness and disrupt their operational processes. Important factors in this field include how nanoparticles move through biofilms because their effectiveness decreases when their size increases and biofilm penetration becomes impossible for nanoparticles larger than 50 nanometers because biofilm self-diffusion coefficients decrease exponentially when the square of nanoparticle diameter increases (Noronha *et al*., 2017). People use plant extracts because they possess extensive and easily accessible natural resources that exist throughout the world and their safe usage possibilities and their wide selection of metabolites which have strong reducing capabilities and their production process generates minimal waste and energy expenses (Souza *et al*., 2018).

The process of plaque biofilm development leads to the emergence of dental health problems. Researchers added AgNPs to dental biomaterials because of its antibacterial properties which help prevent biofilm development. AgNPs demonstrated more effective control over Gram-negative bacteria which caused periodontal disease according to the research findings of Xu *et al*., (2020). Researchers have created multiple treatment methods which target disease-causing microorganisms through the use of natural compounds and plant-based materials in nanoparticle-based buccal membrane systems (Figure 2.4). Nanomaterials serve as delivery systems for both oral fluids and medical drugs which enable medical professionals to treat oral cancers while delivering exceptional dental services. These substances exist in dental products such as toothpaste and mouthwash and various dental care items (Yazdanian *et al*., 2022).

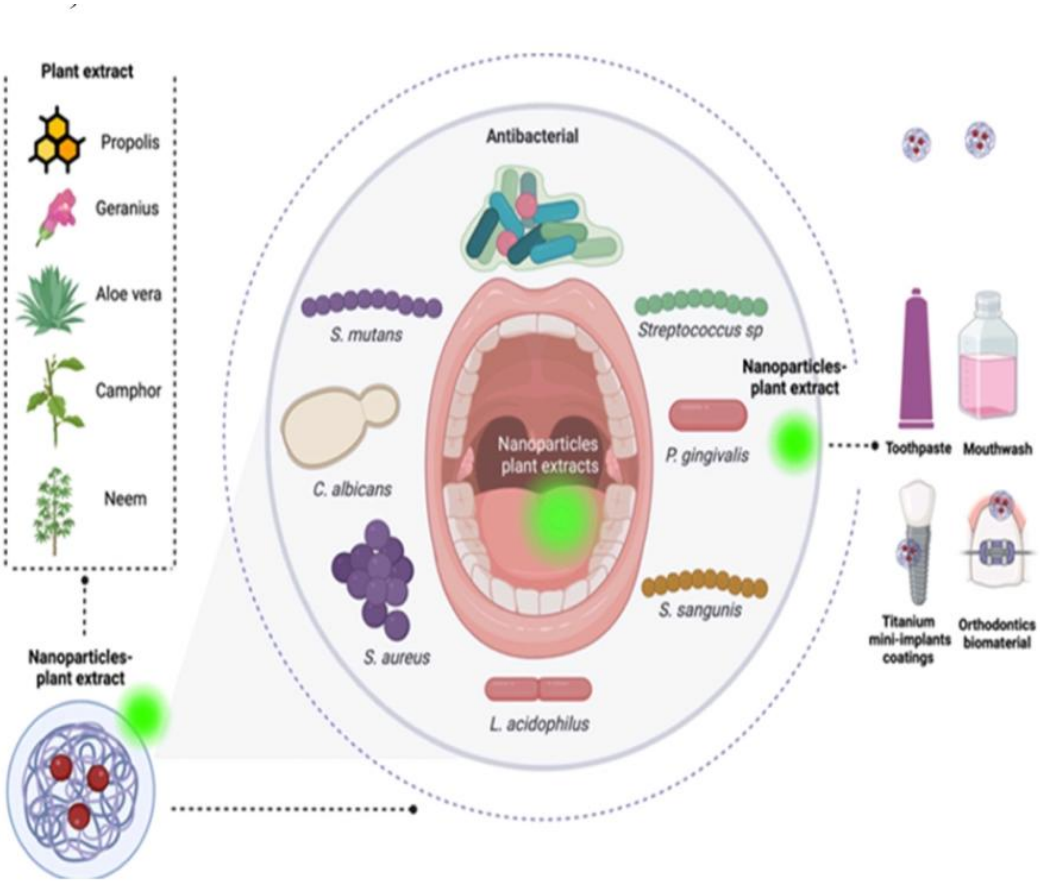

Figure 2.4 Types of Plant-based Nano particles used in Treatment of Oral Diseases.

Source: Gloria-Garza *et al*., 2025.

2.12. The Use in terms of Future Directions and Associated Limitations

As per Milia *et al*., (2023), plants of ethnobotanical (medicinal) properties have known potential to treat dental caries. The majority of research studies show that dental practitioners can use herbal medicines to treat patients because these products provide similar benefits to conventional medications without causing any of their side effects yet researchers still need to establish proof that these products are safe for use and compatible with human tissues while showing their medical effectiveness. The existing research has primarily taken place through in vitro experiments and preclinical studies which creates a critical requirement for researchers to increase their funding and for the establishment of research initiatives that assess the safety, efficacy, cost-effectiveness and the pharmacological characteristics of these natural product compounds in regards to clinical trials. The limited knowledge about how phytotherapeutic agents affect oral tissues and how they function and what side effects they cause presents a main obstacle to their use in dental procedures according to Shaalan and El-Rashidy, (2023). The global health situation makes this situation especially important because alternative and herbal treatments prove particularly useful in low-income areas which lack proper access to standard dental services according to Mishra *et al*., (2019). Human communities across the world still practice using plants as a source of medicine (Milian *et al*., 2023), while plants continue to serve as vital components for traditional medical systems (Khoramian Tusi *et al*., 2020). Herbal medicines show multiple biological effects which produce antimicrobial properties and antioxidant effects and anti-inflammatory benefits that take place through oral and intravenous consumption (Delimont and Carlson, 2020). The increasing demand for dental treatment alternatives makes plant-based substances an essential treatment choice because they provide

effective results with minimal harmful effects (Mishra *et al*., 2019). Phytocompounds found in plants function as an essential antibacterial agent that provides treatment for common dental problems. The medicinal plants establish their antibacterial properties through multiple intricate mechanisms which assist in preventing bacteria from acquiring treatment resistance (Milian *et al*., 2023). Numerous phytochemicals exhibiting combined effects in one plant extract proves most effective in eliciting better therapeutic effects in comparison to just what one single compound could do. Researchers need to investigate the effects that phytochemicals have when they combine with both plant-derived substances and existing dental treatment medications (Shaalan & El-Rashidy, 2023). The natural substances provide many benefits yet they can produce severe negative reactions which need complete evaluation. The safe therapeutic application of medicinal plants depends on accurate identification of their botanical source and proper quality control of extracted compounds through established quantitative and qualitative standards.

Research in the future needs to investigate developed nations because these countries now face two public health threats of increasing bacterial and viral resistance while showing persistent high levels of dental diseases. Resulting effects of herbal and phytotherapeutic products in clinical studies needs proper verification through large-scale population-based studies because existing trials have produced mixed results according to Delimont & Carlson, (2020). This study results show that alternative and herbal therapies provide effective treatment methods which improve worldwide dental health for people who live in remote areas. (Mishra *et al*., 2019). The scientific discoveries provide a basis which researchers can use to create new pharmaceutical compounds and antimicrobial agents. (Khoramian Tusi *et al*., 2020).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of Plant Specimens

Fresh young stem of guava (*Psidium guajava*) and Neem (*Azadirachta indica*) were collected from Thinker's Corner, Emene, Enugu State, at respective coordinates 167° S, 6°28'7"N, 7°31'37"E and 208° SW, 6°28'8"N, 7°31'38"E. The stems were harvested, cleaned to remove dirt, and transported in sterile polythene bags.

3.2 Equipment Used

The equipment employed in this study included beakers, syringes, centrifuge, test tubes, conical flasks, sterile Petri dishes, Whatman No. 1 filter papers, weighing balance, measuring cylinders, cork borers, micropipettes, bijou bottles, spatulas, swab sticks, pipette tips, Bunsen burner, refrigerator, autoclave, and aluminum foil. The usage of these instruments followed standard microbiological operating procedures as noted in (Medina *et al.,* 2019).

3.3 Chemicals and Reagents

All chemicals used were of analytical grade. These included dimethyl sulfoxide (DMSO), ethanol, barium chloride, sulphuric acid, sodium chloride, distilled water, Gram staining reagents, Fehling's solution, Benedict's reagent, ferric chloride, aluminum chloride, lead subacetate, picric acid, and ammonia solution. Chemical handling and preparation adhered to safety protocols documented (Ibrahim and Saidu (2020).

3.4 Media Preparation

All culture media were prepared strictly according to manufacturers' instructions. Each medium was weighed using an analytical balance, dissolved in distilled water, homogenized by heating to 100°C, and sterilized at 121°C for 15 minutes at 15 psi in an autoclave. Sterility testing was performed by incubating unused plates for 24 hours. This procedure followed the media preparation principles outlined by Medina and Torres, (2019)

3.4.1 Nutrient Agar Preparation

Nutrient agar was used for maintaining the organisms. The preparation involved dissolving 28 g of nutrient agar powder in 1000 ml of distilled water before homogenization and sterilization. This method follows (Adeoye and Abiodun, 2019).

3.4.2 Nutrient Broth

Thirteen grams of nutrient broth powder were dissolved in 1000 ml of distilled water and homogenized before sterilization. The broth was used for growing the organisms used for downstream tests.

3.4.3 MRS Agar (Selective for *Lactobacillus*)

To prepare MRS agar, 62 g of the medium powder were dissolved in 1000 ml distilled water, homogenized at 100°C, and sterilized. This medium supports the growth of *Lactobacillus* species as described by (Akande *et al*., 2020).

3.4.4 Blood Agar Preparation (for *Streptococcus* Isolation)

A total of 2.8 g of nutrient agar powder was dissolved in 100 ml distilled water, sterilized, allowed to cool to 45–50°C, and enriched with 5 ml sterile defibrinated human blood. Proper mixing yielded blood agar suitable for *Streptococcus* isolation. This method is consistent with (Ezechi *et al.*, 2020).

3.4.5 Mueller–Hinton Agar (for Sensitivity Testing)

Mueller–Hinton agar was prepared by dissolving 38 g of powder in 1000 ml distilled water and sterilizing the mixture according to NCCLS-validated procedures as outlined by (Ogundele *et al.*, 2020).

3.5 Isolation of Test Organisms

3.5.1 Isolation of *Lactobacillus* spp.

Oral swab samples were streaked onto MRS agar plates and incubated at 37°C for 24 to 48 hours under microaerophilic conditions. Colonies exhibiting creamy, convex morphology were subcultured repeatedly for purity. These procedures align with reports by (Akande *et al*., 2019).

3.5.2 Isolation of *Streptococcus* spp.

Samples were streaked onto freshly prepared 5% blood agar plates and incubated at 37°C for 24 hours. Colonies exhibiting hemolysis were selected and purified. This method follows protocols used by (Oladipupo *et al*., 2020).

3.6 Gram Staining

Gram staining was performed to confirm the Gram-positive nature of *Lactobacillus* and *Streptococcus* species. Smears were heat-fixed and stained sequentially with crystal violet for one minute, iodine for one minute, alcohol decolorizer for 10–15 seconds, and safranin counterstain for 30 seconds before microscopic observation. This method follows (Onwuakor *et al.,* 2019).

3.6.1. Biochemical Tests to confirm test isolates

3.6.2. Catalase test

On a sterile glass slide surface, clumps of bacteria isolates were placed using sterile wire loop and flooded with a drop of 3% hydrogen peroxide. The solution was mixed well using the

wireloop. A positive catalse result is indicated by bubble formation, whereas a negative result shows absence of bubble formation (Mannan *et al*., 2017).

3.6.3. Oxidase test:

A sterile glass slide was used as the isolates were placed on its surface and reacted with the cytochrome oxidase reagent. The formation of blue-purple coloration indicates positive oxidase whereas formation of pink coloration indicates oxidase negative result. The bacteria being positive or negative is based on its change in color (Dimri *et al*., 2020).

3.6.4. Indole test:

In a test tube containing 5ml of peptone water, the test organism was inoculated and placed in an incubator at 35-37 °C for 24 hours to enable the organism adsorb the indole. After 24 hours, approximately 0.5ml of Kovac's reagent was introduced into the tube. A red colour appearance at the topmost layer indicated positive while a yellow colouration depicted negative for the test (Edem *et al.,* 2022).

3.6.5 Citrate utilization test

10ml of Citrate medium was added into several test tubes and was placed in an autoclave at a temperature of 121°C. It was left for 15 minutes so that the medium can be properly sterilized. The organism used for test was transferred to the sterilized media by inoculation method and was incubated for a period of 48 hours at 37 °C. The presence of a deep-blue colour showed that the reaction was positive (Edem *et al*., 2022).

3.6.6. Coagulase test

Blood serum was added to a clean glass slide in drops. The isolate was picked with a sterile wire loop and was mixed into the serum drop. The presence of agglutination indicated a positive

reaction and an absence of agglutination showed that the reaction was negative (Edem *et al.,* 2022).

3.7 Storage of Pure Cultures

Pure isolates were stored on nutrient agar slants. Six to seven milliliters of molten nutrient agar were dispensed into sterile bijou bottles, sterilized, and slanted to solidify. Each slant was inoculated with a pure colony, incubated for 24 hours at 37°C, and stored at 4°C. This storage procedure is consistent with (Ibrahim and Saidu, 2020).

3.8 Preparation of Plant Extracts

Fresh plant stems were washed thoroughly and dried at room temperature for five days until moisture was eliminated. The dried stems were ground into fine powder using a mechanical grinder and stored in airtight containers until use. Extract preparation followed the approach used by (Nwachukwu *et al.*, 2020).

3.8.1 Ethanolic Extraction

A total of 30 g of powdered stem material was placed into a 500 ml clean airtight container, and 400 ml of ethanol were introduced. The mixture was left at room temperature and agitated intermittently for 48 hours to allow complete extraction of bioactive compounds. Filtration was carried out using clean white cloth, followed by Whatman No. 1 filter paper. The filtrate was evaporated to dryness on stainless trays at room temperature. This method follows the extraction protocols reported (Adepoju *et al.*,2019).

3.8.2 Extract Storage

Dried extracts were collected using clean spatulas, transferred into labelled universal containers, and stored in the refrigerator for further use, as recommended (Nwachukwu *et al.,*2020).

3.9 Standardization of Extracts

The ethanolic extract was standardized by dissolving 2 g of the dried extract in 10 ml of 1% DMSO to obtain a stock concentration of 200 mg/ml. From this stock, different concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml) were prepared using a two-fold serial dilution. Five sterile test tubes were labeled and each received 2 ml of distilled water. Two millilitres of the stock extract were added into the first test tube to obtain 100 mg/ml. Two millilitres from the first tube were transferred into the second to achieve 50 mg/ml. The procedure continued up to the fifth tube, resulting in 6.25 mg/ml.

This method follows the extract dilution techniques described by (Adepoju *et al.,* 2019*)*.

3.10 Preparation of 0.5 McFarland Standard

A 0.5 McFarland turbidity standard equivalent to 1.5×10^8 CFU/ml was prepared by mixing 0.05 ml of 1% barium chloride solution with 9.95 ml of 1% sulphuric acid. The solution was allowed to stand to form a uniform milky suspension. The 1% barium chloride solution was prepared by dissolving 0.1 g of $BaCl_2$ crystals in 10 ml of distilled water, while the 1% sulphuric acid solution was prepared by mixing 1 ml concentrated $H_2 SO_4$ with 9 ml of distilled water.

This procedure follows (Amadi *et al*., 2019).

3.11 Centrifugation of Organisms to Obtain Pure Cells

A total of 120 ml of nutrient broth was prepared and dispensed aseptically into two conical flasks labeled for *Lactobacillus* and *Streptococcus*. After autoclaving and cooling, a loopful of each isolate from its slant culture was inoculated into the respective flask and incubated at 37°C for 48 hours to obtain full growth. Ten millilitres of the broth culture were transferred into sterile centrifuge tubes and centrifuged at 3000 rpm for 10 minutes. The supernatant was decanted, and

the cell pellets were washed three times by suspending in sterile normal saline, centrifuging again, and decanting. The washed cells were then used for dilution and further experiments.

The centrifugation protocol follows (Akande *et al.*, 2019).

3.12 Standardization of Microorganisms

A three-fold serial dilution of each standardized cell suspension was made. Three sterile test tubes were each filled with 2 ml of normal saline. One millilitre of the washed cell suspension was added into the first tube and mixed thoroughly, giving 3 ml. From this mixture, 1 ml was transferred into the second tube; from the second tube, 1 ml was transferred to the third. Each tube was compared visually with the prepared 0.5 McFarland standard to select the matching turbidity suitable for antimicrobial testing.

This dilution technique follows the methods of (Akanbi *et al.*, 2020).

3.13 Antimicrobial Screening Test

The antimicrobial screening test was carried out to determine the activity of the extract against the test organisms. For each organism, five plates were prepared. Each plate contained 15 ml of molten nutrient agar into which 2 ml of the extract (at stock concentration), 2 ml of 10% DMSO, 2 ml of 10% ethanol, or nutrient agar only (negative control) were incorporated before pouring. The plates were allowed to gel and then incubated at 37°C for 24 hours to confirm sterility. After incubation, each plate was streaked with the standardized test organism aseptically and incubated again at 37°C for 24 hours. Growth was recorded as no growth (–), growth (+), medium growth (++), or heavy growth (+++).

This incorporation-based screening method follows the procedures described by (Ogundele *et al.,* 2020).

3.14 Minimum Inhibitory Concentration (MIC)

The MIC was determined using the broth dilution method. Five sterile test tubes were each filled with 10 ml nutrient broth and labeled according to the different extract concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml). A volume of 0.05 ml of the standardized organism was added to each tube, followed by 0.5 ml of the appropriate extract concentration. The tubes were mixed gently and incubated at 37°C for 24 hours. After incubation, tubes were examined for turbidity. The MIC was recorded as the lowest extract concentration showing no visible growth.

This MIC procedure follows the methods of (Adeoye and Abiodun, 2019).

3.15 Sensitivity Screening Test (Agar Well Diffusion Method)

The agar well diffusion technique was conducted to evaluate the antibacterial potency of the different extract concentrations. A total of 300 ml of sterile Mueller–Hinton agar was prepared and dispensed as 25 ml per plate into twelve sterile Petri dishes (two plates per concentration and per organism). The plates were incubated for 24 hours at 37°C to check for sterility. After incubation, 0.05 ml of standardized organism suspension was spread uniformly on each plate using a sterile swab stick.

A sterile cork borer (8 mm diameter) was used to punch six wells on each plate. Five wells received 0.04 ml of the different concentrations of the extract, while the remaining well received gentamicin, used as a positive control. The plates were allowed to stand for one hour to permit diffusion of the extract into the agar and then incubated for another 24 hours at 37°C. Zones of inhibition were measured in millimeters.

This agar diffusion procedure is based on the NCCLS guidelines and adapted from (Ogundele *et al.,* 2020).

3.16 Phytochemical Analysis

Phytochemical screening was carried out on the extracts to determine the presence of biologically relevant secondary metabolites. Tests conducted included alkaloids, glycosides, saponins, tannins, flavonoids, resins, proteins, steroids, and terpenoids following the classical detailed methods reproduced in the document and supported by (Nwachukwu *et al*., 2020).

3.16.1 Test for Alkaloids

Two grams of powdered sample were extracted with 20 ml of 5% ethanol by heating in a boiling water bath for 10 minutes. The mixture was filtered, and portions of the filtrate were treated with Mayer's, Wagner's, and Dragendorff's reagents to observe precipitates characteristic of alkaloids.

3.16.2 Test for Glycosides

Five millilitres of dilute sulphuric acid were added to 0.1 g of extract and heated. After neutralization with potassium hydroxide, Fehling's I and II were added and boiled for 5 minutes. Brick-red precipitate indicated glycosides.

3.16.3 Test for Saponins

Filtrate from 0.25 g extract boiled in 20 ml of water was used. Frothing and emulsion formation tests were performed to confirm saponins.

3.16.4 Test for Tannins

A portion of extract solution was tested with ferric chloride and lead subacetate. Blue-black precipitate indicated tannins.

3.16.5 Test for Flavonoids

One millilitre of 1% aluminium chloride was added to the extract solution. A yellow colour indicated flavonoids.

3.16.6 Test for Resins

The extract dissolved in ethanol was poured into water. Resin presence is indicated by precipitate.

3.16.7 Test for Proteins

Million's reagent, xanthoproteic reaction, and picric acid solution were used to detect proteins.

3.16.8 Test for Steroids

Steroid presence was tested by chloroform extraction and sulphuric acid layer reaction.

3.16.9 Test for Terpenoids

Evaporated chloroform extract was heated with concentrated sulphuric acid; grey colour confirmed terpenoids.

3.17 Molecular Identification of Test Organisms

3.17.1 DNA Extraction

Genomic DNA was extracted according to the protocol below:

1. 50 - 100 mg (wet weight) of bacterial cells that had been resuspended in up to 200 ul of water or isotonic buffer were added to a ZR BashingBead ™ Lysis Tube (0.1 mm & 0.5 mm). 750 pl BashingBead ™ Buffer was added to the tube

2. It was secured in a bead beater fitted with a 2 ml tube holder assembly and processed at maximum speed for ≥ 5 minutes.

3. The ZR BashingBead ™ Lysis Tube (0.1 & 0.5 mm) was centrifuged in a microcentrifuge at 10,000 x g for 1 minute.

4. 400 ul supernatant was transferred to a Zymo-Spin™ III-F Filter in a collection tube and centrifuged at 8,000 x g for 1 minute.

5. 1,200 ul of Genomic Lysis Buffer was added to the filtrate in the collection tube from Step 4.

6. 800 ul of the mixture from Step 5 was transferred to a Zymo-Spin™ IICR Column® in a collection tube and centrifuged at 10,000 x g for 1 minute.

7. The flow through from the collection tube was discarded and Step 6 repeated.

8. 200 ul DNA Pre-Wash Bufferwqs added to the Zymo-Spin™ ICR column in a new collection tube and centrifuged at 10,000 x g for 1 minute.

9. 500 ul of g-DNA Wash Buffer was added to the Zymo-Spin™ IICR Column and centrifuged at 10,000 x g for 1 minute.

10. Zymo-Spin™ IICR Column was transferred to a clean 1.5 ml microcentrifuge tube and 100 pl (35 ul minimum) DNA Elution Buffer was added directly to the column matrix and centrifuged at 10,000 x g for 30 seconds to elute the DNA.

This extraction method follows Ibrahim & Saidu (2020) and Ezechi *et al.* (2020).

3.17.2 PCR Amplification of 16S rRNA Gene

PCR amplification used primers 27F and 1492R. Each 25 µl reaction contained 12.5 µl master mix, 1 µl of each primer, 2 µl template DNA, and 8.5 µl water. The cycling conditions were: 95°C for 5 minutes; 30 cycles of 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 minute; and final extension at 72°C for 7 minutes.

CHAPTER FOUR

RESULTS

4.1. Morphological and Biochemical Features of Isolates

The isolates streaked directly from oral swabs displayed typical morphological and biochemical characteristics of the expected organisms as observed in Figure 4.1. The isolates belong to the *Streptococcus* sp. and *Lactobacillus* sp. as observed in Table 4.1.

4.2. Antimicrobial Screening Test

The antimicrobial screening was done to determine the susceptibility of the bacteria isolates to the stock ethanoic extracts, 10%DMSO solution, 10% ethanoic solution and nutrient media as shown in Table 4.2. The ethanoic extract of the neem and guava was effective against *Streptococcus* sp. but was a bit ineffective against the *Lactobacillus* sp. the 10% DMSO and ethanol plus nutrient agar both served as control samples.

4.3. Antibacterial Susceptibility Test

The antibacterial sensitivity to the extracts in comparison to a control (Gentamicin) is visualized in Figure 4.2. The ethanoic extracts had inhibition zone diameters at the lowest inhibitory concentrations of 14mm at 12.5mg/mL, 16mm at 25mg/mL, 17 mm at 50mg/mL and 19 mm at 100mg/mL for guava, and 18mm at 25mg/Ml, 20mm at 50mg/Ml, and 22mm at 100mg/Ml for neem respectively which was far in range from the control Gentamicin at 24 mm, 24 mm, 25 mm and 26 mm respectively as shown in Table 4.2.

4.4. Minimum Inhibitory Concentration (MIC) Analysis

The minimum Inhibitory concentration of the extracts as presented in Table 4.3 shows that the ethanol Guava extract against *Streptococcus* sp. had the least inhibitory effect against the isolate at 6.25 mg/mL, followed closely by the Guava extract against *Lactobacillus* sp. and the Neem extract against Streptococcus sp. at both 12.5 mg/mL respectively. The Neem extract against *Lactobacillus* sp. had the most inhibitory effect at 25 mg/mL in comparison to the other 3 extracts. Figure 4.3 also shows the test tubes for the MIC analysis.

4.5. Phytochemical Analysis of Plant Extracts

The analysis of bioactive compounds of the plant extracts indicated positive for the presence of alkaloids, flavonoids, glycosides, saponins, resins, proteins, tannins, steroids, and terpenoids in the neem Stem bark extract, but only the absence of saponins and tannins in the Guava stem extract as indicated in Table 4.3. These various phytochemical compounds give the plant extract most of its antimicrobial activity and suggests it's use as a potential natural borne agent in the treatment of dental caries.

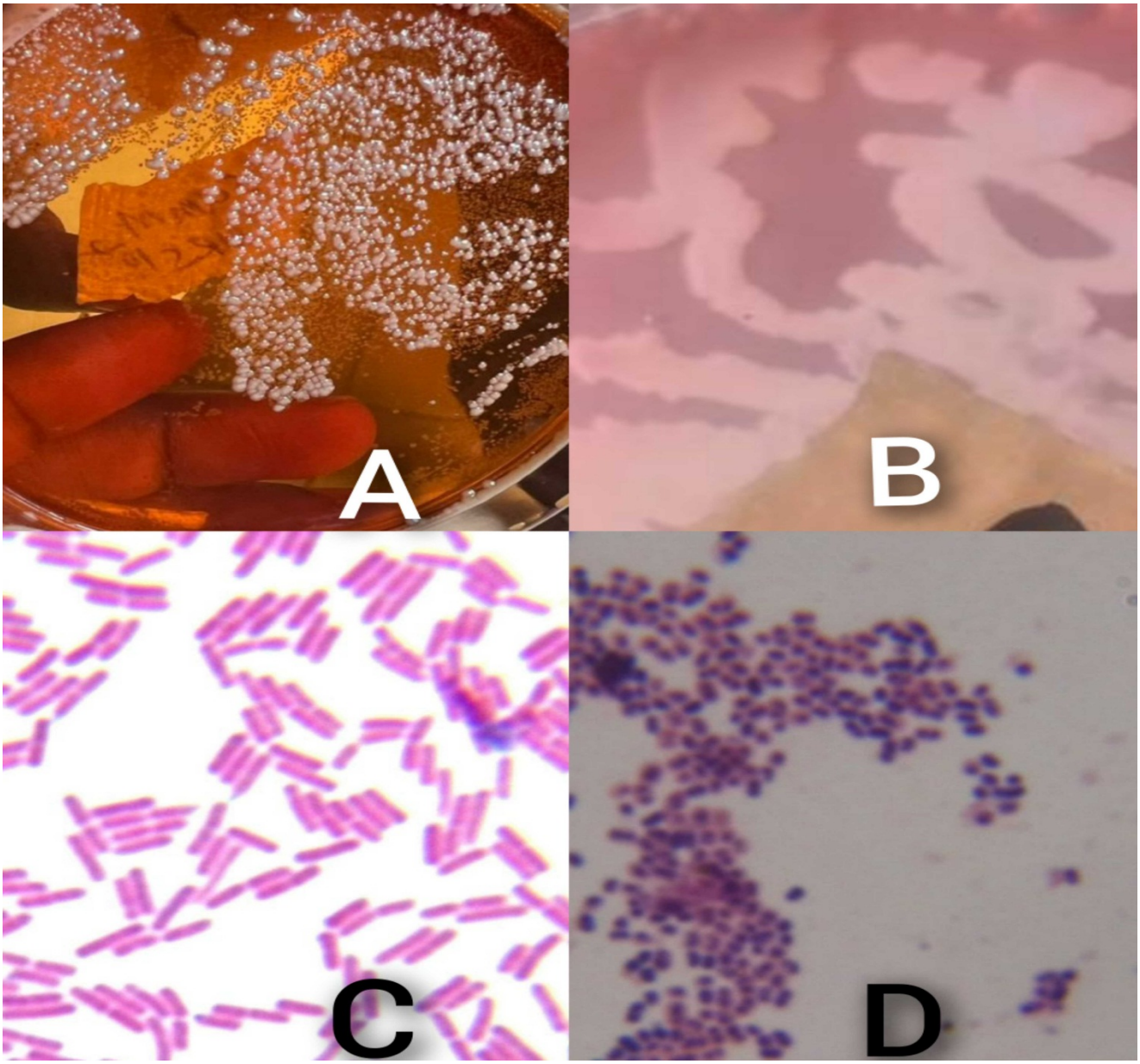

Figure 4.1. Morphology and Gram stain characteristics of Bacteria isolates, (A): *Lactobacillus* sp. morphology; (B): *Streptococcus* sp. morphology; (C): *Lactobacillus* Gram-positive rods structure; (D): *Streptococcus* Gram-positive cocci in chains structure.

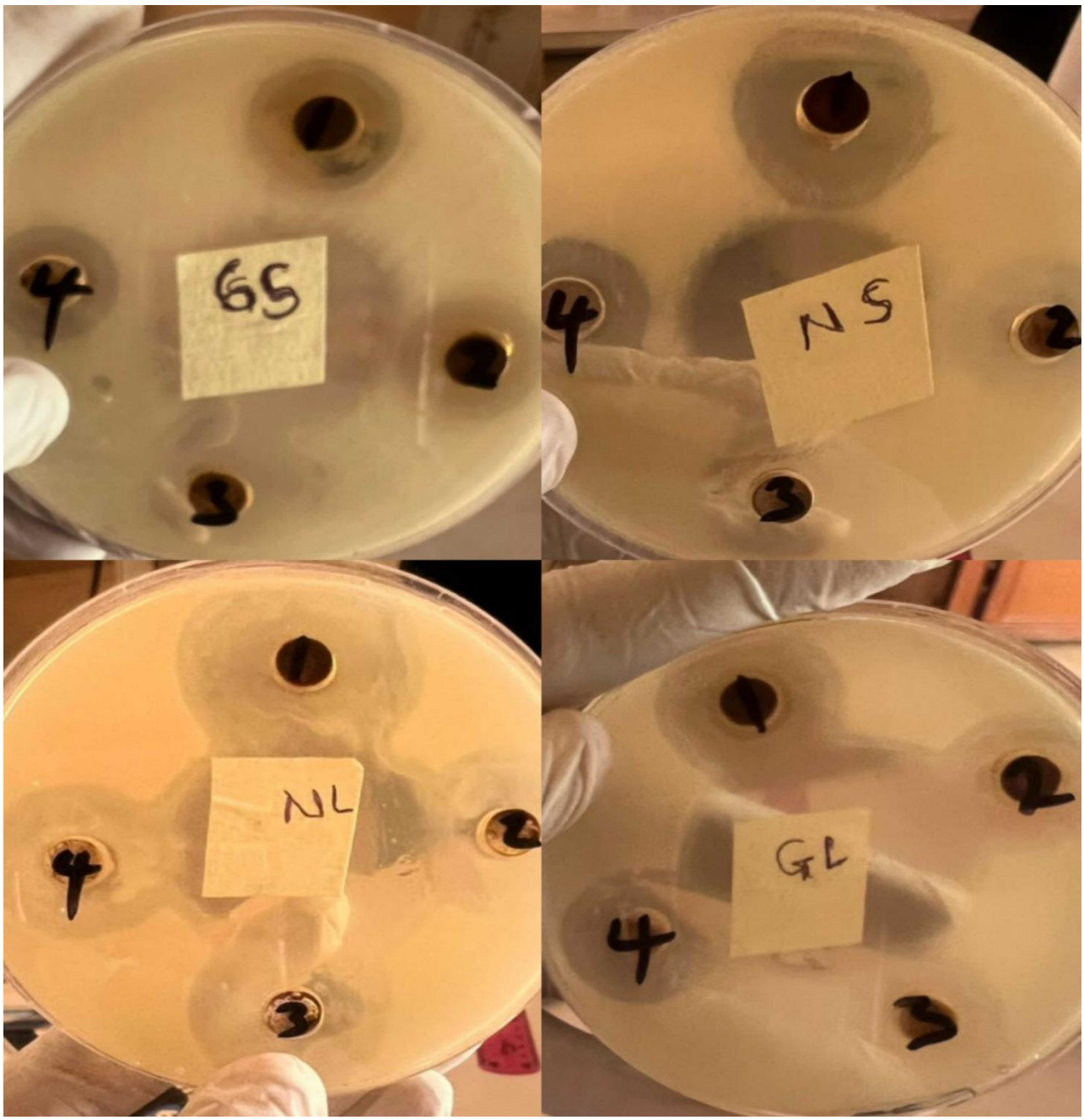

Figure 4.2. Antibiotic Sensitivity Screening via Agar well diffusion

Figure 4.3. Test Tubes for MIC analysis

Table 4.1. Morphological and Gram Stain Characteristics of Bacteria Isolates

Features	***Streptococcus* sp.**	***Lactobacillus* sp.**
Gram Reaction	Gram +ve	Gram +ve
Appearance	Large mucoid colonies	Small circular colonies
Color formation	Pale Grey colouration	Creamy white colouration
Cell arrangement	Cocci in chains	Long Rod shapes
Elevation	Convex and rough	Convex and smooth
Texture	Dry like matt texture	Slimy and glistening texture

Key: (Gram +ve) Gram positive**.**

Table 4.2. Antimicrobial Sensitivity Screening Test for Ethanolic Plant Extracts (mm)

Test organisms	**Plant Extract**	**100mg/ml**	**50mg/ml**	**25mg/ml**	**12.5mg/ml**	**6.25mg/ml**	**Gen**
Streptococcus	Neem	22	20	18	0	0	24
sp.	Guava	19	17	16	14	0	24
Lactobacillus	Neem	21	19	0	0	0	25
sp.	Guava	18	16	0	0	0	26

Key: (+) Inhibition of growth; (0) No inhibition; (Gen) Gentamicin antibiotic.

Table 4.3. Minimum Inhibitory Concentration (MIC) of Ethanolic plant Extracts

Test organisms	Plant Extract	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	Gen
Streptococcus	Neem	+	+	+	_	_	+
sp.	Guava	+	+	+	+	_	+
Lactobacillus	Neem	+	+	_	_	_	+
sp.	Guava	+	+	_	_	_	+

Key: (+) Inhibition of growth; (-) No inhibition; (Gen) Gentamicin antibiotic.

Table 4.4. Phytochemical Test of the Plant Extracts

Phytochemicals	**Guava Stem Bark**	**Neem Stem Bark**
Alkaloids	+	+
Glycosides	+	+
Saponins	-	+
Tannins	-	+
Flavonoids	+	+
Resins	+	+
Proteins	+	+
Steroids	+	+
Terpenoids	+	+

Key: (+): Presence of phytochemical compound; (-): Absence phytocmical compound.

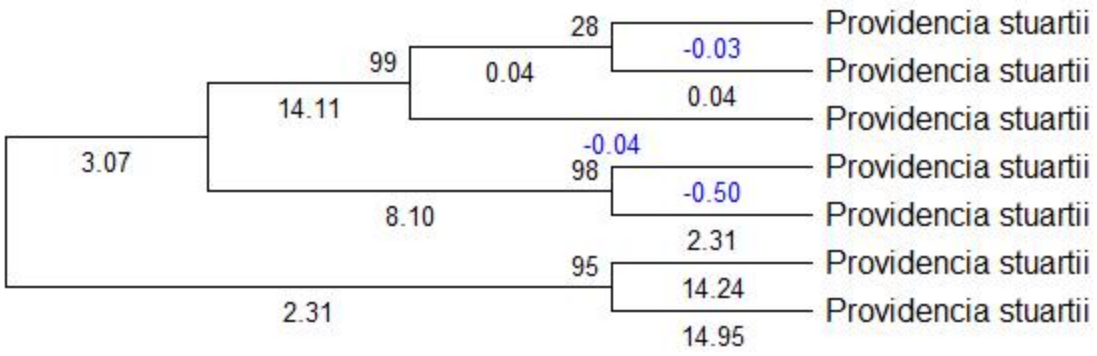

Figure 4.4: Phylogenetic tree of bacteria species.

Interpretation

The phylogenetic analysis of the bacterial species, the isolate showed close relationship with *lactobacillu*s species strong bootstrap values(99-100), and short branch length indicate reliable results with minimal genetic variation.The amplified was sequenced and analyzed using BLAST. The sequence showed highest similarity to *lactobacillus* species with 99.84% identity and 94% query coverage. The closet related sequence had accession number PV809825.1

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1. Discussion

This study aimed at extracting phytochemical compounds from both the Neem and Guava stem respectively and testing their antibacterial efficacy against oral pathogens namely *Streptococcus* sp. and *Lactobacillus* sp. known for causing dental caries, and this was duly achieved. The two bacteria were collected using sterile swab sticks from the oral cavity (mouth) of patients plagued with the dental caries infection as done by a certified dentist. The samples were subcultured constantly and pure isolates with typical morphological and Gram stain characteristics were obtained as seen from the results before they were concentrated and washed to get a high cell purity. These bacteria served as test organisms for the antagonistic activity of the two plant extracts.

On observation of the antimicrobial activity of the obtained extracts from the plants against the bacteria isolates, the synergy of the extracts with the positive control Gentamicin antibiotic disc and shows the ethanolic extracts of the Neem plant had top inhibition zone diameters of 22 mm against *Streptococcus* sp. and 21 mm against *Lactobacillus* sp. respectively. These were both far in range to the positive control antibiotic disc which had zones of 24 mm and 25 mm respectively against same bacteria isolates. This was also higher than the zone diameters of the guava plant which recorded diameters of 19 mm against *Streptococcus* sp. and 18 mm against *Lactobacillus* sp., with its synergistic positive antibiotic disc having 24 mm and 26 mm zone diameters respectively. The difference in the zone widths across the two plant extracts could be as a result of the nature of the phytochemical compounds found naturally in these individual plants. The neem plant contains a vast concentration of over 300 phytochemical compounds present all

around the plant with some extra antibacterial activity hence the result of higher clearance zones for bacteria inhibition. The results of the inhibition zones of the guava plant here against both the *Lactobacillus* sp. and *Streptococcus* sp. are consistent to the work of a similar study by KASHARI *et al*., (2020) who recorded inhibition zones of 19 mm against *Lactobacillus* sp. and 18 mm against *Streptococcus mutans* respectively utilising methanol as the solvent of extraction. In comparison to the positive control sample, this had a much wider inhibition diameter due to the broad spectrum of activity nature of this antibiotic disc which is also very potent against Gram-positive bacteria particularly.

The minimum Inhibitory concentration of the extracts shows the guava stem extract against *Streptococcus* sp. had the least inhibition at a 6.25 mg/mL concentration in comparison to the other 2 extracts which had equal inhibitions at 12.5 mg/mL for the guava extract against the *Lactobacillus* sp. and the neem extract against *Streptococcus* sp. respectively. Only the neem extract against *Lactobacillus* sp. had the most inhibition at 25 mg/mL. The lower the concentration in this case proves the effectiveness of the plant extract in clearing the inoculated bacteria isolates at the lowest dose which is an excellent feature and comparison for determining its positive activity for use as a natural antibacterial agent. The guava stem extract against the *Streptococcus* sp. at 6.25 mg/mL being the least recorded concentration amongst all tested concentrations is consistent with the same work of similar study by KASHARI *et al*., (2020) who also recorded a low MIC value of 2.188 mg/mL against *Streptococcus mutans* using a methanol guava stem extract, whereas the same extract against *Lactobacillus* sp. recorded double the MIC value at 4.375 mg/mL against *Lactobacillus* sp. For the neem extract, there's a similar trend of the ethanolic extract being more effective against the *Streptococcus* sp. here at 12.50 mg/mL, than at 25 mg/mL against the *Lactobacillus* sp. This may be as a result of the nature of the Lactobacillus

bacteria isolate being recalcitrant to alcohol based inhibition as they undergo adaptive evolutionary stress responses. This modifies their cell membrane fatty acid composition and triggers the production of stress-shock proteins, allowing the bacterial population to develop increased resistance to higher, previously lethal concentrations of alcohol.

The preliminary screening for phytochemical compounds in both plants revealed the presence of some important phytochemicals present in both samples which were alkaloids, terpenoids, proteins, steroids, flavonoids, resins and glycosides. The neem plant extracts showed presence of all the listed phytochemicals and saponins and tannins as well whereas the Guava extract showed absence of these two tested compounds. An important fact is that the phytochemicals found around different plant parts may vary across each plant, but some similar bioactive compounds may be found but at different concentration quantities. The neem plant due to the nature of the phytocmical compounds found here, showcases a broad spectrum antibacterial activity as seen in the inhibition zones recorded. The concentration of the tannin in guava parts like leaves and the stem bark influences the effectiveness of antibacterial compounds present in there. The higher the levels of tannin the more the antibacterial activity will increase as seen in the results with guava plants showing faint presence or complete absence of tannins here and in the lower inhibition zone diameters exhibited here (Pereira *et al*., 2023).

5.2. Conclusion

In conclusion, the stem of both the Guava and Neem trees proved efficient as ethanolic extracts in suppressing the growth of two probable dental caries causing bacteria *Streptococcus* sp. and *Lactobacillus* sp. respectively in this study. This could suggest the possible development of these plant based therapeutics as an alternative and possibly safer remedy to treating dental caries, especially in rural areas with less spending power.

5.3. Recommendation

Based on the findings of this study, it could be recommended that these two plant extracts may be further studied with the full knowledge of the phytochemical compounds found within these plants, to be able to utilise them to their full potential. These plants therefore pose a promising future for ethnobotanical application and medicinal application in dentistry and orthodontics. With the complications surrounding the consistent use of commercial mouthwashes which could potentially deplete all beneficial microbiota of the oral cavity, these plants could be synthesized into specific formulations that particularly target plaque forming and dental caries causing microbes which would lead to tooth decay on the long run. It's important to know that the beneficial microbiota of the mouth help sustain life and good gut health of an individual and should therefore not be cleared out for an individual's survival.

REFERENCES

Abdullahi, A. (2021). "Phytochemical screening of guava leave extract". *International Journal of Pure and Applied Science Research*. 12(2): 89-95.

Ahamad, A. and Ansari, S. (2022). A Review on Multipurpose Medicinal Properties Of Traditionally Used *Psidium Guajava* Leaves. *Asian Journal of Pharmaceutical and Clinical Research.* 15(8), 9-22.

Ahmad, R., Misra, A., Trivedi, A. and Khan, M. A. (2017). Evaluation of In Vitro Cytotoxic Activity of Ethanolic Extract of *Azadiracta indica* Leaves as a Function of pH on Human Breast Cancer Cell Line MDA-MB 231. *Journal of Basic and Clinical Pharmacy.* 8:S72-S79.

Ahmed, S. K., Hussein, S., Qurbani, K., Ibrahim, R. H., Fareeq, A., Mahmood, K. A. and Mohamed, M. G. (2024). Antimicrobial resistance: Impacts, challenges, and future prospects. *Journal of Medicine, Surgery, and Public Health.* 2, 100081.

Ancuceanu, R., Anghel, A. I., Ionescu, C., Hovanet, M. V., Cojocaru-Toma, M. and Dinu, M. (2019). Clinical Trials with Herbal Products for the Prevention of Dental Caries and Their Quality: A Scoping Study. *Biomolecules.* 9, 884.

Banas, J. A. and Drake, D. R. (2018). Are the *mutans Streptococci* still considered relevant to understanding the microbial etiology of dental caries? *BMC Oral Health.* 18, 129.

Ben-Zaken, H., Kraitman, R., Coppenhagen-Glazer, S., Khalifa, L., Alkalay-Oren, S., Gelman, D., Ben-Gal, G., Beyth, N. and Hazan, R. (2021). Isolation and Characterization of *Streptococcus mutans* Phage as a Possible Treatment Agent for Caries. *Viruses* 13, 825.

Bowen, W .H., Burne, R. A., Wu, H. and Koo, H. (2018). Oral Biofilms: Pathogens, Matrix, and Polymicrobial Interactions in Microenvironments. *Trends in Microbiology*. 26, 229–242.

Bowen, W. H., Burne, R. A., Wu, H. and Koo, H. (2018). Oral biofilms: pathogens, matrix, and polymicrobial interactions in microenvironments. *Trends Microbiol.* 26, 229–242.

Braga, T. M., Rocha, L., Chung, T. Y., Oliveira, R. F., Pinho, C., Oliveira, A. I., Morgado, J. and Cruz, A. (2020). Biological Activities of Gedunin-A Limonoid from the Meliaceae Family. *Molecules (Basel, Switzerland),* 25(3), 493.

Chatzopoulos, G. S., Karakostas, P., Kavakloglou, S., Assimopoulou, A., Barmpalexis, P. andTsalikis, L. (2022). Clinical Effectiveness ofHerbal Oral Care Products in Periodontitis Patients: A Systematic Review. *International Journal of Environmental Research and Public Health.* 19, 10061.

Chaudhary, S., Kanwar, R. K., Sehgal, A., Cahill, D. M., Barrow, C. J., Sehgal, R. and Kanwar, J. R. (2017). Progress on *Azadirachta indica* Based Biopesticides in Replacing Synthetic Toxic Pesticides. *Frontiers in plant science.* 8, 610.

Chen, X., Daliri, E. B. -M., Kim, N., Kim, J. -R., Yoo, D. and Oh, D. -H. (2020). Microbial Etiology and Prevention of Dental Caries: Exploiting Natural Products to Inhibit Cariogenic Biofilms. *Pathogens.* 9, 569.

da Costa Rosa, T., de Almeida Neves, A., Azcarate-Peril, M. A., Divaris, K., Wu, D., Cho, H., Moss, K., Paster, B. J., Chen, T. B. and Freitas-Fernandes, L. (2021). The bacterial microbiome and metabolome in caries progression and arrest. *Journal of Oral Microbiology.* 13, 1886748.

Daswani, P. G., Gholkar, M. S. and Birdi, T. J. (2017). *Psidium guajava*: A Single Plant for Multiple Health Problems of Rural Indian Population. *Pharmacognosy Reviews*. 11(22):167-174.

DeAssis, E. L., Silveira, F. D., Da Ponte, A. V. A. and Regis, R. R. (2022). A Systematic Review of the Potential Effects of *Lippia sidoides* on Dental Plaque and Periodontal Diseases. Planta Medica. 88, 341–355.

Delimont, N. M. and Carlson, B. N. (2020). Prevention of Dental Caries by Grape Seed Extract Supplementation: A Systematic Review. *Nutrition and Health.* 26, 43–52.

Eltay, E. G., Gismalla, B. G., Mukhtar, M. M. and Awadelkarim, M. O. A. (2021). *Punica Granatum* Peel Extract as Adjunct Irrigation to Nonsurgical Treatment of Chronic Gingivitis: *Punica granatum* Peel Extract as Adjunct Oral Irrigation. *Complementary Therapies in Clinical Practice.* 43, 101383.

Fishbein, S. R. S., Mahmud, B. and Dantas, G. (2023). Antibiotic perturbations to the gut microbiome. *Nature Reviews Microbiology.* 21, 772–788.

Furquim dos Santos Cardoso, V., Amaral Roppa, R. H., Antunes, C., Silva Moraes, A. N., Santi, L. and Konrath, E. L. (2021). Efficacy of Medicinal Plant Extracts as Dental and Periodontal Antibiofilm Agents: A Systematic Review of Randomized Clinical Trials. *Journal of Ethnopharmacology*. 281, 114541.

Gilbert, J.A.; Blaser, M. J., Caporaso, J. G., Jansson, J. K., Lynch, S. V. and Knight, R. (2018). Current understanding of the human microbiome. *Nature Medicine.* 24, 392–400.

Gloria-Garza, M. A., Reyna-Martínez, G. R., Jiménez-Salas, Z., Campos-Góngora, E., Kaˇcániová, M., Aguirre-Cavazos, D. E., Bautista-Villarreal, M., Leos-Rivas, C. and

Elizondo-Luevano, J. H. (2025). Medicinal Plants Against Dental Caries: Research and Application of Their Antibacterial Properties. *Plants.* 14, 1390.

Grabek-Lejko, D. and Hyrchel, T. (2023). The Antibacterial Properties of Polish Honey against *Streptococcus mutans*—A Causative Agent of Dental Caries. *Antibiotics.* 12, 1640.

Gralka, M., Szabo, R., Stocker, R. andCordero, O. X. (2020). Trophic Interactions and the Drivers of Microbial Community Assembly. *Current Biology.* 30, R1176–R1188.

Haji, A. S., Maurya, S. R. and Shah, N. (2023). *Azadirachta indica* A. Juss.: Ethnobotanical knowledge, phytochemical studies, pharmacological aspects future prospects. *Plants and Environment.* 5(1): 1-15.

Handa, S. S. & Khanuja, S. P. S., Longo, G. and Rakesh, D. D. (2018). Extraction technologies for medicinal and aromatic plants. *International centre for science and high technology*. 21-25.

Havsed, K., Stensson, M., Jansson, H., Carda-Diéguez, M., Pedersen, A., Neilands, J., Svensäter, G. and Mira, A. (2021). Bacterial Composition and Metabolomics of Dental Plaque from Adolescents. *Frontiers in Cellular and Infection Microbiology.* 11, 716493.

Heliawati, L., Lestari, S., Hasanah, U., Ajiati, D. and Kurnia, D. (2022). Phytochemical Profile of Antibacterial Agents from Red Betel Leaf (*Piper crocatum* Ruiz and Pav) against Bacteria in Dental Caries. *Molecules.* 27, 2861.

Homayouni Rad, A., Pourjafar, H. and Mirzakhani, E. A. (2023). Comprehensive Review of the Application of Probiotics and Postbiotics in Oral Health. *Frontiers in Cellular and Infection Microbiology*. 13, 1120995.

Huang, X., Browngardt, C. M., Jiang, M., Ahn, S. -J. and Burne, R. A. (2018). Nascimento, M.M. Diversity in Antagonistic Interactions between Commensal Oral *Streptococci* and *Streptococcus mutans*. *Caries Research.* 52, 88–101.

Hurley, E., Barrett, M. P. J., Kinirons, M., Whelton, H., Ryan, C. A., Stanton, C., Harris, H. M. B. and O'Toole, P. W. (2019). Comparison of the salivary and dentinal microbiome of children with severe-early childhood caries to the salivary microbiome of caries-free children. *BMC Oral Health.* 19, 13.

Hussain S. Z., Naseer, B., Qadri, T., Fatima, T. and Bhat, T. A. (2021). Guava (*Psidium guajava*)-Morphology, Taxonomy, Composition and Health Benefits. *InFruits Grown in Highland Regions of the Himalayas: Nutritional and Health Benefits.* 16 : 257-267. Cham: Springer International Publishing.

Isola, G. (2020). Current Evidence of Natural Agents in Oral and Periodontal Health. *Nutrients.* 12, 585.

Jiang, Q., Liu, J., Chen, L., Gan, N. and Yang, D. (2018). The Oral Microbiome in the Elderly with Dental Caries and Health. *Frontiers in Cellular and Infection Microbiology.* 8, 442.

Jiang, S., Gao, X., Jin, L. and Lo, E. C. M. (2016). Salivary Microbiome Diversity in Caries-Free and Caries-Affected Children. *International Journal of Molecular Sciences.* 17, 1978.

Kakad, A. V., Laddha, U. D., Kshirsagar, S. J. and Khairnar, S. J. (2022). Traditional Herbal Remedies for Periodontitis. *Biosciences Biotechnology Research Asia.* 19(4),1079-1091.

Kalpana, B., Prabhu, P., Bhat, A. H., Senthilkumar, A., Arun, R. P., Asokan, S., Gunthe, S. S. and Verma, R. S. (2020). Bacterial diversity and functional analysis of severe early childhood caries and recurrence in India. *Scientific Reports.* 10, 21248.

Kassebaum, N. J., Smith, A. G. C., Bernabé, E., Fleming, T. D., Reynolds, A. E., Vos, T. and Murray, C. J. L. (2017). Marcenes, W. Global, Regional, and National Prevalence, Incidence, and Disability-Adjusted Life Years for Oral Conditions for 195 Countries, 1990–2015: A Systematic Analysis for the Global Burden of Diseases, Injuries, and Risk Factors. *Journal of Dental Research.* 96, 380–387.

Kermeoglu, F., Aksoy, U., Kalender, A., Oztan, M. D., Oguz, E. I. and Kıyan, M. (2018). Determination of the Minimum Inhibitory Concentrations of Alexidine and Chlorhexidine against *Enterococcus faecalis* and *Candida albicans*: An In Vitro Study. *Cureus.* 10, e2221.

Khare, P. and Kamble, S. (2025). Antimicrobial Properties of Neem (*Azadirachta Indica*): A Comprehensive Review of Phytochemicals and Mechanisms of Action. *International Journal of Scientific Research and Technology.* 2(4), 646-654.

Khoramian Tusi, S., Jafari, A., Marashi, S. M. A., Faramarzi Niknam, S., Farid, M., and Ansari, M. (2020). The Effect of Antimicrobial Activity of *Teucrium polium* on Oral *Streptococcus mutans*: A Randomized Cross-over Clinical Trial Study. *BMC Oral. Health.* 20, 130.

Kassebaum, N. J., Smith, A. G. C., Bernabé, E., Fleming, T. D., Reynolds, A. E., Vos, T., Murray, C. J. L., Marcenes, W., & GBD 2015 Oral Health Collaborators. (2017). Global, regional, and national prevalence, incidence, and disability-adjusted life years for oral conditions for 195 countries, 1990–2015: A systematic analysis for the Global

Burden of Diseases, Injuries, and Risk Factors. Journal of Dental Research, 96(4), 380–387.

Kilani-Morakchi, S., Morakchi-Goudjil, H. and Sifi, K. (2021). Azadirachtin-Based Insecticide: Overview, Risk Assessments, and Future Directions. *Frontiers in Agronomy.* 3.

Kim, B. -S., Han, D. -H., Lee, H. and Oh, B. (2018). Association of Salivary Microbiota with Dental Caries Incidence with Dentine Involvement after 4 Years. *Journal of Microbiology and Biotechnology.* 28, 454–464.

Koul, O., Walia, S. and Dhaliwal, G. S. (2018). Neem (*Azadirachta indica*) in pest management. *BioControl.* 53(2):237-253.

Kumar, M., Prakash, S., Radha, Kumari, N., Pundir, A., Punia, S., Saurabh, V., Choudhary, P., Changan, S. and Dhumal, S. (2021). Beneficial Role of Antioxidant Secondary Metabolites from Medicinal Plants in Maintaining Oral Health. *Antioxidants.* 10, 1061.

Kumari, P., Mankar, A., Karuna, K., Homa, F., Meiramkulova, K. and Siddiqui, M. W. (2020). Mineral composition, pigments, and postharvest quality of guava cultivars commercially grown in India. *Journal of Agriculture and Food Research.* 1: 2:100061.

Lathakumari, R. H., Vajravelu, L. K., Satheesan, A., Ravi, S. and Thulukanam, J. (2024). Antibiotics and the gut microbiome: Understanding the impact on human health. *Medical microbiology.* 20, 100106.

Leiton-Ramírez, Y. M., Ayala-Aponte, A. and OchoaMartínez, C. I. (2020). Physicochemical properties of guava snacks as affected by drying technology. *Processes.* 8(1):106.

Lemos, J. A., Palmer, S. R., Zeng, L., Wen, Z. T., Kajfasz, J. K., Freires, I. A., Abranches, J., and Brady, L. J. (2019). The Biology of *Streptococcus mutans*. *Microbiology spectrum,* 7(1), 10.1128/microbiolspec.gpp3-0051-2018.

lSasi, M., Kumar, S., Kumar, M., Thapa, S., Prajapati, U., Tak, Y., Changan, S., Saurabh, V., Kumari, S. and Kumar, A. (2021). Garlic (*Allium sativum* L.) Bioactives and Its Role in Alleviating Oral Pathologies. *Antioxidants.* 10, 1847.

Luo, Y., Peng, B., Wei, W., Tian, X. and Wu, Z. (2019). Antioxidant and anti-diabetic activities of polysaccharides from guava leaves. Molecules. 5;24(7):1343.

Mandava, K., Batchu, U. R., Kakulavaram, S., Repally, S., Chennuri, I., Bedarakota, S. and Sunkara, N. (2019). Design and Study of Anticaries Effect of Different Medicinal Plants against *S. mutans* glucosyltransferase. BMC Complement. *Alternative Medicine*. 19, 197.

Mazur, M., Ndokaj, A., Jedlinski, M., Ardan, R., Bietolini, S. and Ottolenghi, L. (2021). Impact of Green Tea (*Camellia sinensis*) on Periodontitis and Caries. Systematic Review and Meta-Analysis. *Japanese Dental Science Review.* 57, 1–11.

Mei, M. L., Yan, Z., Duangthip, D., Niu, J. Y., Yu, O. Y., You, M., Lo, E. C. M. and Chu, C. H. (2020). Effect of silver diamine fluoride on plaque microbiome in children. *Journal of Dentistry.* 102, 103479.

Milia, E. P., Sardellitti, L. and Eick, S. (2023). Antimicrobial Efficiency of *Pistacia lentiscus* L. Derivates against Oral Biofilm-Associated Diseases—A Narrative Review. *Microorganisms.* 11, 1378.

Milutinovici, R. A., Chioran, D., Buzatu, R., Macasoi, I., Razvan, S., Chioibas, R., Corlan, I. V., Tanase, A., Horia, C. and Popovici, R. A. (2019). Vegetal Compounds as Sources of Prophylactic and Therapeutic Agents in Dentistry. *Plants.* 10, 2148.

Mishra, P., Marwah, N., Agarwal, N., Chaturvedi, Y. and Suohu, T. (2019). Comparison of *Punica granatum, Terminalia chebula,* and *Vitis vinifera* Seed Extracts Used as Mouthrinse on Salivary *Streptococcus mutans* Levels in Children. *Journal of Contemporary Dental Practice.* 20, 920–927.

Mitwalli, H., Mourao, M. D. A., Dennison, J., Yaman, P., Paster, B. J. and Fontana, M. (2019). Effect of Silver Diamine Fluoride Treatment on Microbial Profiles of Plaque Biofilms from Root/Cervical Caries Lesions. *Caries Research.* 53, 555–566.

Moga, M. A., Bălan, A., Anastasiu, C. V., Dimienescu, O. G., Neculoiu, C. D., and Gavriş, C. (2018). An Overview on the Anticancer Activity of Azadirachta indica (Neem) in Gynecological Cancers. *International Journal of Molecular Sciences.* 19(12), 3898.

Moghadam, E. T., Yazdanian, M., Tahmasebi, E., Tebyanian, H., Ranjbar, R., Yazdanian, A., Seifalian, A. and Tafazoli, A. (2020). Current Herbal Medicine as an Alternative Treatment in Dentistry: In Vitro, In Vivo and Clinical Studies. *European Journal of Pharmacology.* 889, 173665.

Mosaddad, S. A., Tahmasebi, E., Yazdanian, A., Rezvani, M. B., Seifalian, A., Yazdanian, M. and Tebyanian, H. (2019). Oral Microbial Biofilms: An Update. *European Journal of Clinical Microbiology and Infectious Diseases.* 38, 2005–2019.

Nagini, S., Nivetha, R., Palrasu, M. and Mishra, R. (2021). Nimbolide, a Neem Limonoid, Is a Promising Candidate for the Anticancer Drug Arsenal. *Journal of Medicinal Chemistry.* 64 (7), 3560–3577.

Naseer, S., Hussain, S., Naeem, N., Pervaiz, M. and Rahman, M. (2018). The phytochemistry and medicinal value of *Psidium guajava* (guava). *Clinical phytoscience.* 4(1), 1-8.

Noronha, V. T., Paula, A. J., Durán, G., Galembeck, A., Cogo-Müller, K., Franz-Montan, M. and Durán, N. (2017). Silver Nanoparticles in Dentistry. Dental Materials. 33, 1110–1126.

Nowaczyk, P. M., Bajerska, J., Lasik-Kurdy´s, M., Radziejewska-Kubzdela, E., Szwengiel, A. and Wo´zniewicz, M. (2021). The Effect of Cranberry Juice and a Cranberry Functional Beverage on the Growth and Metabolic Activity of Selected Oral Bacteria. *BMC Oral Health.* 21, 660.

Otálora, M. C, Wilches-Torres, A. and Gómez Castaño, J. A. (2022). Spray-drying microencapsulation of pink guava *(Psidium guajava*) carotenoids using mucilage from opuntia ficus-indica cladodes and Aloe vera leaves as encapsulating materials. *Polymers.* 13;14(2): 310.

Pagano, S., Lombardo, G., Orso, M., Abraha, I., Capobianco, B. and Cianetti, S. (2020). Lasers to prevent dental caries: A systematic review. *BMJ Open.* 10, e038638.

Pang, L., Wang, Y., Ye, Y., Zhou, Y., Zhi, Q. and Lin, H. (2021). Metagenomic Analysis of Dental Plaque on Pit and Fissure Sites With and Without Caries Among Adolescents. *Frontiers in Cellular and Infection Microbiology.* 11, 740981.

Parker, E., Ogechukwu, C., Emmanuel, S., Emmanuel, C., Samson, A. O., Tochukwu, N. O. and Okwesilieze, F. C. N. (2022). Antidiabetic and Hepatoprotective Effects of *Psidium guajava* L Fruit Puree on Alloxaninduced Diabetes in Wistar Rats: Tropical. *Journal of Natural Product Research*. 6(5):795-800.

Pasquoto-Stigliani, T., Campos, E. V. R., Oliveira, J. L., Silva, C. M. G., Bilesky-José, N., Guilger, M., Troost, J., Oliveira, H. C., Stolf-Moreira, R., Fraceto, L. F. and de Lima, R. (2017). Nanocapsules Containing Neem (*Azadirachta Indica*) Oil: Development, Characterization, and Toxicity Evaluation. *Scientific reports,* 7(1), 5929.

Pellerin, G., Bazinet, L. and Grenier, D. (2021). Deacidification of Cranberry Juice Reduces Its Antibacterial Properties against Oral *Streptococci* but Preserves Barrier Function and Attenuates the Inflammatory Response of Oral Epithelial Cells. *Foods.* 10, 1634.

Pitts, N.B., Twetman, S., Fisher, J. and Marsh, P. D. (2021). Understanding dental caries as a non-communicable disease. *British Dental Journal.* 231, 749–753.

Saleem, S.,Muhammad, G., Hussain, M. A. and ukhari, S. N. A. (2018). A Comprehensive Review of Phytochemical Profile, Bioactives for Pharmaceuticals, and Pharmacological Attributes of *Azadirachta indica*. *Phytotherapy Research.* 32 (7), 1241–1272.

Sasi, M., Kumar, S., Kumar, M., Thapa, S., Prajapati, U., Tak, Y., Changan, S., Saurabh, V., Kumari, S., and Kumar, A. (2021). Garlic (*Allium sativum* L.) Bioactives and Its Role in Alleviating Oral Pathologies. *Antioxidants.* 10, 1847.

Sedigh-Rahimabadi, M., Fani, M., Rostami-chijan, M., Zarshenas, M. M. and Shams, M. (2017). A Traditional Mouthwash (*Punica granatum var pleniflora*) for Controlling Gingivitis of Diabetic Patients: A Double-Blind Randomized Controlled Clinical Trial. J. Evid.-Based Complement. *Alternative Medicine.* 22, 59–67.

Shaalan, O. and El-Rashidy, A. (2023). Antibacterial Effect of Miswak Herbal Toothpaste Compared to Fluoride Toothpaste in High Caries Risk Patients: Randomized Clinical Trial. *Journal of Clinical and Experimental Dentistry: JCED.* 15, e526–e534.

Shetty, S., Shetty, R. M., Rahman, B., Vannala, V., Desai, V. and Shetty, S. R. (2020). Efficacy of *Psidium guajava* and *Allium sativum* Extracts as Antimicrobial Agents against Periodontal Pathogens. *Journal of Pharmacy and Bioallied Sciences.* 12, S589–S594.

Souza, J. A. S., Barbosa, D. B., Berretta, A. A., Do Amaral, J. G., Gorup, L. F., De Souza Neto, F. N., Fernandes, R. A., Fernandes, G. L., Camargo, E. R. and Agostinho, A. M. (2018). Green Synthesis of Silver Nanoparticles Combined to Calcium Glycerophosphate: Antimicrobial and Antibiofilm Activities. *Future Microbiology.* 13, 345–357.

Suleiman, W. B. (2020). InVitro Estimation of Superfluid Critical Extracts of Some Plants for Their Antimicrobial Potential, Phytochemistry, and GC-MSAnalyses. *Annals of Clinical Microbiology and Antimicrobials.* 19, 29.

Takenaka, S., Ohsumi, T. and Noiri, Y. (2019). Evidence-Based Strategy for Dental Biofilms: Current Evidence of Mouthwashes on Dental Biofilm and Gingivitis. *Japanese Dental Science Review.* 55, 33–40.

Tibebu A., Haile G. and Kebede A. (2017). Review on medicinal value and other application of neem tree: Senior seminar on animal health. *Arc Journal of Immunology and Vaccines.* 2(2): 16-24.

Toma, M. and Luchian, V. (2019). Morphological and anatomical study of *Psidium guajava* Linn. *(guava)-a new fruit tree and medicinal plant researched in Romania.* 13(2) ;2285-5653.

Uzzaman S. (2020). Pharmacological activities of Neem (*Azadirachta indica*): A review. *International Journal of Pharmacognosy and Life Science*. 1(1): 38-41.

Valadas, L. A. R., Gurgel, M. F., Mororó, J. M., da Cruz Fonseca, S. G., Fonteles, C. S. R., de Carvalho, C. B. M., Fechine, F. V., Rodrigues Neto, E. M., de França Fonteles, M. M. and Chagas, F. O. (2019). Dose-Response Evaluation of a Copaiba-Containing Varnish against *Streptococcus mutans* In Vivo. *Saudi Pharmaceutical Journal.* 27, 363–367.

Valenti, C., Pagano, S., Bozza, S., Ciurnella, E., Lomurno, G., Capobianco, B., Coniglio, M., Cianetti, S. and Marinucci, L. (2021). Use of the Er:YAG Laser in Conservative Dentistry: Evaluation of the Microbial Population in Carious Lesions. *Materials.* 14, 2387.

Wolfoviz-Zilberman, A., Kraitman, R., Hazan, R., Friedman, M., Houri-Haddad, Y. and Beyth, N. (2021). Phage Targeting *Streptococcus* mutans In Vitro and In Vivo as a Caries-Preventive Modality. *Antibiotics.* 10, 1015.

Xu, H., Tian, J., Hao, W., Zhang, Q., Zhou, Q., Shi, W., Qin, M., He, X. and Chen, F. (2018). Oral Microbiome Shifts from Caries-Free to Caries-Affected Status in 3-Year-Old Chinese Children: A Longitudinal Study. *Frontiers in Microbiology*. 9, 2009.

Xu, L., Wang, Y. Y., Huang, J., Chen, C. Y., Wang, Z. X. and Xie, H. (2020). Silver Nanoparticles: Synthesis, Medical Applications and Biosafety. *Theranostics.* 10, 8996–9031.

Yadav, M., Kaushik, M., Roshni, R., Reddy, P., Mehra, N., Jain, V. and Rana, R. (2017). Effect of Green Coffee Bean Extract on Streptococcus mutans Count: A Randomised Control Trial. *Journal of Clinical and Diagnostic Research*. 11, ZC68–ZC71.

Yang, S. Y. and Kang, M. K. (2020). Biocompatibility and Antimicrobial Activity of *Reynoutria elliptica* Extract for Dental Application. *Plants.* 9, 670.

Yang, S. Y., Choi, Y. R., Lee, M. J. and Kang, M. K. (2020). Antimicrobial Effects against Oral Pathogens and Cytotoxicity of *Glycyrrhiza uralensis* Extract. Plants. 9, 838.

Yazdanian, M., Rostamzadeh, P., Rahbar, M., Alam, M., Abbasi, K., Tahmasebi, E., Tebyaniyan, H., Ranjbar, R., Seifalian, A. and Yazdanian, A. (2022). The Potential Application of Green-Synthesized Metal Nanoparticles in Dentistry: A Comprehensive Review. *Bioinorganic Chemistry and Applications.* 2022, 2311910. .

Zhang, Y., Chen, Y., Chen, C., Zhu, Y., Liu, M. and Chen, J. (2024). The enhancement mechanisms of mucin and lactoferrin on α-amylase activity in saliva: Exploring the interactions using QCM-D and molecular docking. *International Journal of Biological Macromolecules.* 257, 128710.

Zhu, C., Yuan, C., Ao, S., Shi, X., Chen, F., Sun, X. and Zheng, S. (2018). The Predictive Potentiality of Salivary Microbiome for the Recurrence of Early Childhood Caries. *Frontiers in Cellular and Infection Microbiology*. 8, 423.

Zhu, Y., Wang, Y., Zhang, S., Li, J., Li, X., Ying, Y., Yuan, J., Chen, K., Deng, S. and Wang, Q. (2023). Association of Polymicrobial Interactions with Dental Caries Development and Prevention. *Frontiers in Microbiology*. 14, 1162380.