

**PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF
Guizotia scabra LEAF, STEM BARK AND ROOT EXTRACTS FOR DENTAL
CARIES CONTROL**

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DECLARATION

Declaration by the Candidate

This research project is my original work and has not been submitted for any academic award in any institution; and shall not be reproduced in part or full, or in any format without prior written permission from the author and/or University of Eldoret.

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Declaration by the Supervisors

This thesis has been submitted with our approval as University supervisors.

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DEDICATION

I dedicate this work to my dear husband Victor, and our children, Joy and Alpha, your support, patience, and encouragement kept me going. To my parents, who nurturing independent thought as well as my friends and colleagues for their unwavering moral support they gave me.

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ABSTRACT

Medicinal plants offer promising alternatives for treating oral infections. This study investigated the phytochemical composition and antibacterial activity of *Guizotia scabra* tender leaf, stem bark, and root extracts against *Streptococcus mutans* and *Streptococcus sobrinus*, key pathogens in dental caries. Ethanol and acetone extracts were subjected to Standard laboratory tests for phytochemicals analysis, High-performance liquid chromatography (HPLC) for quantification of flavanoids, Fourier-transform infrared spectroscopy (FTIR) for determination of functional groups and antibacterial bioassays to assess antibacterial activity. Phytochemical tests revealed the presence of anthraquinones, alkaloids, terpenoids, flavonoids, steroids, saponins, and quinones. HPLC analysis confirmed the presence of myricetin and quercetin, with leaf extracts showing the highest concentrations (myricetin: 7.0013 ppm; quercetin: 0.901 ppm). Stem bark lacked myricetin but contained quercetin (2.4991 ppm), while root extracts had moderate levels of both (myricetin (2.0627 ppm) and quercetin (0.7027 ppm). FTIR spectra identified key functional groups, including O-H/N-H (3441 cm^{-1})¹ linked to alkanoids (Quercetin) (N-H /O-H group), C=O (1643 cm^{-1}) which correlate to terpenoids, C-H/C-O (1396 cm^{-1}) linked to alkaloids structure having N /O-H bond, 2000 cm^{-1} to terpenoids /flavanoids and possible metal-ligand vibrations (538 cm^{-1}). There is also a distinct C-N stretch (1250 cm^{-1}) likely due to C-N stretching in alkaloids showed by leaf and root extracts. Antibacterial evaluation demonstrated that ethanol leaf extract had the highest inhibition against *Streptococcus mutans* (5.0 mm, 62.5%) and *Streptococcus sobrinus* (4.0 mm, 50%), followed by root extracts (3.0 mm, (37.5%) and 2.5 mm, (31.25%) respectively. Acetone extracts showed similar trends with the leaf extract at 4.6 mm, (57.5%) and 4.3 mm, (53.75%). However, commercial toothpastes showed superior inhibition (up to 7.0 mm, 87.5%) likely due to fluoride content and other additives. These findings suggest that *Guizotia scabra* possesses bioactive compounds with potential for natural oral healthcare applications. Further studies should optimize extraction; evaluate compound synergy, ensuring safety and efficacy for possible formulation of dental products.

Keywords: *Guizotia scabra*, phytochemicals, antibacterial activity, dental infections, *Streptococcus mutans* & *Streptococcus sobrinus* bacteria, natural oral care.

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LIST OF ACRONYMS

CC	Column Chromatography
DNA	Deoxyribonucleic Acid
FTIR	Fourier Transform Infrared Spectroscopy
HPLC-UV	High Performance Liquid Chromatography-Ultraviolet
HIV/AIDS	Human Immunodeficiency Virus / Acquired Immunodeficiency Syndrome
IJARCS	International Journal of Advanced Research in Chemical Science
IR	Infrared
JPP	Journal of Pharmacognosy and Phytochemistry
JSTOR	Journal Storage (digital library)
KEMRI	Kenya Medical Research Institute
KEPHIS	Kenya Plant Health Inspectorate Service
MAE	Microwave-Assisted Extraction
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
NIST	National Institute of Standards and Technology
QE	Quercetin Equivalent
RNA	Ribonucleic Acid
Rf	Retention Factor
SFE	Supercritical Fluid Extraction
TCM	Traditional Chinese Medicine
TCS	Triclosan

TLC	Thin Layer Chromatography
UAE	Ultrasound-Assisted Extraction
WJPR	World Journal of Pharmaceutical Research
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Medicinal plants continue to play a vital role in global healthcare, offering accessible and cost-effective remedies for a wide range of health conditions, particularly in regions with limited access to conventional medical services (Ahmad & Karmakar, 2023). In many rural and underserved communities, plant-based treatments are often the primary or only form of healthcare available (Abdel-Aziz *et al.*, (2018). The World Health Organization (WHO) recognizes traditional medicine, including herbal remedies, as a key component of primary healthcare systems worldwide (WHO, 2023). Recent studies affirm that approximately 80% of the global population, including individuals in both developing and industrialized nations depend on traditional medicine for their health needs (Alqethami *et al.*, 2022; Kedir *et al.*, 2021). Moreover, there is growing interest in harnessing the therapeutic potential of medicinal plants for modern drug development, as they represent a rich source of bioactive compounds with significant pharmacological potential (Aremu *et al.*, 2019; Tadesse *et al.*, 2020).

In countries like India, there are over 20,000 plant species known for their medicinal benefits, with 15–20% of these plants playing a key role in traditional medicine globally (Gupta *et al.*, 2012). Similarly, Africa's diverse plant species have long been central to the healthcare practices of its people. In South Africa, medicinal plants are in high demand due to the high prevalence of diseases such as HIV/AIDS, compounded by socio-economic challenges like poverty and unemployment. However, unsustainable harvesting practices

have put many plant species at risk of extinction (Abdel-aziz *et al.*, 2018; Grace *et al.*, 2002). The use of medicinal plants extends across Africa, including in West and East Africa, where traditional healers rely on these plants for treating diseases such as malaria and gastrointestinal disorders (Attah *et al.*, 2021). *Artemisia annua*, native to China but found in parts of Africa, is an example of a plant with promising potential for malaria treatment (Jansen *et al.*, 2015).

In North America, the popularity of herbal medicine continues to rise, with commonly used plants such as *Echinacea*, *Panax ginseng*, and *Hypericum perforatum* (St. John's Wort) frequently employed for managing colds, fatigue, and mild depression (Zhou *et al.*, 2019; Kennedy *et al.*, 2020). This growing interest in natural therapies has fuelled the expansion of the natural health product industry, prompting increased scientific exploration into the role of medicinal plants in preventing chronic diseases such as cancer, cardiovascular conditions, and diabetes (Luo *et al.*, 2020; Salehi *et al.*, 2018). In Asia, traditional medical systems like Traditional Chinese Medicine (TCM), Ayurveda, and Kampo have integrated herbal remedies for centuries and continue to influence contemporary pharmacological research and drug development (Fan *et al.*, 2020; Ekor, 2021). In Southeast Asia, countries rich in biodiversity such as Malaysia, Indonesia, and Thailand remain at the forefront of ethnopharmacological research, using indigenous plants for both curative and preventive healthcare, including the promotion of general wellness and longevity (Nasri *et al.*, 2022; Ahmad *et al.*, 2019).

In Japan and South Korea, traditional practices like Kampo remain deeply embedded in the culture, and plants such as Ginseng (*Panax ginseng*) have been extensively studied for their

potential to enhance immune function and combat stress (Zhou *et al.*, 2019). Ginseng is used for treating conditions like chronic fatigue syndrome in both Asia and Western countries (Reay *et al.*, 2005). In Latin America, indigenous knowledge of medicinal plants is invaluable. Herbs such as Lapacho (*Tabebuia impetiginosa*) in the Andes have been used for their anti-inflammatory and anti-cancer properties (Basso *et al.*, 2007). In Brazil, the Amazon rainforest's diverse flora offers vast potential for discovering new medicinal compounds, many of which are currently being studied for their role in modern pharmaceuticals (Pereira *et al.*, 2017).

The global importance of medicinal plants extends beyond their cultural significance to their biological activity. Many plants contain bioactive compounds like alkaloids, flavonoids, terpenoids, and steroids, which have been shown to possess antimicrobial, antibacterial, anticancer, anti-inflammatory, and antioxidant properties (Biradar & Rachetti, 2013; Yadav & Agarwala, 2011). These properties make them viable alternatives to synthetic drugs, particularly for treating chronic diseases. For instance, in Africa, herbal remedies are often used to manage pain, infections, and skin diseases (Medi & Jasprica, 2007; Megersa *et al.*, 2019a).

As dental issues like tooth pain, dental caries, and gum disease increase worldwide, medicinal plants have found a growing role in oral care. The World Health Organization's 2012 Oral Health Fact Sheet highlighted the global burden of oral diseases, which impact millions, leading to pain, dysfunction, and social stigma (Liao *et al.*, 2013; Petersen, 2003). Plants such as *Aloe vera*, neem, and clove are frequently used for their analgesic, antibacterial and antimicrobial properties. In India, neem is used as a natural toothbrush,

while *Aloe vera* is commonly applied to treat gum disease and mouth ulcers (Nor Amiyah *et al.*, 2016). In the United States, peppermint and echinacea are incorporated into oral care products, reflecting a shift toward plant-based alternatives in dental hygiene (Song *et al.*, 2018). Herbal plants have long served as primary sources of traditional medicine, especially in rural communities.

Preliminary general studies indicate that *Guizotia scabra* extracts exhibit notable antibacterial effects against Gram-positive bacteria, particularly *Streptococcus* species (Bekele *et al.*, 2022). Bioassay experiments using disc diffusion and minimum inhibitory concentration (MIC) tests have demonstrated significant antibacterial activity, which is likely due to the presence of phenolic and flavonoid compounds (Teshome *et al.*, 2023).

The primary microorganisms responsible for dental caries are mutans streptococci, particularly *Streptococcus mutans*, *Streptococcus sobrinus*, and lactobacilli (Dhillon *et al.*, 2015). These pathogens form the foundation for many dental related issues. In response to the increasing prevalence of dental caries, herbal remedies have been proposed as effective treatments due to their demonstrated antibacterial, antifungal, and antiviral properties (Parsaei *et al.*, 2013).

This study aimed to extract and determine the bioactive compounds from *Guizotia scabra* tender leaf, stem bark and root extracts, as a potential for treating dental infections, and promoting oral health. The findings will contribute to the on-going global search for effective, locally sourced remedies, thus providing a natural alternative to synthetic pharmaceuticals in dental care (Fyhrquist *et al.*, 2002).

1.2 Statement of the Problem

Access to affordable and effective dental care remains a significant public health challenge, particularly in low resource settings where government allocation to oral health services is often inadequate. As a result, many individuals are unable to afford or access conventional dental treatments, leading to increased prevalence of oral health issues such as tooth decay and gum disease (Bapilus & Mb, 2018). In such contexts, the reliance on natural remedies for managing oral infections has become both a cultural norm and a necessity (Shah & Shelar, 2018). Although the global oral health industry has increasingly integrated herbal ingredients into commercial products, there is still limited scientific validation for many locally used plants. One such plant is *Guizotia scabra*, commonly found on farms, which has been traditionally used in some communities for various medicinal purposes (Paul *et al.*, 2025).

1.3 Objectives

1.3.1 General Objective

To determine phytochemical composition and antibacterial potential of *Guizotia scabra* plant parts extracts against dental caries bacteria.

1.3.2 Specific objectives

- i. To determine and compare the extracted percentage yields of *Guizotia scabra* (tender leaf, stem bark, and root) ethanol and acetone extracts .

- ii. To determine the phytochemical constituents of *Guizotia scabra* (tender leaf, stem bark and root) ethanol and acetone extracts using standard laboratory test procedures.
- iii. To assess phytochemical constituents of *Guizotia scabra* ethanol and acetone extracts bioactive groups using TLC, HPL-UV and FTIR.
- iv. To evaluate antibacterial activity of *Guizotia scabra* extracts against *Streptococcus mutans* and *Sobrinus* bacterial strain and compare antibacterial activity of the extracts with that of commercial toothpaste products against *Streptococcus mutans* and *Streptococcus sobrinus*.

1.3.3 Research Questions

- i. How does the extracted percentage yields of *Guizotia scabra* (tender leaf, stem bark, and root) ethanol and acetone extracts compare?.
- ii. What phytochemicals are present in the tender leaf, stem bark and root ethanol and acetone extracts of *Guizotia scabra* as determined by standard laboratory test procedures?
- iii. What are the characteristic features of phytochemicals present in *Guizotia scabra* extracts as analyzed by TLC, HPLC and FTIR?
- iv. Does the *Guizotia scabra* extracts exhibit antibacterial activity and how does the antibacterial activity of *Guizotia scabra* extracts compare with that of commercial toothpaste products against *Streptococcus mutans* and *Streptococcus sobrinus*?

1.4 Significance of the Study

This study offers valuable insights into the potential of *Guizotia scabra* as a natural alternative for oral healthcare, particularly in underserved regions where access to commercial dental products is limited. By establishing the phytochemical composition and antibacterial activity of its tender leaf, stem bark, and root extracts, the research contributes to scientific knowledge on underutilized medicinal plants and supports the development of affordable, safe and accessible plant-based dental treatments. The demonstrated antibacterial effects against *Streptococcus mutans* and *Streptococcus sobrinus* highlight its potential for incorporation in locally available natural antimicrobial agents into natural oral herbal formulations care products like toothpaste and mouthwashes for dental caries prevention.

1.5 Justification of the Study

Dental caries continues to be a major global public health problem, with particularly high prevalence in developing countries due to limited access to modern dental care and lifestyles patterns. Synthetic antimicrobial agents, though effective, are associated with adverse effects such as tooth staining, taste alteration, and microbial resistance

The growing global interest in natural healthcare solutions highlights the need to explore alternative remedies which are cheaper and available. *Guizotia scabra*, though commonly found in agricultural regions, remains under-researched in terms of its medicinal potential, particularly for oral health. Given the growing interest in phytomedicine as a safer and more cost-effective alternative to synthetic drugs (Janakiram *et al.*, 2020; Banu & Cathrine, 2015), there is a need to scientifically evaluate the bioactive compounds of *Guizotia*

scabra, particularly in its leaves, stem bark, and roots as well as determining their antibacterial properties against oral pathogens. Without such knowledge, valuable natural medicinal resources may remain underutilized, and populations lacking access to formal healthcare will continue to face preventable oral health issues. This study, therefore, sought to fill this gap by identifying the key phytochemicals present in *Guizotia scabra* with potential antibacterial activity, which can be used by the populace as an affordable and natural treatment for bacterial infections affecting oral health.

The study addresses that gap by evaluating the plant's bioactive compounds and assessing their potential to combat oral infections like dental caries, conditions commonly linked to *Streptococcus mutans* and *Streptococcus sobrinus*. Hence contributing to the advancement of natural and sustainable healthcare practices.

1.6 Scope of the Study

This study investigated the phytochemical composition and antibacterial activity of *Guizotia scabra* extracts collected from farms in Uasin Gishu County. Only ethanolic and acetone extracts of the leaf, stem bark, and root were analyzed under controlled laboratory conditions. Phytochemical screening was carried out using TLC, HPLC-UV, and FTIR spectroscopy to identify key bioactive compounds such as flavonoids, alkaloids, saponins, and terpenoids. The antibacterial potential of the extracts was evaluated in vitro using agar disc diffusion bioassays against *Streptococcus mutans* and *Streptococcus sobrinus*, bacteria associated with dental caries and gum disease. The study was limited to laboratory-based, in vitro testing and did not extend to in vivo experiments or clinical trials

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Dental caries continues to be one of the most widespread and persistent infections globally, often resulting in pain, tooth loss, and a range of complications when left untreated (Parsaei *et al.*, 2013). The primary microorganisms responsible for dental caries are particularly *Streptococcus mutans*, *Streptococcus sobrinus*, and *lactobacilli* ssp. These pathogens form the foundation for many dental related infections. In response to the increasing prevalence of dental caries, herbal remedies have been proposed as effective treatments due to their demonstrated antibacterial, antifungal, and antiviral properties (Parsaei *et al.*, 2013). As the global shift toward natural alternatives continues, many dental care products, such as toothpaste, mouthwashes, sprays, creams, and gels, are now incorporating plant based ingredients that possess anti-inflammatory and antibacterial properties, aligning with the growing interest in natural remedies (Dhillon *et al.*, 2015).

In parallel, synthetic dental care products have raised significant concerns, particularly with ingredients such as triclosan (TCS), which is widely used in toothpaste and other personal care products. Triclosan, a chlorophenol compound, is known for its antibacterial, antifungal, and antiviral effects (Levy, 2001). However, evidence suggests that Triclosan may pose potential risks to human health, including cancer and skin irritations, due to its ability to interfere with sensory nerve endings (Dhillon *et al.*, 2015). . This can result in desensitization to pain and adverse skin reactions, including facial spots (Dhillon *et al.*, 2015). Furthermore, triclosan contributes to environmental pollution; approximately 95%

of triclosan from consumer products is washed into wastewater treatment plants, which are unable to remove it. As a result, triclosan has become one of the most frequently detected contaminants in aquatic ecosystems, particularly in countries like the United States and Switzerland (Snyder *et al.*, 2019; Kunz *et al.*, 2020). These environmental concerns underscore the need for more available, accessible and safer alternatives in oral health care.

Beyond triclosan, fluoride based products have also attracted controversy, especially regarding their use in children under the age of six. Ingredients such as sodium fluoride (NaF), Ammonium fluoride (NH₄F), Stannous fluoride (SnF), and Sodium monofluorophosphate (Na₂PO₃F) (Stachurski *et al.*, 2023) are known to help reduce dental caries, but excessive exposure during tooth development can lead to dental fluorosis. This condition, caused by the over deposition of fluoride on developing enamel, leads to reduced mineralization (Denbesten & Li, 2011). Studies have shown that while fluoride concentrations ranging from 1000 to 1500 ppm are effective in preventing caries, higher concentrations can cause mottled enamel and other undesirable effects, such as changes in taste and tooth discoloration (Everett, 2011; Padi, 2019). These findings raise important questions about the safety of fluoride, particularly for young children, and highlight the need for continued exploration of alternative, less harmful dental care options.

Other chemical agents such as bleach and peroxide, commonly used in whitening toothpaste, can cause irritation to the oral mucosa and skin, and may even lead to severe chemical burns in large doses. Additionally, synthetic flavourings and fragrances used in oral care products are often derived from petrochemicals, further raising health and environmental concerns (Chowdhury *et al.*, 2015).

In the light of the issues discussed above, there is a growing inclination towards natural ingredients, such as plant extracts and special mineral salts, as promising alternatives. Plant-based ingredients like lemon, eucalyptus, rosemary, chamomile, and minerals like sodium chloride and sodium fluoride have gained attention for their antimicrobial and antiseptic properties, which can help address cariogenic microorganisms in the oral cavity in a safer and more sustainable manner (Hassid *et al.*, 2025). In particular, the *Asteraceae* family of plants has been recognized for its numerous contributions to traditional medicine and oral health care. Many species in this family are naturally available and have been used for centuries, although their potential remains largely underappreciated in modern contexts. Among these, *Guizotia scabra* stands out as a plant with remarkable therapeutic potential (Paul *et al.*, 2025). Research by Print *et al.* (2016) demonstrates that the phytochemicals present in the aerial parts of *Guizotia scabra* exhibit strong antioxidant activity, supporting its traditional use as a source of powerful antioxidants. This finding positions *Guizotia scabra* as a promising candidate for use in pharmaceuticals, cosmetics, and even food preservation.

Given the growing interest in natural alternatives, the current study aimed to determine the phytochemicals present in *Guizotia scabra* extracts and evaluate their antibacterial properties, particularly against the bacterial strains commonly responsible for dental caries, such as *Streptococcus mutans*. The findings from this study could contribute to the development of safer, more effective natural remedies for dental care, aligning with global efforts to reduce the reliance on synthetic chemicals in health care products (Hassid *et al.*, 2025). Future research should focus on isolating and characterizing the active compounds

responsible for their antimicrobial effects of phytochemicals to develop new, natural therapeutic agents.

2.2 Phytochemicals found in various herbal plants

Phytochemicals are bioactive compounds found in plants, which can be extracted from leaves, bark, seeds, seed coats, flowers, roots, and pulps. These compounds often serve as the origin of direct therapeutic agents (Banu & Cathrine, 2015). Phytochemistry identifies and characterizes several secondary metabolic compounds exhibited by herbal plants. These plants act as reservoirs of natural chemical compounds with structurally distinct bioactive molecules (Latha *et al.*, 2013). The extraction and analytical estimation of bioactive compounds from plants are crucial for modern biomedical research. These bioactive molecules have potential applications in drug manufacturing and agrochemicals, serving as lead compounds for the development of more effective therapeutic agents (Kaur *et al.*, 2017; Vizhi *et al.*, 2016).

Globally, there was significant research into herbal plants with pain-relieving properties, attributed to their unique chemical compositions (Smullen *et al.*, 2012; Bhardwaj & Bhardwaj, 2012; Mizan *et al.*, 2018; Philip *et al.*, 2018). Studies have reported valuable components in these plants, including tannins, flavonoids, and phenolic compounds, which exhibit remarkable physiological effects on the human body (Shai *et al.*, 2008; Sharafati-Chaleshtori *et al.*, 2011).

Despite advancements in conventional medicine, challenges such as weak health systems, dynamic lifestyles, high costs of oral hygiene products, and the emergence of drug resistant diseases hinder efforts to combat dental infections (Taheri *et al.*, 2011). Herbal plants

present a viable alternative as they are cost effective, locally available, and generally safe for strengthening oral health (Amaral *et al.*, 2013). These plants offer a combination of compounds that work synergistically against microbes, a feature that has been recognized in the pharmaceutical industry for their broad structural diversity and extensive curative potential (Liu *et al.*, 2019; Calixto, 2019).

Various plant extracts have shown promising antimicrobial properties against oral pathogens, making them viable alternatives to conventional antibiotics. For instance, the leaves of *Drosera peltata* (*Droseraceae*) have been used in local treatments for dental caries. A study by Palombo (2011) found that chloroform-based extracts from the plant's leaves exhibited broad spectrum antibacterial action, particularly against *Streptococcus mutans* and *Streptococcus sobrinus*. The active compound responsible for this activity was identified as plumbagin. Additionally, Philip *et al.* (2018) conducted a study on 27 medicinal herbal extracts and found several that significantly inhibited the growth of oral *Streptococci*.

Azadirachta indica (Neem) has also been extensively studied for its antimicrobial properties. Neem sticks reduce the adherence of *Streptococcus* species, making them effective in oral disease management (Prashant *et al.*, 2007). The aqueous extract of Neem has demonstrated significant antibacterial activity against *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis*, and *Streptococcus anginosus* (Chandana *et al.*, 2017). Furthermore, Lekshmi *et al.*, (2012) highlighted Neem leaves as a promising candidate for future dental hygiene solutions.

Another widely used herbal remedy is *Salvadora persica* L. (Miswak), an evergreen shrub from the Salvadoraceae family. In many parts of the world, especially in developing countries, Miswak sticks are traditionally used as natural toothbrushes (Ahmad & Rajagopal, 2014b). Studies have shown that regions where Miswak is used experience lower incidences of tooth loss in adults, likely due to its antibacterial compounds that help control dental caries and gingivitis (Khalessi *et al.*, 2004; Szyszkowska *et al.*, 2010).

The Miswak plant contains flavonoids, saponins, alkaloids, volatile oils, steroids, terpenoids, and carbohydrates, all of which contribute to its antimicrobial activity (Abdillahi *et al.*, 2010). The World Health Organization (WHO) supports the use of Miswak as a strong agent for dental care due to its beneficial compounds, including vitamin C, fluoride, chloride, and salvadorine (Kemoli *et al.*, 2001).

Several other medicinal plants have demonstrated significant antibacterial activity against *Streptococcus* species. *Anthemis nobilis* L. (Roman Chamomile), native to Europe, Australia, and North America, contains flavonoids, coumarins, and essential oils, which provide anti-inflammatory and antibacterial properties (Woldeab *et al.*, 2018). Similarly, *Euphrasia* L. (Eyebright), a plant genus with over 450 species, contains aucubin, a compound that effectively inhibits bacterial growth and prevents histamine release (Parsaei *et al.*, 2013; Szyszkowska *et al.*, 2010).

Arnica montana, a perennial herb found in mountainous regions of Europe, has been identified for its antiseptic, anti-inflammatory, and antibacterial properties. Its essential oil, flavonoids, and lactones contribute to its effectiveness against oral pathogens (Woldeab *et al.*, 2018; Medi & Jasprica, 2007). Another plant, *Mentha piperita* (Peppermint), is a hybrid

of watermint and spearmint and is widely recognized for its high menthol content. Peppermint oil provides a cooling sensation and exhibits antimicrobial activity against oral bacteria (Rita & Animesh, 2011; Szyszkowska *et al.*, 2010).

Sage (Salvia officinalis), native to the Mediterranean region, has long been used for its antibacterial and antioxidant properties. The plant contains essential oils, flavonoids, tannins, and carnosol, which are beneficial in oral health. Sage decoctions are commonly used as mouth rinses for treating oral inflammation and infections (Yousefzadi *et al.*, 2007; Sciences, 2016). Likewise, *Thymus vulgaris* (Thyme) is another Mediterranean herb with strong antibacterial properties due to its essential oil content, particularly thymol and phenolic acids (Parsaei *et al.*, 2013).

Oak (*Quercus lobata*) bark, rich in tannins, exhibits strong antibacterial effects by inhibiting bacterial growth and neutralizing toxins. Oak bark infusions have been used for treating oral and throat infections, moderate bleeding, and skin irritations (Sciences, 2016). Similarly, *Uncaria tomentosa* (Vilcacora), a vine native to South and Central America, has shown antibacterial activity against *Streptococcus mutans*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Citrobacter freundii* (Sciences, 2016).

Several other plants have been explored for their antimicrobial and antibacterial properties. *Plantago major* (Plantain) and *Plantago lanceolata* (Ribwort Plantain) have long been used in herbal medicine due to their tannins, flavonoids, and mucilage content, which exhibit antibacterial and anti-inflammatory effects (Sciences, 2016). *Calendula officinalis* (Marigold) contains flavonoids, triterpenes, and essential oils, which contribute to its pain-relieving and wound-healing properties (Okoh *et al.*, 2008; Sciences, 2016).

Garlic (*Allium sativum*) is well known for its strong antibacterial properties. Holding crushed garlic in the mouth for a few minutes has been shown to disinfect the oral cavity, with freshly prepared garlic juice capable of killing *Streptococcus pyogenes* and *Corynebacterium diphtheriae* within minutes (Ríos & Recio, 2005).

Aloe vera (*Aloe arborescens* Mill.) and red betel (*Piper betle*) extracts have also demonstrated potent antimicrobial and immune-modulating properties. The presence of essential vitamins, minerals, flavonoids, tannins, and alkaloids contributes to their antibacterial effectiveness (Alfarabi *et al.*, 2010; Azadmehr *et al.*, 2011; Kaur *et al.*, 2017). Additionally, *Eucalyptus saligna* and *Eucalyptus globulus* mouth rinses have been traditionally used to treat toothaches, sore throats, and bad breath due to their disinfectant properties against *Escherichia coli* and *Staphylococcus aureus* (Sciences, 2016).

Moringa oleifera roots have also demonstrated antibacterial activity, particularly against *Staphylococcus aureus*, *Vibrio cholerae*, and *Escherichia coli*. In Cameroon, Moringa root is commonly applied directly to the affected area to alleviate toothache., *Cocos nucifera* (Coconut) root extract has been used as a mouthwash to treat dental pain and hypersensitivity, with its lauric acid content showing significant antibacterial effects against *Streptococcus mutans* (Anzaku, 2017).

Similarly *Datura stramonium*, a common weed, has exhibited antibacterial activity against Gram-positive bacteria. Its methanol extract has demonstrated effectiveness against *Staphylococcus aureus*, while its ethanol extract has shown strong inhibitory effects on bacterial growth (Gaire & Subedi, 2013). These findings suggest that medicinal plants hold

great potential as alternative treatments for oral infections, particularly those caused by antibiotic-resistant *Streptococcus* species.

In Kenya, the diverse array of over 700 medicinal plant species plays an essential role in local healthcare, treating conditions like fever, wounds, respiratory disorders, and gastrointestinal problems (Wekesa *et al.*, 2019). The Maasai people, for example, use *Acacia* species for wound healing, while the Kikuyu people use plants like *Bidens pilosa* and ginger (*Zingiber officinale*) for digestive issues (Mbavhalelo *et al.*, 2017). One of the notable medicinal plants in Kenya is *Moringa oleifera*, known for its antimicrobial, anti-inflammatory, and antioxidant properties. It is commonly used to manage conditions like diabetes, hypertension, and skin disorders (Obanda *et al.*, 2013).

Kenya's use of medicinal plants extends into modern medical research. The Kenya Medical Research Institute (KEMRI) has conducted studies on the pharmacological properties of local plants, including *Aloe vera*, which has shown significant antimicrobial as well as antibacterial effects (Mugo *et al.*, 2014). *Siphonochilus aethiopicus*, native to Kenya, has been studied for its potential in treating respiratory conditions like asthma (Muchiri *et al.*, 2016). The commercialization of medicinal plants in Kenya has led to a growing herbal medicine industry, with the Kenya Plant Health Inspectorate Service (KEPHIS) working to ensure quality control and standardization of these products for both local and international markets (Oduor *et al.*, 2015). However, overexploitation due to unsustainable harvesting practices poses a threat to biodiversity and conservation efforts are underway to protect endangered plant species (Ouma *et al.*, 2020).

Incorporating medicinal plants into Kenya's public health strategy is also a priority. The government's Kenya National Traditional Medicine Policy, launched in 2008, recognized the role of traditional medicine and medicinal plants in improving health outcomes, particularly for diseases like malaria, HIV/AIDS, and tuberculosis (Kenya Government, 2008). This policy seeks to integrate herbal treatments into the national healthcare system.

The global demand for medicinal plants is increasing, driven by cultural preferences and scientific research. In this context, research into plants like *Guizotia scabra* is vital for understanding their therapeutic potential. Phytochemical determination, using techniques like TLC, HPLC-UV and FTIR, will help determine the bioactive compounds responsible for the medicinal effects of these plants in the goal to discover plant-based treatments that are not only effective but also sustainable and accessible, hence contributing to global healthcare advancements (Shah & Shelar, 2018; Koparde, 2017).

Medicinal plants exhibit a wide range of beneficial properties, such as cell growth suppression, antioxidant, anticancer, antiseptic, antifungal, antibacterial, antiviral, and antimicrobial plaque inhibiting activity. They also prevent histamine release, display anti-haemolytic activity, and aid in managing microbial plaque in gingivitis and periodontitis, thereby boosting immunity (Maridass & De Britto, 2008).

This current study aims to explore the medicinal value of *Guizotia scabra* (locally considered a weed), which grows rapidly on farms throughout the year and its tender leaves have been traditionally used to alleviate toothache (Gupta *et al.*, 2023). The research will scientifically validate its potential as a natural remedy for dental care, and in future as a pain relief, as a potential ingredient in the formulation of natural toothpaste. By

investigating the phytochemical composition and antibacterial activity of *Guizotia scabra* tender leaf, stem bark and root extracts, this study seeks to contribute to the global search for affordable, safe, and effective treatments with broad-spectrum activity. Additionally, the study seeks to propose the possibility of domesticating this plant, transforming it from a perceived menace into a valuable economical resource for farmers and the pharmaceutical industry.

2.3 Overview of *Guizotia scabra*

2.3.1 Distribution of *Guizotia scabra*

Guizotia is a genus of African herbs belonging to the family Asteraceae, commonly known as sunflecks or as wild niger (Tesfaye *et al.*, 2020) (plate 2.1). The most well-known species, *Guizotia abyssinica*, is primarily cultivated for its oil rich seeds but can occasionally be found growing outside of cultivation in regions of Europe, North America, and Asia. The genus comprises of several species, including *Guizotia arborescens*, *Guizotia candussioi*, *Guizotia jacksonii*, *Guizotia scabra*, *Guizotia schimperi*, *Guizotia villosa*, *Guizotia villosula*, and *Guizotia zavattarii*. These species are primarily distributed across Africa and are adapted to various ecological conditions, contributing to the biodiversity of the Asteraceae family (Adane *et al.*, 2021).



Plate 2.1 (a) Tender *Guizotia scabra* plant

(b) Mature *Guizotia scabra* plant

Source: Author (2024)

2.3.2 Scientific classification, Botanical and Morphological description of *Guizotia scabra*

According to (Mulatu Geleta, 2007a); (Mulatu Geleta *et al.*, 2010) and (Murthy *et al.*, 1995), *Guizotia* is an African genus that has about 6 or 7 known species which are found growing in eastern African mountains, it is a humble Afro-montane predominant genus belonging to the Asteraceae family.

Kingdom: Plantae
 Clade: Tracheophytes
 Clade: Angiosperms
 Clade: Eudicots
 Clade: Asterids
 Order: Asterales
 Family: Asteraceae or Compositae
 Subfamily: Asteroideae

Tribe: Millerieae
Subtribe: Milleriinae
Genus: Guizotia
Cass. 1829

Botanical name: *Guizotia scabra*.

English name: Sun fleck.

Local names: Luo: Nyamnina, Onina

Sambaa: Mpuishi.

Nandi: Chepisali, Arap Misoi,

2.3.3 Ecology of *Guizotia scabra*

Guizotia scabra is a hardy, fast growing perennial herb that can reach heights of 1 to 2 meters, and in some cases, up to 4 meters. It thrives in moist environments, particularly swampy areas, stream banks, and regions with high rainfall (Ssenku *et al.*, 2023). This adaptability has allowed it to spread across various African countries, including Ethiopia, Kenya, Tanzania, Uganda, Sudan, Nigeria, and Mozambique, among others (Tesfaye *et al.*, 2020).

In Ethiopia, the plant is one of the most widespread broad leaved weeds, commonly found at elevations between 1,400 and 2,800 meters. It is often seen growing along roadsides, in open fields, and in agricultural lands, making use of disturbed soils to establish itself (Ssenku *et al.*, 2023). Its ability to colonize different habitats, resist herbicides suggests that it is quite resilient to environmental changes and human activities.

While *Guizotia scabra* can sometimes be viewed as an invasive weed in farmlands, competing with crops for nutrients and space, it also offers ecological benefits. The plant

helps stabilize soil, preventing erosion in sloped areas (JSTOR Plants.). Additionally, it serves as a valuable nectar source for pollinators, attracting bees, butterflies, and other beneficial insects (Zambia Flora).

Its reproductive strategy makes it especially persistent; it spreads through both seeds and vegetative growth, allowing it to rapidly colonize new areas (Zambia Flora). However, like many wild plants, it faces threats from deforestation, habitat loss, and land conversion for agriculture. Despite this, *Guizotia scabra* continues to play an important role in its native ecosystems, contributing to soil health and supporting local biodiversity (JSTOR Plants; Bekele *et al.*, (2022).

2.3.4 Uses of *Guizotia scabra*

Guizotia scabra is a plant deeply embedded in the traditions of many African communities, valued for its culinary, medicinal, and cultural significance. The plant is mostly undomesticated and is collected from its habitat for native value as a food, medicine, and for making strands and threads. In Nigeria, its seeds are roasted and ground into a flavourful spice, often added to meat dishes and vegetable based meals to enhance their taste (Tesfaye *et al.*, 2020). The leaves are sometimes consumed as vegetables, and in traditional remedies, crushed leaves are applied to relieve toothaches. Different ethnic groups have their own names and unique uses for the plant. The Berom people call it "*Diaraliu*" and use it in ceremonial foods, while the Ron people, who refer to it as "*Fwalal*," mix it with local beans for festive meals (Wondimu & Mekonnen, 2022).

Beyond its role in cooking, *Guizotia scabra* has been widely used in traditional medicine. In Rwanda, it is believed to help with intestinal worms, digestive issues, and certain skin

conditions (INES Rwanda, n.d.). In Uganda, some local healers use a preparation made from burnt leaves mixed with other plant roots for medicinal purposes, including its use in traditional reproductive health treatments (Ssenku *et al.*, 2023).

Studies show that the essential oils extracted from *Guizotia scabra* possess antibacterial properties, making it effective against bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa*, and *Salmonella typhi* (Thieme Connect, n.d.). The plant has also been traditionally used to treat ailments like syphilis, stomach-aches, and gonorrhoea (JSTOR Plants, n.d.).

The wide ranging uses of *Guizotia scabra* reflect its importance in food, medicine, and cultural practices across Africa. Whether enhancing meals, providing natural remedies, or playing a role in local traditions, this plant continues to be a valuable part of many communities.

In African ethnomedicine, *Guizotia scabra* has been utilized for various medicinal applications, including the treatment of gastrointestinal disorders, wound healing, and respiratory infections (Gebrehiwot *et al.*, 2019). Its use in oral health, particularly for managing toothaches and bacterial infections, has been documented in Ethiopian traditional medicine (Wondimu & Mekonnen, 2022). The plant's potential antibacterial properties make it a candidate for further investigation in dental care applications.

2.4 Phytochemical Composition of herbal *plants in general*

2.4.1 Classes of Phytochemicals

Research on herbal plants has uncovered a range of bioactive compounds, including flavonoids, tannins, alkaloids, saponins, and terpenoids, Anthroquinones, steroids all known for their antimicrobial, antibacterial, anti-inflammatory, and antioxidant effects (Fekadu *et al.*, 2021). These natural compounds play a crucial role in the plant's potential medicinal benefits, particularly in oral health care.

Studies by Ogboji *et al.* (2018) and George *et al.* (2009) emphasize the significance of these bioactive compounds in herbal toothpaste formulations, which have been effective in reducing plaque and gingivitis. For example, naringin, a flavonoid found in citrus fruits, has demonstrated the ability to inhibit periodontal pathogens and other harmful oral microorganisms. Similarly, ethanol extracts of *Helichrysum italicum*, a plant from the Asteraceae family, has shown antibacterial activity against *Streptococcus mutans*, *Streptococcus sanguis*, and *Streptococcus sobrinus* (Palombo, 2011). Ríos and Recio (2005) further explain that these bioactive compounds go through a detailed process of identification, isolation, purification, and analysis to assess their therapeutic potential.

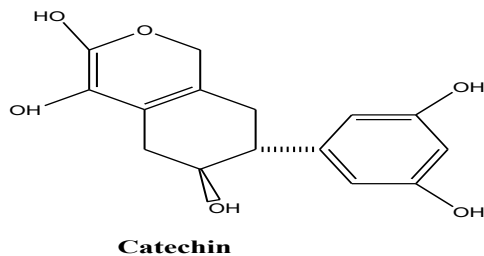
Various herbal phytochemicals, such as flavonoids, coumarins, iridoid glycosides, phenolic acids, resins, triterpenes, choline, carotenoids, tannins, vitamins, mineral salts, and essential oils, have been identified (Latha *et al.*, 2013). Among these, flavonoids and essential oils are particularly valued for their strong biological activities (Noori, 2012).

2.4.2 Flavonoids

Flavonoids are a diverse group of polyphenolic compounds widely found in plants, contributing to the vibrant pigmentation of many fruits, flowers, and vegetables. Their health benefits have been extensively researched, particularly in oral medicine. Among the most commonly occurring flavonoids are quercetin, kaempferol, and quercetrin, which are present in nearly 70% of plant species (Medi & Jasprica, 2007). Research suggests that quercetin plays a key role in preventing and managing oral diseases such as periodontal disease, oral lesions, tooth decay, and bacterial infections (Doughari, 2014).

As a potent antioxidant, quercetin helps neutralize harmful free radicals that cause cellular damage. Studies have highlighted its wide ranging pharmacological properties, making it effective in preventing and treating dental caries, gingivitis, and various oral infections. Its anti-inflammatory, antibacterial, and potential anti-cancer properties further reinforce its value in dental health care (Corega *et al.*, 2014).

Flavonoids are classified into various sub categories, including flavonols, dihydroflavonones, flavonols, anthocyanidins, proanthocyanidins, chalcones, catechins, and leucoanthocyanidins (Venketeshwer, 2012). Among these, catechins (Compound 1 below) have gained attention for their strong antibacterial activity, particularly against Gram-positive bacteria. Chemically described as (2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol, catechins(compound 1 below) have been found effective in combating oral infections, including dental caries and periodontal disease, by inhibiting bacterial growth (Rasouli *et al.*, 2017).



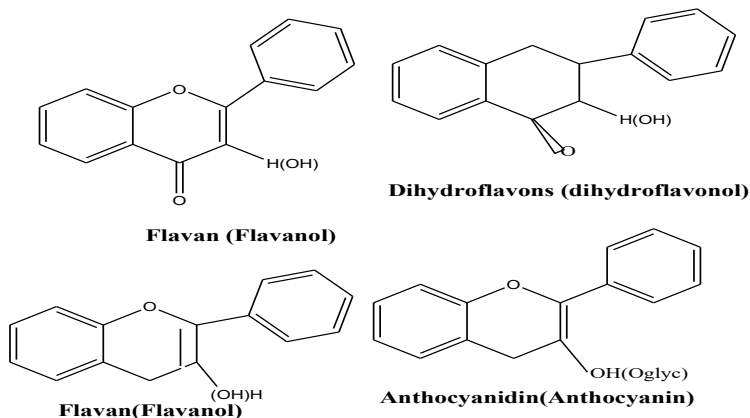
Compound 1

Flavonoids are natural compounds found in plants that play a key role in giving fruits, vegetables, and flowers their vibrant colours. Their core structure consists of 15 carbon atoms arranged in a pattern that connects two benzene rings through a three carbon bridge, forming a unique shape. This structure (C6-C3-C6) classifies them as flavonoids or bioflavonoids (Albert *et al.*, 2023).

These plant-based compounds are widely known for their powerful antioxidant properties, helping to protect body cells from damage caused by unstable molecules, commonly known as free radicals. Research suggests that flavonoids contribute to overall health by reducing oxidative stress, which in turn lowers the risk of chronic diseases like heart disease, neurodegenerative disorders, and even some cancers. Flavonoids come in different types, including flavones, flavonols, flavanones, flavanols, isoflavones, and anthocyanins, each offering unique health benefits. They are naturally present in a variety of foods, such as citrus fruits, berries, apples, onions, kale, tea, cocoa, and red wine (Zheng *et al.*, 2025).

Beyond their antioxidant effects, flavonoids have anti-inflammatory and antimicrobial properties, supporting the immune system and promoting better health. They also influence key biological processes in the body, including the regulation of inflammation and cell

metabolism. Since their absorption and effectiveness depend on how they are broken down in the body, factors like diet, gut health, and food preparation can impact their benefits. With their broad range of protective properties, flavonoids are essential for maintaining long term health and are a valuable part of a balanced diet (Roy *et al.*, 2022).



Compounds (2),(3), (4),(5)

Flavonoids have been recognized for their incredible ability to support the body's natural defenses. Madziga, *et al.*, (2010) describe them as "nature's biological response transformers" because they help regulate how the body reacts to allergies, viruses, and harmful mutations. Similarly, Yamamoto and Gaynor (2001) highlight their impressive range of benefits, including their ability to fight allergies, inflammation, infections, and even cancer.

One of the ways flavonoids work is by disrupting bacterial cell processes. They block the enzymes responsible for DNA and RNA production, preventing bacteria from multiplying

and spreading (Parsaei *et al.*, 2013). This makes flavonoids a natural and effective alternative to antibiotics.

Research by Tichy and Novak (1998), as well as Wu-Yuan, Green, and Birch (2009), further supports their antimicrobial power, showing that flavonoids can help combat infections caused by *Staphylococcus aureus* and harmful bacteria in the digestive system. Their ability to fight cancer related viruses and mutagens also make them a promising tool for long term health and disease prevention.

2.4.3 Polyphenolic Compounds

Polyphenols are natural compounds found in plants, known for their wide range of health benefits. These compounds have unique structures made up of multiple phenol groups attached to benzene rings, giving them strong antioxidant properties. To be classified as a polyphenol, a compound must contain at least two phenolic hydroxyl (-OH) groups linked to one or more benzene rings. In nature, polyphenols help plants grow, fight off diseases, and even determine their colour and flavour. When consumed, they provide significant health benefits for humans.

Polyphenols are grouped based on the number of phenol rings they contain and how these rings are connected. The main types include flavonoids, phenolic acids, stilbenes, and lignans (Vladimir-Knežević & Blažeković, n.d.).

Flavonoids are the most common type of polyphenols, found in a variety of fruits, vegetables, tea, and chocolate. They are further divided into flavanols, flavonols, anthocyanidins, flavones, flavanones, and chalcones, each with unique properties that

contribute to health. Non-flavonoids include compounds like stilbenes (such as resveratrol, found in red wine and grapes), phenolic acids, tannins, and saponins, (compounds (5,6,7) all of which offer protective benefits.

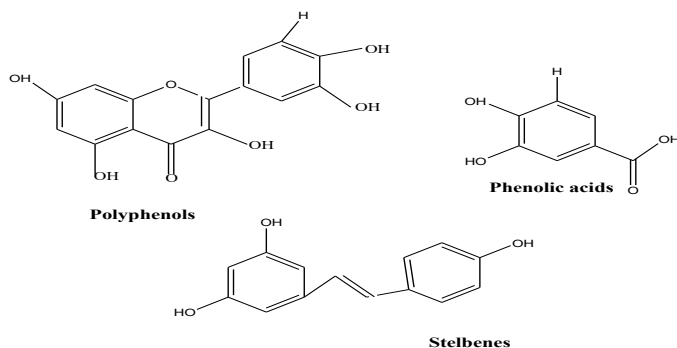
Scientists have found that polyphenols act as powerful antioxidants, helping to fight oxidative stress, a condition linked to aging, heart disease, and other chronic illnesses (Stagos, 2020). Some polyphenols, like vescalagin, have been identified for their ability to prevent harmful sugar-related damage in the body, which is particularly beneficial for people with diabetes (Woldeab *et al.*, 2018). Natural stilbenoids, such as resveratrol, are widely studied for their anti-aging, heart-protective, and anti-inflammatory properties.

Beyond their role as antioxidants, polyphenols have antiviral, antibacterial, antifungal, anti-cancer, and even anti-inflammatory effects. Their ability to neutralize harmful free radicals helps protect cells from damage, preventing illnesses linked to oxidative stress.

One key group, phenolic acids, is especially effective at fighting oxidative damage. Their strength comes from the number and position of hydroxyl (-OH) groups in their structure, which makes them powerful at neutralizing harmful molecules (Castellano, 2012). Additionally, these compounds block enzymes responsible for producing free radicals, further reducing the risk of diseases like cancer, heart disease, and neurodegenerative disorders.

In short, polyphenols are essential plant-based compounds that play a crucial role in maintaining health and preventing disease. By including polyphenol rich foods such as

berries, tea, red wine, dark chocolate, and leafy greens in your diet, you can support your body's natural defence system and promote long term wellness.



Compounds (5) ,(6),(7)

2.4.4 Essential Oils

Essential oils, derived from various herbal plants, are packed with powerful natural compounds that give them a wide range of health benefits. They are often called "volatile phytoncides" due to their strong bioactivity. Each essential oil is made up of a complex mixture of around 300 different organic compounds (Azadmehr *et al.*, 2011; Ardalan *et al.*, 2013; Szyszkowska *et al.*, 2010). In total, researchers have identified about 3,000 compounds in essential oils, mainly consisting of isoprenoids, benzene derivatives, sulphur based, and nitrogen containing compounds (Palombo, 2011).

Some of the most well-known active ingredients in essential oils include carvacrol, thymol, and eugenol which have been shown to have antioxidant, anti-tumor, antimicrobial, and stress relieving effects (Fu *et al.*, 2013). These compounds work against a wide range of

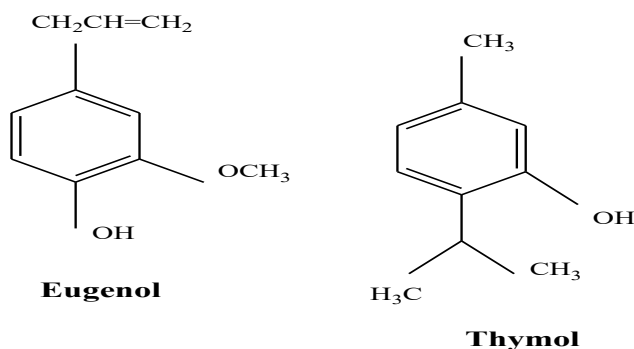
harmful organisms, including viruses, bacteria, yeasts, moulds, and protozoans (Zhou *et al.*, 2019). What makes essential oils even more valuable is their ability to fight antibiotic resistant bacteria. Unlike conventional antibiotics, which bacteria can become resistant to over time, essential oils offer a natural multi target approach that makes it difficult for bacteria to develop resistance (Ardalan *et al.*, 2013).

For centuries, essential oils have been used as natural remedies to treat infections and protect against diseases. In the past, people even relied on them to ward off "bad air" during outbreaks. Today, they continue to be widely used for both prevention and treatment of various ailments (Szyszkowska *et al.*, 2010; Ardalan *et al.*, 2013).

One particularly powerful compound, eugenol (compound 8), is the main active ingredient in dianthus oil. It is known for its strong antibacterial and pain relieving properties, making it a key ingredient in dental treatments. Eugenol is commonly mixed with root canal cement, temporary fillings, and medicated pastes to help treat tooth infections and pulp inflammation (Woldeab *et al.*, 2018). It is also used to sterilize root canals in cases of pulp necrosis and plays a role in treating dentin with silver nitrate to prevent further decay (Parsaei *et al.*, 2013).

Another essential oil component, thymol (compound 9), is found in thyme, oregano, and Satureja oils. It has potent antibacterial and antifungal effects, making it a natural disinfectant. Thymol appears as clear, white crystals with a strong thyme scent and is often used in alcohol based tinctures (1%–10%) to sterilize root canals and treat pulp infections (Vieura & Skorupa, 1993).

Additionally, aliphatic alcohols and leaf aldehydes, which are responsible for the fresh, green scent of many plants, also have strong antibacterial and antifungal properties. These natural compounds contribute to the medicinal, therapeutic, and aromatic benefits of essential oils, making them a popular choice for holistic healing, skincare, and antimicrobial applications (Chandana *et al.*, 2017).

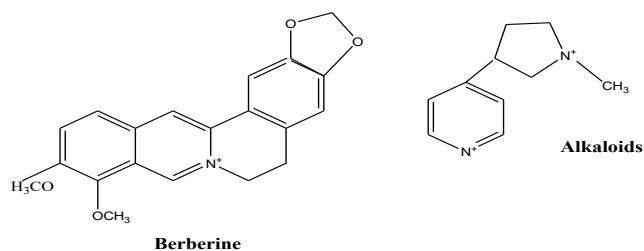


Compounds (8), (9)

2.4.5 Alkaloids

Alkaloids are a diverse group of naturally occurring compounds found in plants, recognized for their powerful medicinal properties. These nitrogen based compounds originate from amino acids and have unique chemical structures that make them highly active in the human body (Doughari 2009; Pradeep *et al.* 2014). Their effects range from pain relief and muscle relaxation to antimicrobial and sedative actions, making them essential in both traditional healing practices and modern medicine. Some of the best known alkaloids include morphine and codeine, widely used for pain management, tubocurarine, a muscle relaxant often used during surgeries, and sanguinarine and berberine (compound 10), which

have strong antimicrobial and antiseptic properties. Another important alkaloid, hyoscine (scopolamine), is commonly used to prevent motion sickness and nausea, while quinine has long been valued for its ability to treat malaria. Additionally, everyday stimulants like caffeine and theobromine found in coffee and chocolate are also alkaloids that boost energy and enhance focus. Beyond their immediate medicinal uses, some alkaloids (compound 11) have anticancer potential, as they can disrupt DNA replication and slow the growth of harmful cells (Vizhi *et al.* 2016). Despite their vast presence in nature with over twelve thousand identified alkaloids across twenty percent of plant species, only a small percentage have been fully explored for therapeutic applications, leaving many with untapped potential (Doughari 2009). This is mainly due to difficulties in extraction, potential toxicity, and the need for more research on their effects. However, as scientists continue to study these natural compounds, new possibilities emerge for alkaloid based treatments making them an exciting area of modern medicine.



Compounds (10),(11)

2.4.6 Tannins

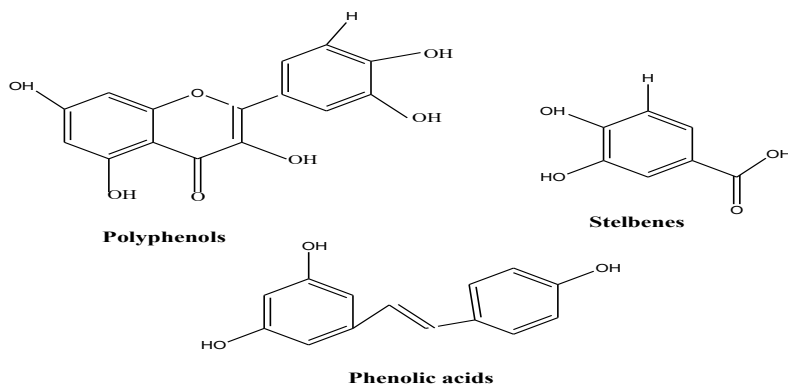
Tannins are natural plant compounds found in leaves, bark, fruits, and seeds, playing a vital role in defending plants against bacteria, fungi, insects, and herbivores. These compounds

belong to a broader category of polyphenols (compound 12) and are classified into four main types: gallotannins, ellagitannins, complex tannins, and condensed tannins, based on their structure (Khanbabaee & Ree, 2014). Among them, condensed tannins, also known as proanthocyanidins, consist of flavonoid molecules linked together in different ways, contributing to their strong antioxidant and protective effects. Some well-known tannins that easily break down through hydrolysis include aflavins (found in tea), daidzein, genistein, and glycitein (Doughari, 2014).

Tannins have been widely used in traditional medicine for their antibacterial, antiviral, and antiseptic properties (Akiyama et al., 2001; Funatogawa *et al.*, 2004). They work by disrupting microbial metabolism, which helps slow down the growth of harmful bacteria and viruses (Doughari, 2014). Because of their phenolic structure, tannins also have powerful antioxidant effects, protecting cells from oxidative damage and reducing inflammation. Research suggests that these properties make them useful in preventing infections, supporting immune health, and even reducing the risk of certain chronic diseases such as heart conditions and cancer. Their ability to bind proteins also explains their astringent effects, which help with wound healing, digestive issues, and skin conditions.

Due to their wide range of health benefits, tannins are commonly used in herbal medicines, skincare products, and food preservation. Their antiviral, antibacterial, and antitumor properties make them an exciting area of research in the development of natural treatments and pharmaceuticals. As scientists continue to explore their potential, tannins are gaining

recognition as powerful bioactive compounds that could play a significant role in modern medicine and wellness therapies



Compounds (12), (13), (14)

2.5 Analysis of Phytochemicals

Advanced analytical methods, including TLC, HPLC-UV, and FTIR were employed to determine phytochemicals present in the plant extracts (Khambabare C., & Kulothungan, S. (2014). Recent studies on *Guizotia scabra* have for other therapeutic use revealed the presence of bioactive compounds with antimicrobial properties, supporting its potential use in dental care formulations (Kebede *et al.*, 2023).

2.5.1 Solvent Extraction of bioactive compounds in Plant Extracts

Solvent extraction is a widely used technique for separating bioactive compounds from plant materials due to its efficiency, simplicity, and versatility. It is particularly favoured for its ability to provide clean separation compared to precipitation methods, which often

result in unwanted co-precipitation. One of its biggest advantages is the use of a separating funnel, which makes the process straight forward and quick, allowing for faster extraction times (Altemimi *et al.*, 2017).

The selection of solvents plays a crucial role in ensuring the accuracy and effectiveness of the extraction process. Different solvents with varying polarities are chosen based on the nature of the targeted compounds, ensuring that phenolic compounds, flavonoids, alkaloids, and essential oils are efficiently isolated from plant materials. For instance, polar solvents such as methanol, ethanol, acetone and water are commonly used for extracting hydrophilic compounds like phenolic acids, while non-polar solvents like hexane and chloroform are suitable for extracting lipophilic compounds such as essential oils and certain alkaloids (Albert *et al.*, 2023).

Modern solvent extraction methods, including ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE), have further improved the efficiency of the process. These techniques enhance the yield, reduce extraction time, and minimize solvent consumption, making them more environmentally friendly. The optimization of solvent ratios, extraction time, and temperature plays a vital role in achieving high purity extracts that can be used for medicinal, cosmetic, and food applications (Roy *et al.*, 2022).

2.5.2 Thin-Layer Chromatography for compounds determination in Plant Extracts

It operates on the principle of differential adsorption, where a small drop of sample is spotted on TLC plate and a suitable solvent is used to develop. The sample then interacts with both a stationary phase (a thin layer of adsorptive material such as silica gel, alumina,

or cellulose) and a mobile phase (a solvent system that moves the sample along the plate) (Hahn-Deinstrop, 2007).

This analyte movement is driven by capillary action, resulting in distinct spots on the TLC plate, which is visualized under UV light or through staining agents such as iodine vapor (Biradar & Rachetti, 2013). Under UV light at 366 nm, different plant metabolites exhibit characteristic fluorescence, flavonoids appear as orange-yellow bands, while phenolic acids show blue fluorescent bands (Males & Medic-Saric, 2001; Yrjönen, 2004).

TLC is widely used for qualitative and semi quantitative analysis, particularly in herbal medicine and phytochemical research. It requires only a few micrograms of the sample, making it ideal for analysing complex plant extracts to determine the number, identity, and purity of bioactive components. In combination with advanced techniques such as high-performance thin-layer chromatography and densitometry, TLC has become an essential tool in pharmaceutical quality control, food safety, and natural product research.

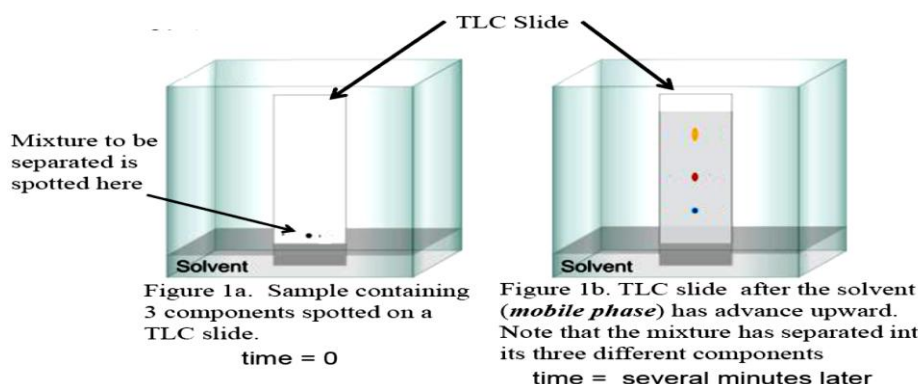


Plate 2.2: TLC slide sample

(S. Kumar & Prasad, 2020)

TLC is used to aid in the singling out the components in a mixture as each give distinct spot at a unique distance, visualizing the TLC plate under UV light then collating the calculated Rf values found with Rf values of the recognized standard components (S. Kumar & Prasad, 2020), then comparing their Rf values and color reactions with specific reagents. The retention factor is then calculated using the formula:

$$R_f = \frac{\text{stretch moved by solute}}{\text{stretch moved by solvent}}$$

Table 2.1 below shows various adsorbent that can be employed to separate many components (Vidyapeeth *et al.*, 2017).

Table 2.1: Adsorptive material used to isolate different components

Adsorptive material	Compound separated
Silica gel	Amino acids, alkaloid, sugars, lipid, etc
Alumina	Alkaloids, phenols, steroids, and carotenes.
Celite	Steroids and inorganic cations
Cellulose powder	Amino acids, food dyes, alkaloids

By combining Rf values, color and fluorescence patterns as well as reagent specific reactions classes of different phytochemicals can be determined (e.g. alkaloids gives orange brown color on Dragendoffs reagent (Harbone (1998).

If the RF values are less than 0.03 about the fingerprint values then confirmation of purity and identification of isolated compounds (Tradit *et al.*, 2007 ; S. Kumar & Prasad, 2020). To other chromatographic methods, TLC will offer accurate confirmation of the purity in

natural products, a simple, reliable, quick, and cheaper mode of detecting many components in a mixture of natural product, as well as less sample clean-up and equipment (Nyireddy, 2002; Grygierczyk *et al.*, 2008); (Medi & Jasprica, 2007).

2.5.3 High-Performance Liquid Chromatography-Ultra Violet for compounds determination in Plant Extracts

HPLC-UV is a powerful technique used to separate, identify, and quantify bioactive compounds found in plant extracts. It works by passing a liquid mixture through a tightly packed column under high pressure (up to 400 bars), allowing different compounds to be separated based on their chemical properties (Karpagasundari & Kulothungan, 2014).

Once separated, the compounds pass through a UV detector, which measures how much light each one absorbs. This helps scientists determine both the identity and concentration of each compound in the sample. The technique is especially useful for analysing heat sensitive and non-volatile compounds, making it ideal for phytochemical research, herbal medicine, and food analysis.

HPLC is widely used for fingerprinting plant extracts, particularly for detecting phenolic compounds, flavonoids, and other antioxidants known for their antibacterial, antifungal, and anti-inflammatory properties. By applying Beer-Lambert's law, scientists can accurately measure the concentration of these compounds, ensuring high precision and reliability. Thanks to its sensitivity, accuracy, and ability to handle complex mixtures, HPLC-UV is a go to method for natural product research, pharmaceutical development, and quality control in plant based medicines and supplements.

2.5.4 Fourier transform-infrared spectroscopy in determination of compounds in Plant Extracts

FTIR is one of the most reliable methods for identifying and analysing the chemical composition of plant extracts. It works by passing infrared light through a sample and measuring how different wavelengths are absorbed or transmitted, revealing the unique molecular fingerprint of the compounds present (Rahalison et al., 1991).

When analysing a liquid sample, a small drop is placed between polished salt plates (such as NaCl, AgCl, or KCl), forming a thin layer that allows infrared light to pass through (Sasidharan *et al.*, 2011). The infrared beam interacts with the sample, causing some wavelengths to be absorbed while others pass through. This absorption pattern helps identify functional groups and determine the structure of the molecules in the extract.

The data collected is processed using Fourier Transform algorithms, which convert the raw information into an interpretable spectrum. By comparing this spectrum with reference databases, scientists can accurately identify bioactive compounds, secondary metabolites, and structural modifications in plant extracts (Koparde, 2017). This technique is widely used in pharmaceutical research, herbal medicine, and quality control to ensure the purity and effectiveness of natural compounds.

2.5.5 Bioassay for antibacterial property of plant extract

Tradit *et al.* (2007) mentions that Bioautography is an important method in determining the antibacterial property of plant extract. It is a simple, fast and relatively cheap way of directly targeting and separating the active compounds on the developed chromatogram.

Conventionally, the bioautographic method has been used in the determination of the antibacterial activity of extracts on a TLC plate as it shows inhibition on the growth of microorganisms. It entails sprinkling the microorganism broth inoculant directly on the developed TLC plate (Homans and Fuchs, 1970) and incubating in conducive conditions to promote the growth of the microorganism. The spots of inhibited zones presented on TLC plates are visualized and the R_f values of the active compounds which are antibacterial are determined and compared with the TLC fingerprint (Homans and Fuchs, 1970). The inhibited growth of microbes after appropriate incubation will be noticed as the microorganism change the tetrazolium salts forming colored formazan products. (Tichy and Novak, 1998); Rasmussen, Bush, Tally, 1993). The Bioautography agar overlay method is preferred over the others as little sample is used, it is simple and has a better resolution of biologically active components (Rahalison *et al.*, 1991).

2.6 Antibacterial Activity of *Guizotia scabra* extracts against Streptococci strains

2.6.1 The Role of Streptococci in Oral Infections

Certain bacteria, particularly *Streptococcus mutans* and *Streptococcus sobrinus*, play a major role in causing dental problems like plaque build-up, cavities, and gum disease (Smith & Johnson, 2020); (plate 2.3 b). These bacteria thrive in the mouth by sticking to teeth and forming biofilms thin layers of bacteria and other microorganisms. When we consume sugary foods, these bacteria break down the sugars, releasing acids that erode tooth enamel and lead to decay.

Among the most problematic bacteria are *S. mutans*, *S. sobrinus*, and *Lactobacillus*, which are commonly linked to cavities (Ihsan & Jabuk, 2016). Even though our mouths host a

diverse range of bacteria, only a few species are directly responsible for dental caries, particularly those that affect the roots of the teeth (plate 2.3 b); (Shah & Shelar, 2018; Medi & Jasprica, 2007).

As Singh (2015) explains, bacteria gather around the teeth and gums, forming plaque, which can lead to tooth decay if not removed. Certain areas of the mouth, such as the deep grooves of molars and the spaces between teeth, are especially prone to plaque build-up (Carvalho *et al.*, 2016). If plaque is left untreated, it can harden into tartar, making it even harder to remove and increasing the risk of gum disease.

Plaque can develop in two ways, above the gumline (supragingival plaque) in which the plaque is mainly associated with *S. mutans* and *S. sobrinus*, which contribute to cavities and below the gumline (subgingival plaque) which harbors bacteria that can cause gum disease and infections (Waldner-Tomic *et al.*, 2014; Liao *et al.*, 2013).

Dental cavities are one of the most widespread chronic diseases worldwide, and if left untreated, they can cause severe pain, tooth loss, and even serious infections that spread beyond the mouth (Liao *et al.*, 2013). In fact, poor oral health has been linked to other health problems, including heart disease, diabetes, and complications during pregnancy (Carvalho *et al.*, 2016).

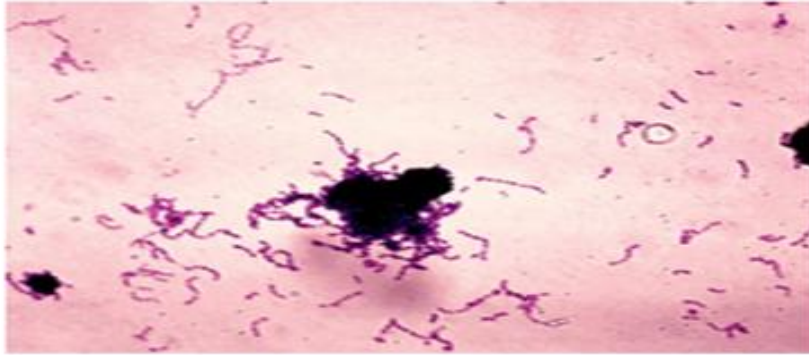


Plate 2.3 (a): Morphology Gram stain of *Streptococcus mutans*)

(Smith & Johnson, 2020).

It is rod-like with chains when cultured, which leads to sub-acute bacterial endocarditis and dental caries.



Plate 2.3 (b): Dental caries /root decay: shows tooth destroyed by cervical decay as a result of dental caries (Shah & Shelar, 2018; Medi & Jasprica, 2007).

With antibiotic resistance on the rise, researchers are looking for alternative ways to fight harmful oral bacteria. Natural treatments, including plant based antibacterials and

traditional herbal remedies, are gaining attention for their ability to prevent bacterial growth, stop biofilm formation, and reduce acid production. These alternative approaches could offer effective, long term solutions for maintaining oral health.

2.6.2 Antibacterial Studies on *Guizotia scabra*

Preliminary studies indicate that *Guizotia scabra* extracts exhibit notable antibacterial effects against Gram-positive bacteria, particularly *Streptococcus* species (Bekele *et al.*, 2022). Bioassay experiments using disc diffusion and minimum inhibitory concentration (MIC) tests have demonstrated significant antibacterial activity, which is likely due to the presence of phenolic and flavonoid compounds (Teshome *et al.*, 2023).

2.7 Research Gaps

Although *Guizotia scabra* has been the subject of widespread use as medicinal plant in some regions, its scientific data remain scarce, especially regarding comparative analysis of phytochemical composition and bioactivity work on the leaf, stem bark, and root. Studies available have focused on *Guizotia* species general antibacterial properties and nutritional role, like seeds for oil production.

Dental caries research has also primarily emphasized synthetic agents such as fluoride and chlorhexidine (Carvalho *et al.*, 2016), with limited exploration on locally available medicinal plants as safer alternatives on specific oral pathogens such as *Streptococcus mutans* and *Streptococcus sobrinus*, which are key contributors to dental caries.

Studies rarely have integrated chromatographic techniques (TLC/HPLC) with antibacterial assays, which is essential to isolate and determine phytochemicals as well as their

bioactivity. Also there is no clear published comparative analysis of different plant parts (leaf, stem bark, root) of *Guizotia scabra* with respect to solvent extraction, phytochemical diversity, and antibacterial activity against oral pathogens.

This lack of targeted research hinders the potential development of natural oral healthcare products. Therefore, this study addresses these gaps by evaluating the phytochemical constituents of *Guizotia scabra* leaf, stem bark, and root and evaluating their specific antibacterial activity against streptococci using modern laboratory techniques, thereby laying foundation for future therapeutic and dental applications.

2.8 Conclusion

Guizotia scabra presents promising potential as a natural remedy for dental infections due to its rich phytochemical composition and antibacterial properties. By determining its bioactive compounds and testing its antibacterial effects against *streptococci*, this study seeks to contribute available, valuable insights into the development of plant based dental care solutions.

CHAPTER THREE

METHODOLOGY

3.1 Introduction on methodology

This chapter presents the methodologies employed in investigation of the phytochemical composition and antibacterial properties of *Guizotia scabra*. A purposive approach was adopted for sampling, where the *Guizotia scabra* tender plants were selected in some farms in Kamagut, Elgon view and Chepkoilel located in Uasin Gishu County, Kenya. The farms were chosen based on their accessibility, growth stage of *Guizotia scabra*, as well as the abundance of the plant. Various analytical approaches and techniques were used, to determine and subsequently analyse the plant phytochemicals. This included Qualitative Standard laboratory tests, TLC, HPLC, FTIR and microbial sensitivity bioassays to assess the plant's potential as a natural remedy for dental health applications. These methods were selected for their ability to effectively determine bioactive compounds and evaluate antibacterial activity, providing a comprehensive analysis of *Guizotia scabra*'s medicinal potential.

3.2 Research Design

This study adopted an experimental research design to evaluate the phytochemical composition and antibacterial activity of ethanol and acetone extracts of *Guizotia scabra*. The research was conducted in three main phases, the first involved qualitative phytochemical determination by standard qualitative laboratory tests and by Thin layer chromatography (TLC). This was followed by quantitative analysis of selected phytochemicals using High-Performance Liquid Chromatography-Ultra Violet (HPLC-

UV) based on their availability of their standards as well as their high therapeutic value, and further characterization of qualitatively identified phytochemicals using Fourier transform-infrared spectroscopy (FTIR). The third phase focused on assessing the antibacterial efficacy of the extracts against *Streptococcus mutans* and *Streptococcus sobrinus* through laboratory based bioassays in comparison with a commercial tooth paste

3.3 Identification of Plant Samples Species

Field identification of the *Guizotia scabra* plant was done with the aid of local farmers, herbalists and other residents who knows the plant by a common local name. The identified plant samples were then collected and authenticated at the University of Eldoret, Botanical Herbarium Lab as ,Family; Asteraceae or Composite, English name; Sun fleck and the voucher number for the specimen plant was M.U.H/gS/032/081. The authentication process involved morphological and taxonomic examination to confirm the correct species had been collected.

3.4 Sampling of Plant Samples

Guizotia scabra tender plants were randomly collected from farms in Uasin Gishu County, Kenya. These farms were selected purposively based on ease of accessibility, the presence of tender *Guizotia scabra* plants, which commonly grows as a weed in cultivated agricultural farms.

Purposive sampling technique was used to select plants that were healthy, tender, and free from visible signs of disease or pest infestation. This approach ensured the integrity and quality of plant material intended for phytochemical and antibacterial analyses. Entire

plants were carefully uprooted and transported to the laboratory in clean, ventilated containers to minimize the degradation of bioactive compounds.

The tender leaf, stem bark and root were selected for this study due to their traditional relevance in local medicinal practices, particularly the leaf extract, which is often used by some locals to relieve tooth pain. Studying these parts separately allowed for comparison of bioactive compound concentration and antibacterial activity, contributing to a comprehensive understanding of *Guizotia scabra*'s potential in oral healthcare.

3.5 Preparation of plant Samples

3.5.1 Cleaning, drying and crushing of Plant Samples

At the laboratory, the plant parts were separated systematically, tender leaves were plucked, the stem bark was peeled off, and roots were trimmed carefully and grouped separately. Each plant parts were separately cleaned with distilled water, air-dried for two weeks under shade at room temperature to preserve chemical integrity, and later manually crushed into smaller fragments (Appendix I) to increase the surface area for efficient solvent penetration. To preserve their quality, potency and stability, the processed samples were separately stored in airtight containers in readiness for further analysis.

3.5.2 Extraction procedure

Dried and ground plant materials (tender leaf, stem bark, and root, (Appendix I) were each subjected to solvent extraction using both ethanol and acetone separately in preparation for determination of the phytochemical constituents and antibacterial property evaluation.

Extracted yields for each plant part were determined and calculated for the tender leaf, stem bark, and root of *Guizotia scabra* using both ethanol and acetone as solvents. The percentage yield for each was calculated using the formula:

$$\text{Percentage yield (\%)} = \left(\frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \right) \times 100$$

Acetone extraction was done separately for each plant part, 10 g of finely ground plant material from each part was weighed into a round-bottom flask, and extracted with 50 mL of acetone, 2 mL of concentrated HCl, and 1 mL of a 1% aqueous urotropine solution. The mixture was refluxed on a boiling water bath for 30 minutes, then cooled, filtered, and diluted to 100 mL with acetone. 25 mL portion of this solution was partitioned in a separating funnel with 50 mL distilled water and extracted three times with 15 mL portions of acetone. The combined acetone fractions were washed with water, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The resulting residue was reconstituted in 10 mL of HPLC-grade acetone, passed through 0.45 μm nylon microfilters, and stored in amber vials for further analysis as previously described (Cai *et al.*, 2022).

This extraction process was carried out to extract bioactive phytochemicals from different parts of *Guizotia scabra* (leaf, stem bark, and roots) using acetone as the solvent. The aim was to obtain acetone-based extracts rich in secondary metabolites such as flavonoids, alkaloids, phenolics, tannins, saponins, and terpenoids for further phytochemical and antibacterial analysis. Refluxing with HCl and urotropine helped to release bound

phytochemicals from plant tissues. Partitioning with ethyl acetate improved the extraction of bioactive compounds.

Ethanol extraction was separately carried out on prepared plant parts, where varying masses of each ground plant part was extracted using 100 mL of 95% ethanol (Cai *et al.*, 2022). Each mixture was placed in conical flasks, sealed, and left to macerate for 72 hours with occasional shaking to enhance solvent penetration. After maceration, the extracts were filtered using Whatman No.1 filter paper. The filtrates were concentrated under reduced pressure using a rotary evaporator and then air-dried to obtain ethanol plant based extracts. These were stored at 4°C in sterile, airtight containers for later analysis procedures. (Palaiogiannis *et al.*, 2023).

The above procedure was meant to extract bioactive phytochemicals from *Guizotia scabra* parts using ethanol as the solvent. The goal was to recover a broad spectrum of compounds including flavonoids, alkaloids, phenolics, tannins, saponins, and terpenoids. These extracts were further used to carry out qualitative standard laboratory tests for phytochemicals determination and antibacterial testing. Ethanol is a safe, versatile solvent capable of extracting both polar and moderately non-polar compounds. Maceration allowed for gentle extraction, preserving heat-sensitive compounds. Occasional shaking enhanced solvent penetration. Rotary evaporation removed ethanol efficiently, yielding concentrated crude extract. Cold storage preserved stability and bioactivity of the extracts for further analysis.

3.5.3 Purification of the crude extracts

The crude extracts obtained from the tender leaf, stem bark, and root of *Guizotia scabra* were subjected to purification using gradient elution. Column chromatography was conducted using a glass column (50 cm × 2.5 cm internal diameter) packed with silica gel (mesh size 70–230) using the slurry method in hexane as the packing solvent. Separately, each dried crude extract (ethanol / acetone-based) was pre-adsorbed onto a small amount of silica gel to form a free-flowing mixture, which was then loaded gently onto the top of the silicon (iv) Oxide column and eluted with gradient polarity using hexane and ethyl acetate solvents.

A gradient elution technique was employed, beginning with 100% hexane and gradually increasing polarity by adding ethyl acetate in 5% increments per 100 mL fraction (Palaioiannis *et al.*, 2023). Eluted fractions were collected sequentially in labelled vials and monitored using TLC and further analysed using HPLC-UV and FTIR for phytochemical determination. Fractions showing similar TLC compounds (i.e., identical Retention factor values and spot patterns under UV) were pooled together (Appendix IV).

The pooled fractions were then concentrated under reduced pressure using a rotary evaporator. The resulting semi-purified fractions were stored in amber glass bottles at 4°C and later used for further phytochemical and analytical determination.

3.6 Determination of Phytochemicals constituents by Qualitative Standard laboratory

Tests

The ethanol and acetone crude extracts from the tender leaf, stem bark, and root of *Guizotia scabra* were separately subjected to standard qualitative lab tests using established methods to detect the presence of various bioactive compounds i.e tannins, alkaloids, saponins, terpenoids, flavonoids, steroids, quinones, anthraquinones, and phenols (Dubale *et al.*, 2023).

3.6.1 Alkaloids

The individual extracts (2g) were diluted and warmed in 2% Sulphuric acid for 3mins , filtered, and separately subjected to Mayer's , where the filtrates were added to Mayer's reagent and iodine. A yellow cream-colored precipitate indicated the presence of alkaloids (Sonam et al., 2017).

3.6.2 Anthraquinones

One gram of each extract was boiled with 2 mL of 10% hydrochloric acid on a water bath for 30 minutes. The mixture was filtered and cooled. Two milliliters of chloroform (CHCl_3) was added to the filtrate, followed by a few drops of 10% NH_3 solution upon heating, a rose-pink color was observed, indicating the presence of anthraquinones

3.6.3 Steroids

Approximately 1g of each extract was placed in a test tube, 2 ml of acetic anhydride was added then followed by five drops of concentrated sulphuric acid. The appearance of a violet pigment indicated the presence of steroids.

3.6.4 Quinones

10 mg of each extract was treated with sodium hydroxide (NaOH), quinones produce a deep red due to formation of semiquinone ions, indicating their presence (Vogel, A. I. (2008). (Joshi et al., 2013).

3.6.5 Tannins

Two grams of each extract was added to water, heated over water bath and then filtered. One millilitre of 5% ferric chloride was added to 1 mL of each extract. The absence of a green-black color indicated the absence of tannins (Ayoola et al., 2008).

3.6.6 Terpenoids

In separate boiling tubes, 0.5 g of each extract was mixed with 2 mL of chloroform, followed by 3 mL of 6M sulphuric acid. The mixture was then heated (Salkawisishki test). A red/brown interface confirmed the presence of terpenoids (Salkowski Test). Terpenoids are widely studied for their antimicrobial, anti-inflammatory, and anticancer properties.

3.6.7 Saponins

One millilitre of each extract was placed in a graduated cylinder and 2 mL of distilled water was added. The solution was shaken vigorously for 10 to 15 minutes. The formation of a stable, persistent foam layer of about 0.5 to 1 cm indicated the presence of saponins (Foam Test).

3.6.8 Flavonoids

One millilitre of 2M sodium hydroxide solution was added to 1 mL of each extract separately. After this, 2 ml of 1% hydrochloric acid solution was added. An intense yellow color that turned colorless upon the addition of 1% hydrochloric acid solution indicated the presence of flavonoids.

3.6.9 Determination of Total Flavonoid Content.

The selected flavonoids (myricetin and quercetin) was determined using the aluminium chloride colorimetric method for their concentration in reference to quercetin and myricetin standards. Purified extracts were mixed with aluminium chloride, potassium acetate and methanol, followed by incubation at room temperature for 30 minutes. Absorbance was measured at 415 nm, and flavonoid concentration was expressed as mg quercetin /myricetin equivalent (QE) per gram of extract.

3.7 Initial analysis of phytochemicals in *Guizotia scabra* by TLC.

Thin-Layer Chromatography was employed in this study to assist in isolation of phytochemical compounds present in the extracts of *Guizotia scabra* tender leaf, stem bark, and root samples. TLC enabled preliminary separation of phytochemicals based on their electrophoresis migration behavior and retention factor (R_f) values.

TLC plates were prepared by marking a baseline 1 cm from the bottom using a pencil. Extract samples were dissolved in a 1:1 mixture of acetone and hexane to achieve balanced solvent polarity; this ratio was later adjusted as needed to optimize separation. Small volumes of each sample were carefully applied onto the baseline using a micropipette and

allowed to dry. The plates were then placed in a TLC chamber pre-saturated with the mobile phase, and covered with a lid to minimize solvent evaporation. When the solvent front had traveled approximately three quarters of the plate length, the plates were removed and allowed to air dry.

Separated spots on the TLC plates were visualized under UV light. The mobile phase polarity and volume were fine-tuned during trials to improve the resolution and retention of compounds. Retention factor (Rf) values for each visible spot were calculated using the formula:

$$R_f = \frac{\text{stretch moved by solute}}{\text{stretch moved by solvent}}$$

3.8 Analysis of selected phytochemicals by HPLC.

HPLC analysis was conducted using a Shimadzu HPLC system (model CTO-10AS VP), equipped with a pump (model LC-20AD), a UV detector (model SIL-20A HT), and a reverse-phase C18 column (250 × 4.6 mm, 5 μm particle diameter). The mobile phase consisted of 1% phosphoric acid and acetonitrile in 35:65 ratio. The flow rate was set at 1.0 mL/min, with the column temperature maintained at 30°C. Elution was monitored in the UV range, and quantitative data acquisition was performed at 367 nm. Calibration was carried out within a concentration range of 1 ppm to 10 ppm using mixed standards of myricetin and quercetin. Standard solutions of 1 ppm, 2 ppm, 4 ppm, 8 ppm, and 10 ppm were analysed via HPLC, and their peak areas were recorded. The HPLC method combined with ultraviolet detection and gradient elution was employed to simultaneously determine and quantify components (myricetin and quercetin) in individual extracts. The mobile

phase comprised 0.1% aqueous acetic acid and acetonitrile (Sc *et al.*, 2016). Sample preparation adhered to standard HPLC procedures with slight modifications. The dried purified extract was dissolved in the mobile phase, filtered and subsequently injected into the HPLC system. Calibration was carried out within a concentration range of 1 ppm to 10 ppm using mixed standards of myricetin and quercetin (Appendix V). Standard solutions of 1 ppm, 2 ppm, 4 ppm, 8 ppm, and 10 ppm were analysed via HPLC, and their peak areas and values were recorded. According to Table 4.6, triplicate samples were analysed, that is, leaf extracts in triplets labelled as LAEX1, LAEX2, LAEX3. The extracts in reference to their standard were analysed using HPLC coupled with UV detector to give the HPLC chromatogram (Appendix V). During separation, the total run time for the components was established and the detection wavelength was optimized to achieve maximum absorbance (Cheng *et al.*, 2017). A HPLC fingerprint analysis was conducted to aid in identifying extract components (Sc *et al.*, 2016).

3.9 Functional Group analysis of *Guizotia scabra* extracts by FTIR Spectroscopy.

Fourier Transform Infrared Spectroscopy was used to determine functional groups present in the extracts of *Guizotia scabra* tender leaf, stem bark, and root. Prior to analysis, all the tools including the die set, mortar, and pestle were thoroughly cleaned with chloroform and dried with tissue to eliminate contaminants.

For each purified plant part extract, about 2g of the dried powdered sample was mixed with spectroscopic grade potassium bromide (KBr) in a 100:1 ratio (KBr to sample by weight). The mixture was finely ground using a mortar and pestle for one to two minutes to achieve

homogeneity, then compressed into a transparent pellet using a hydraulic press. The pellet was placed into the FTIR spectrophotometer sample holder.

Infrared (IR) radiation was directed at each sample pellet, and the absorbance spectra were recorded. The background signal was first collected and subtracted from the sample readings. The resulting interferogram was converted into frequency data using inverse Fourier transformation. Spectral data were expressed as percentage transmittance versus wavenumber (cm^{-1}) and characteristic absorption bands were identified. Functional groups corresponding to peaks were interpreted with reference to standard spectra from the Varian FTIR library (Sc *et al.*, 2016). This analysis enabled the determination of key functional groups such as hydroxyl (O–H), carbonyl (C=O), amine (N–H) and others, enabling the identity of the chemical characteristic nature of the bioactive constituents present in the different plant parts of *Guizotia scabra*.

3.10 Antibacterial Activity of the plant extracts

The antibacterial activity of the extracts against *streptococci* strains (gram positive) was determined using the agar well diffusion method and the inhibition zones measured (Ihsan, S., & Jabuk, A. (2016); (Bekele, T., Abebe, G., & Alemu, D. (2022)). The bacterial strains were cultured in Mueller-Hinton agar plates and incubated at 37°C for 24 hours. Wells of 8 mm diameter were created in the agar, and the extracts were introduced. After incubation, the zones of inhibition were measured and recorded to assess antibacterial action.

CHAPTER FOUR

RESULTS

4.1 Extracted yields

The extraction yields varied depending on the plant part and solvent used. The leaf ethanol extract gave the highest yield at 33.50%, followed by the root (27.30 %) and stem bark (25.60 %). Acetone extracts generally gave lower yields across all plant parts with the leaf extract again yielding relatively more (24.0%), followed by the root (23.40%) and stem bark (21.80%) (Table 4.1).

Table 4.1: Percentage Yield of Ethanol and Acetone Extracts from *Guizotia scabra*

Plant part	Solvent used	Weight of Dry Extract (g)	Weight of Plant material (g)	Percentage Yield (%)
Tender leaf	Ethanol	9.35	28.00	33.50
	Acetone	8.41	35.00	24.00
Stem bark	Ethanol	8.56	33.00	25.60
	Acetone	8.18	40.00	21.80
Root	Ethanol	8.73	32.00	27.30
	Acetone	8.34	36.00	23.40

4.2 Phytochemical constituents in the pooled tender leaf, stem bark and root extracts of *Guizotia scabra*

Table 4.2 below summarizes the presence (+) or absence (-) of specific phytochemicals in each extract. Phytochemical analysis of the plant extracts revealed the presence of several key bioactive compounds. Alkaloids, anthraquinones, steroids, quinones, terpenoids, saponins, and flavonoids were all detected, each showing characteristic positive reactions (Table 4.2). Tannins however were not detected in any of the extracts.

Table 4.2: Phytochemical positivity Standard qualitative test Results

Phytochemical	Observation	Result	key
Alkaloids	Cream precipitate observed	Present	(+)
Anthraquinones	Rose-pink coloration	Present	(+)
Steroids	Violet colour changed to blue	Present	(+)
Quinones	Red coloration	Present	(+)
Tannins	No dark-green coloration	Absent	(-)
Terpenoids	Red coloration at interphase	Present	(+)
Saponins	Persistent foaming observed	Present	(+)
Flavonoids	Yellow changed to colourless	Present	(+)

Table. 4.3 Phytochemical Constituents of the separate leaf, Stem bark and Root extracts of *Guizotia scabra* plant as determined by Qualitative standards lab Test

Extracts	Leaf (EtOH)	Leaf (Acetone)	Stem Bark (EtOH)	Stem Bark (Acetone)	Root (EtOH)	Root (Acetone)
Tannins	-	-	-	-	-	-
Anthraquinones	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Saponins	+	-	+	-	+	-
Phenols	-	-	-	-	-	-
Quinones	+	+	+	+	+	+

Key: (+) Present; (-) Absent; EtOH-Ethanol

These findings confirm that ethanol and acetone complement each other in phytochemical extraction, since saponins were only present in ethanol extracts, while most of the other compounds were extracted by both solvents

4.3 Initial analysis of phytochemicals of *Guizotia scabra* plant extracts by TLC.

Thin-layer chromatography was used to determine the phytochemical in extracts of *Guizotia scabra* leaf, stem bark, and root. The TLC analysis aimed at separation of compounds based on their retention factor (R_f) values and spot characteristics. A solvent

system of hexane to acetone or ethanol (1:1) was used across all samples. Different coloured spots indicated the presence of diverse phytochemicals in the extracts and the Rf values obtained reflected the polarity and mobility of these compounds on the TLC plate (Appendix III).

Table 4.4: TLC Analysis of Ethanol extracts using Hexane: Ethanol (1:1) Solvent system.

Species	Solvent System (SS)	DTS (cm)	No. of Spots	Colour	Leaf Rf - Values (cm)	Stem bark Rf - Values (cm)	Root Rf - Values (cm)
Gs	H:E (1:1)	14.2	5	YG	1.00	1.00	1.00
				BY	0.75	0.72	0.70
				O	0.67	0.63	0.65
				LP	0.63	0.59	0.60
				RB	0.56	0.54	0.52

Key: (Gs) -*Guizotia scabra*: Distance travel by the solvent (DTS),(Rf) -Retention factor, (H: EtOH) - Hexane: Ethanol (YG) –Yellow- Green,(BY) –Brown- Yellow, (RB) - Red-Brown,(LP)- Light Purple and (O)- Orange

Table 4.5: TLC Analysis of Acetone extracts using Hexane: Acetone (1:1) Solvent system.

Species	Solvent System (SS)	DTS (cm)	No. of Spots	Colour	Leaf Rf - Values (cm)	Stem bark Rf - Values (cm)	Root Rf - Values (cm)
Gs	H:A (1:1)	14.2	5	YG	1.00	1.00	1.00
				BY	0.73	0.71	0.70
				O	0.65	0.62	0.60
				LP	0.60	0.58	0.57
				RB	0.54	0.53	0.55

Key: (Gs) -*Guizotia scabra*: Distance travel by the solvent (DTS),(Rf) -Retention factor, (H: A) - Hexane: Acetone (YG) –Yellow- Green,(BY) –Brown- Yellow, (RB) - Red-Brown,(LP)- Light Purple and (O)- Orange

Although similar TLC solvent systems were used, the spot profiles in stem bark and root extracts (Table 4.4 and Table 4.5) after staining with iodine for visualization ,showed slightly different Rf values and intensity, suggesting possible differences in compound solubility and extract composition.

The TLC results confirm that the plant extracts of *Guizotia scabra* contain multiple phytochemical constituents. The presence of five distinct spots in each extract indicates the presence of at least five different compounds per plant part. The consistent Rf patterns and colours as visualized under UV light, such as yellow-green, brown-yellow, orange, and blue-green spots suggest diversity of phytochemicals present.

The leaf extract showed relatively higher diverse Rf values , indicating it may be the richest in bioactive compounds (Table 4.3) if compared with literature Rf values (Kumar

& Prasad, 2020). These findings validate the use of TLC as a quick and effective method to isolate plant extracts and support further identification through more advanced techniques like HPLC and FTIR.

4.4 High-Performance Liquid Chromatography Results on selected phytochemicals in extracts of *Guizotia scabra*

The results showed the average concentration of Myricetin at 7.0013 ppm and Quercetin gave an average concentration of 0.856 ppm. The Bark extracts in Table 4.6 (b), labelled BAEX1, BAEX2, BAEX3 showed absence of Myricetin while Quercetin had average concentration of 2.4991 ppm. Subsequently the root extract samples (RAEX1, RAEX2, RAEX3) as per Table 4.6 (c) below showed Myricetin average concentration of 2.0627 ppm and Quercetin average concentration of 0.7027 ppm. The concentrations of these samples were determined based on their peak areas (Appendix V).

Table 4.6: HPLC determination of Myricetin and Quercetin in *Guizoa scabra***(a) Leaf Sample (LAEX 1, LAEX 2, LAEX 3)**

Compound	LAEX 1	LAEX 2	LAEX 3	Ave .Conc.(ppm)
Myricetin	5.168	8.288	7.548	7.0013
Quercetin	1.034	1.602	0.068	0.856

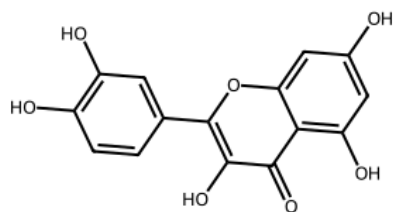
(b) Stem bark Sample (BAEX 1, BAEX 2, BAEX 3)

Compound	BAEX 1	BAEX 2	BAEX 3	Aver. Conc.(ppm)
Myricetin	0.000	0.000	0.000	0.000
Quercetin	2.539	2.932	2.001	2.4991

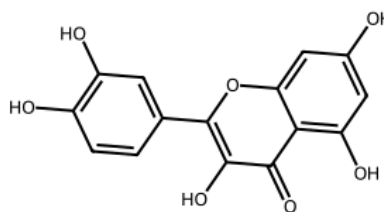
(c) Root Sample (RAEX 1, RAEX 2, RAEX 3)

Compound	RAEX 1	RAEX 2	RAEX 3	Aver. Conc. (ppm)
Myricetin	2.376	3.812	0.005	2.0627
Quercetin	0.924	0.884	0.300	0.7027

The results indicate that myricetin (fig 4.4 (a)below) was most abundant in leaf extracts, while quercetin (fig 4.4 (b)below) was predominantly found in the bark extract as per the peaks and their Rt in Appendix V (Figure 11, 12 and 13).



(a) Myricetin



(b) Quercetin

Plate 4.1 HPLC determination of Myricetin and Quercetin in *Guizotia scabra*

Source: Author (2024)

HPLC peaks at different Rt value, range between 0.6-9.8 indicated different compound present in the extracts (Table 4.7) as also shown by TLC varied Rf value (Table 4.4 and 4.5).

Table 4.7: HPLC Retention Times of Phytochemicals in *Guizotia scabra* Leaf extract.

Peak No.	Retention Time (min)	Peak Intensity (Relative)	Possible Compound Class
1	0.730	Very low	Solvent front / polar impurity
2	1.932	Low	Polar phenolic compound
3	2.696	Very high (major)	Flavonoid / phenolic derivative
4	3.127	Very high (major)	Flavonoid / glycoside
5	3.814	Medium	Flavonoid derivative
6	4.085	Medium	Phenolic / glycoside
7	4.496	Medium	Polyphenol
8	4.674	Medium	Phenolic compound
9	5.466	Low	Flavonoid derivative
10	6.000	Low	Possible phenolic
11	6.686	Low	Polyphenolic
12	7.333	Very low	Terpenoid / lignan
13	7.829	Very low	Terpenoid derivative
14	8.839	Very low	Terpenoid derivative
15	9.917	Low (tailing)	Non-polar compound / late eluting

Compound classes are tentative assignments based on retention time trends at 350 nm (common for flavonoids & polyphenols).

4.5 Fourier Transform Infrared Spectroscopy absorption Frequency Results on functional groups of phytochemicals present in *Guizotia scabra* extracts.

The FTIR spectra of *Guizotia scabra* tender leaf, stem bark, and root extracts (Figure 4.1, Figure 4.2, Figure 4.3) showed strong absorption bands at 3441.01 cm^{-1} (O–H/N–H stretching), 2920 cm^{-1} (C–H stretching), and 1643.35 cm^{-1} (C=O stretching). Additional bands appeared at 2000 cm^{-1} (–C≡N cyano groups), 1396.46 cm^{-1} (C–O, C–H), 1250.0 cm^{-1} (C–N stretching), 1031.92 cm^{-1} (ether/amine, C–O vibration), and 538.14 cm^{-1} (metal–ligand vibrations). The leaf extract showed an extra strong peak at 3759.26 cm^{-1} , weakly present in bark but absent in root. These absorption frequencies confirm the presence of functional groups associated with alkaloids, anthraquinones, quinones, steroids, glycosides, terpenoids, saponins, flavonoids and steroids across all plant part extract (Table 4.8, Table 4.9, Table 4.10)

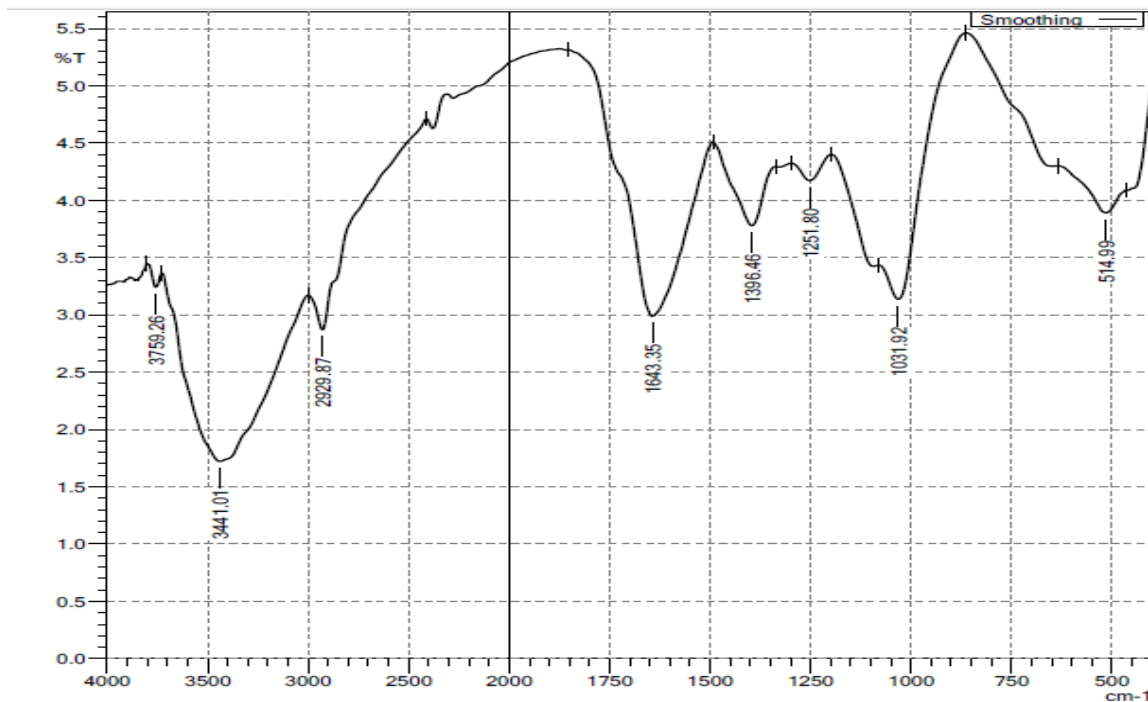


Plate 4.2 Leaves absorption spectrum

Source: Author (2024)

Table 4.8. Leaf absorption Frequency

Absorption Frequency (cm ⁻¹)	Intensity
3759.26	Strong
3441.01	Broad/Strong
2929.87	Small
1643.35	Strong
1396.46	Wide/Small
1251.80	Strong
1031.92	Medium
514.99	Medium

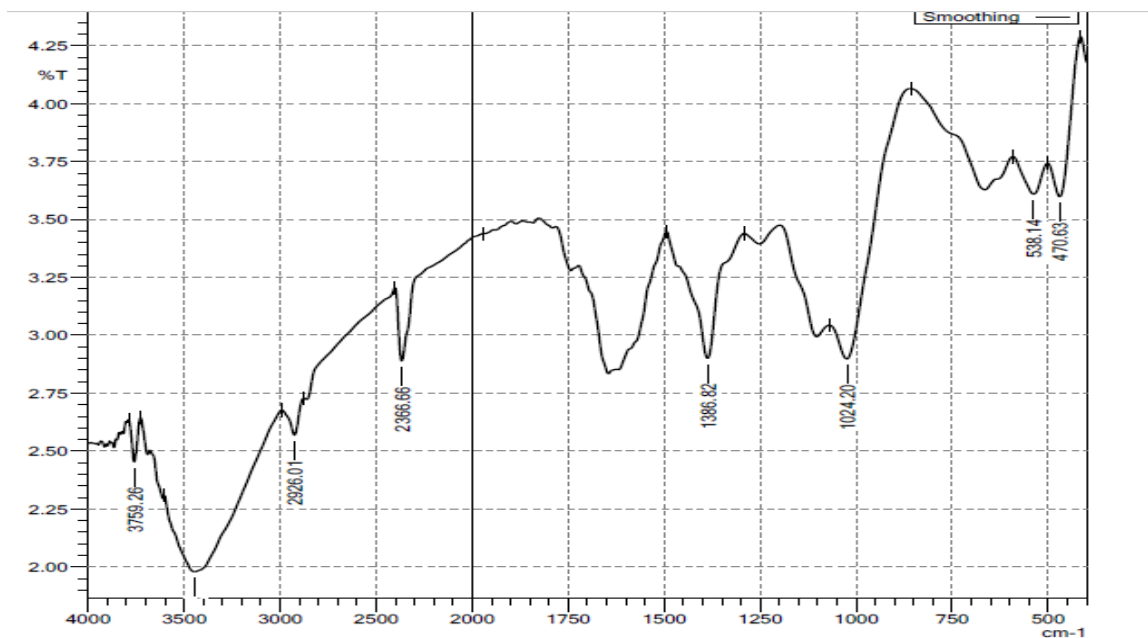


Plate 4.3: Bark absorption spectrum

Source: Author (2024)

Table 4.9: Stem bark absorption Frequency

Absorption Frequency (cm ⁻¹)	Intensity
3759.26	Weak
3441.01	Strong/Wide
2926.01	Small
2366.66	Strong
1643.35	Strong/Wide
1386.82	Narrow/Strong
1024.20	Double /Wide
538.14	Wide
470.63	Wide

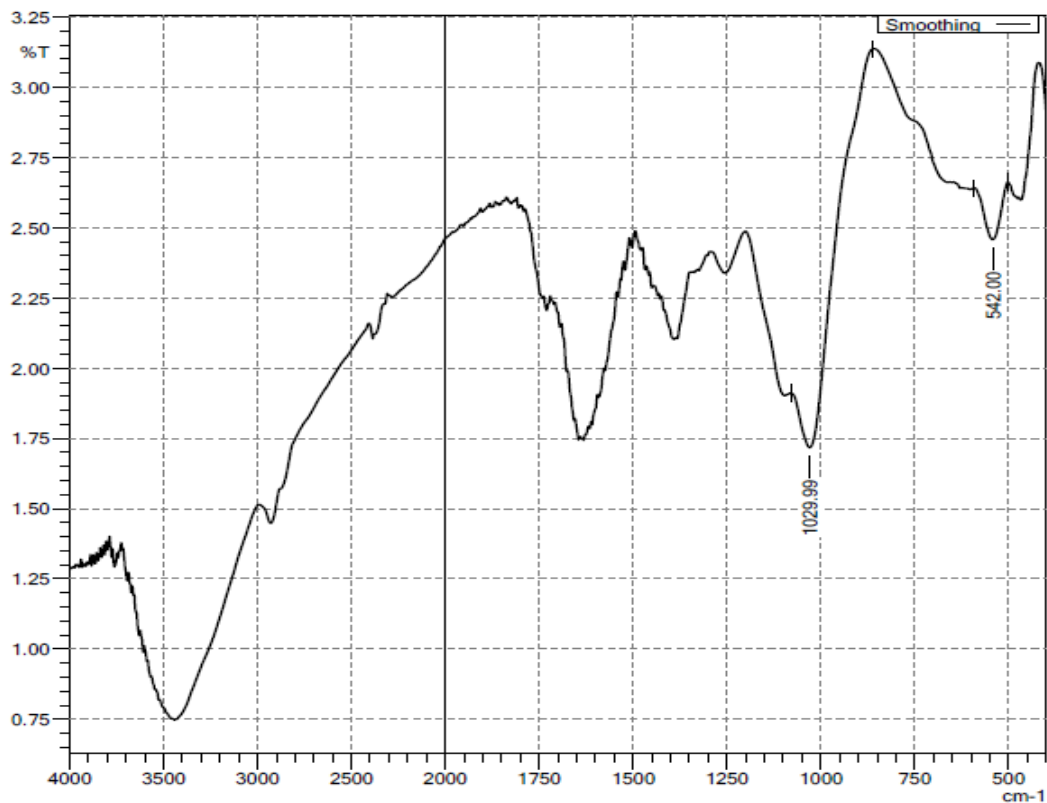


Plate 4.4: Roots Absorption spectrum

Source: Author (2024)

Table 4.10. Root Absorption Frequencies

Absorption Frequency	Intensity
3441.01	Strong/Wide
2929.87	Strong
2366.66	Strong
1643.35	Strong/Wide
1396.46	Strong
1250.0	Strong
1029.99	Double/Strong

4.6 Antibacterial activity of plant extracts of *Guizotia scabra* against using bioassay.

The antibacterial potential of *Guizotia scabra* tender leaf, stem bark and root extracts and commercial toothpastes as the control was tested against *Streptococcus mutans* and *Streptococcus sobrinus* using a bioassay.

4.6.1 Sensitivity Patterns of Ethanol extract and commercial toothpaste against *Streptococcus* bacteria strains as Measured by Zone of Inhibition

The data show notable differences in antibacterial activity between the different extracts and the commercial toothpaste products. The zones were measured in mm then expressed as percentages of 8 mm diameter of the agar wells. The leaf extract of *Guizotia scabra* showed the largest zone of inhibition against *Streptococcus mutans* (5.0 mm, 62.5%), followed by the root extract (3.0 mm, 37.5%). Similarly, the leaf extract exhibited a zone of inhibition of 4.0 mm, (50%) against *Streptococcus sobrinus*, compared to 2.5 mm (31.25%) for the root extract.

When compared with the commercial toothpaste products, both Brand 1 (6.0 mm, 75%) and Brand 2 herbal (7.0 mm, 87.5%) showed substantial antibacterial effects against *Streptococcus mutans*, with Brand 2 showing the highest zone of inhibition overall. For *Streptococcus sobrinus*, both Brand 1 and Brand 2 displayed a 6.0 mm (75%) zone of inhibition, comparable to the leaf extract of *Guizotia scabra* as illustrated in Table 4.11 below.

Table 4.11. *Guizotia scabra* ethanol and commercial toothpastes zones of inhibition in mm and expressed as percentages of 8 mm

Pathogen	<i>Guizotia scabra</i> extracts zones of inhibition in mm and expressed as percentages of 8 mm.			Commercial toothpastes zones of inhibition in mm and expressed as percentages of 8 mm	
	Ethanol extract			Brand 1	Brand 2
	Leaf	Stem bark	Root		
<i>Streptococcus mutans</i>	6.0 mm (75.0%)	2.0 mm (25%)	5.0 mm (62.5%)	3.0 mm (37.5%)	7.0 mm (87.5%)
<i>Streptococcus sobrinus</i>	4.0 mm (50%)	1.5 mm (18.75%)	2.5 mm (31.25%)	6.0 mm (75.0%)	6 mm (75.0%)

The results show the comparative antibacterial activity of ethanol extracts (root, stem, leaf) against *Streptococcus mutans* and *Streptococcus sobrinus*, compared to two commercial toothpaste brands.

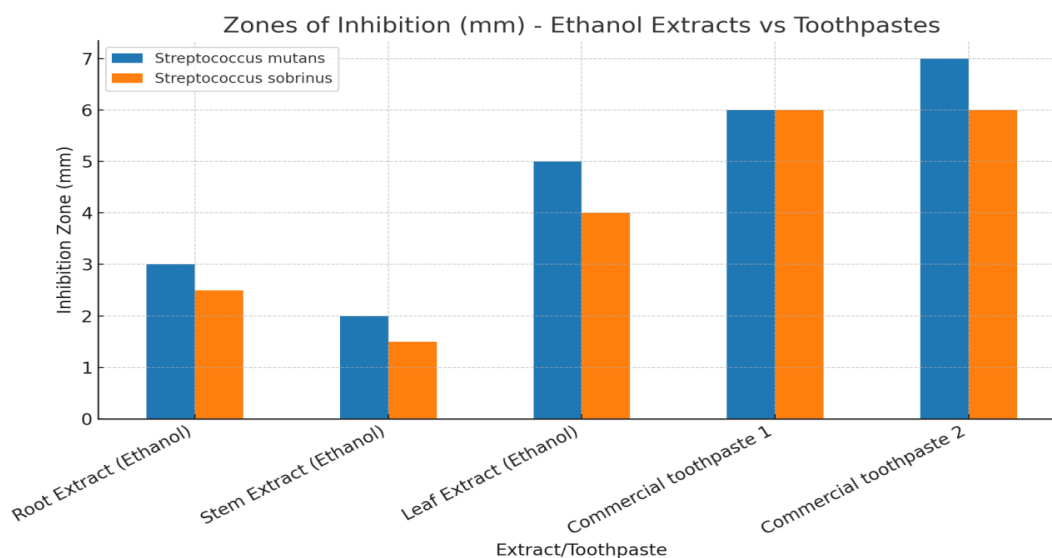


Plate 4.5: Zones of Inhibition (Ethanol Extracts vs Toothpastes)

Source: Author (2024)

4.6.2 Sensitivity Activity of acetone Extract and commercial toothpaste against *Streptococcus* bacteria strains as Measured by Zone of Inhibition

The antibacterial activity of *Guizotia scabra* extracts and commercial toothpastes was tested against *Streptococcus mutans* and *Streptococcus sobrinus*. Among the extracts, the leaf extract stood out with the highest activity, showing inhibition zones of 4.6 mm (57.5%) against *S. mutans* and 4.3 mm (53.75%) against *S. sobrinus*. The root extract followed with moderate activity at 2.8 mm (35.0%) and 2.3 mm (28.75%), while the stem extract showed the lowest activity with inhibition zones, measuring 1.9 mm (23.75%) and 1.7 mm (21.25%) for the respective pathogens. On the other hand, commercial toothpastes performed better overall, with Brand 2 showing the largest zone of inhibition at 7.0 mm (87.5%) against *S. mutans*, while both toothpastes achieved 6.0 cm (75.0%) against *S. sobrinus* as illustrated in Table 4.12.

Table 4.12. *Guizotia scabra* acetone extracts and commercial toothpastes zones of inhibition in mm and expressed as percentages.

Bacterial strain	<i>Guizotia scabra</i> extracts zones of inhibition in mm and expressed as percentages of 8 mm			Commercial toothpastes zones of inhibition in mm and expressed as percentages of 8 mm	
	Acetone Extract			Brand 1	Brand 2
	Leaf	Stem	Root		
<i>Streptococcus mutans</i>	4.6 mm (57.5%)	1.9 mm (23.75%)	2.8 mm (35.0%)	6.0 mm (75.0%)	7.0 mm (87.5%)
<i>Streptococcus sobrinus</i>	4.3 mm (53.75%)	1.7 mm (21.25%)	2.3 mm (28.75%)	6.0 mm (37.5%)	6 mm (75.0%)

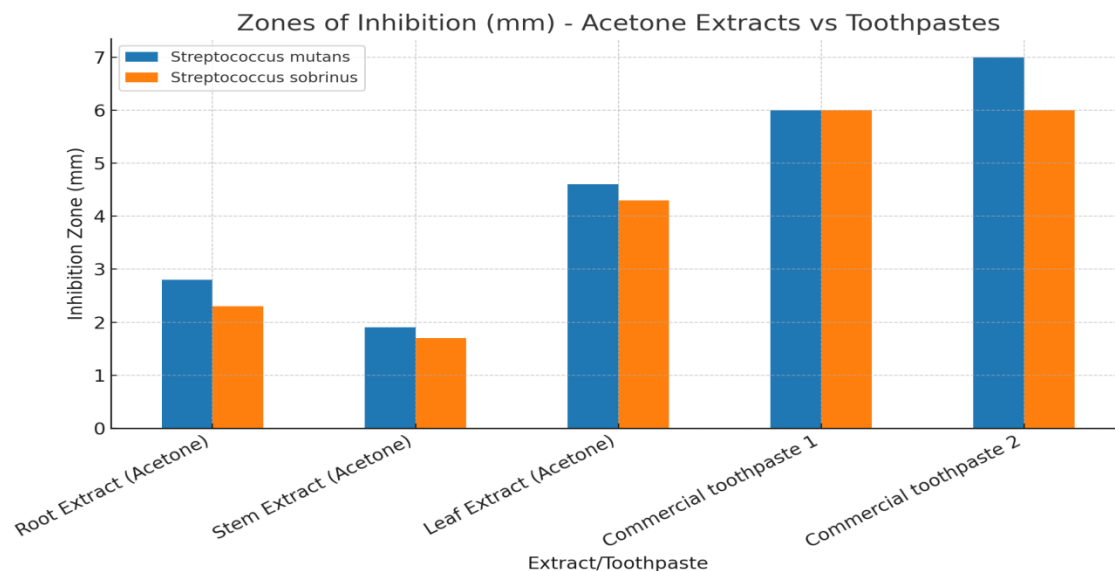


Plate 4.6: Zones of Inhibition of Acetone Extracts vs Toothpastes against *Streptococcus mutans* and *Streptococcus sobrinus*

Source: Author (2024)

Plate 4.6: shows the comparative antibacterial activity of acetone extracts (root, stem, and leaf) against *Streptococcus mutans* and *Streptococcus sobrinus*, compared to the two commercial toothpaste brands.

The bar charts clearly illustrate that leaf extracts of *Guizotia scabra* demonstrated stronger antibacterial activity compared to stem and root extracts regardless of solvent used. Against *Streptococcus mutans*, the leaf ethanol extract achieved 5.0 mm inhibition (62.5% of the 8 mm reference), while the acetone leaf extract reached 4.6 mm (57.5%). Against *Streptococcus sobrinus*, inhibition zones were 4.0 mm (50%) and 4.3 mm (53.75%) for ethanol and acetone leaf extracts respectively.

Commercial toothpastes, however, exhibited consistently higher inhibition zones (6–7 mm; 75–87.5%), confirming their greater efficacy. Root and stem extracts showed relatively weak antibacterial activity, with inhibition zones below 3 mm (<37.5%). Overall, the leaf extracts showed remarkable antibacterial activity, approaching commercial standards, especially against *Streptococcus mutans*. Ethanol extracts were generally more effective than acetone extracts, suggesting ethanol as a more suitable solvent for extracting bioactive compounds. This findings highlight the potential application of *Guizotia scabra* leaf extracts as a natural source of antibacterial agents for oral care products. The mode of action of these phytochemicals e.g flavanols, alkaloids, tannins is by disrupting the cell wall and membrane of the bacteria as well as its DNA preventing cell division. Flavonoids and polyphenols prevent biofilm formation (Bekele, Abebe, & Alemu, 2022).

CHAPTER FIVE

DISCUSSIONS

5.1 Extraction Yields for Ethanol and Acetone Extracts

The extraction yields of *Guizotia scabra* varied notably based on both the plant part used and the type of solvent employed. Among the three parts examined, tender leaf, stem bark and root, the ethanol extract of the leaf produced the highest yield (33.50%), followed by the root (27.30 %) and stem bark (25.60 %). Acetone extracts demonstrated generally lower yields across all plant parts, with the leaf still yielding the most (24.10%) , followed by the root (23.40%) and stem bark (21.80%) (Table 4.1).

Extraction yields indicated ethanol as a more effective solvent compared to acetone, likely due to its polarity and ability to dissolve a wide range of polar and semi-polar phytochemicals, such as flavonoids, alkaloids, saponins, and glycosides (Pavithra *et al.*, 2010; Azwanida, 2015). These findings are consistent with previous studies which indicate that ethanol, as a polar solvent is more efficient in extracting a wide range of phytochemicals. The higher yield from the leaf may be attributed to their rich content of secondary metabolites, which are generally more extractable compared to the lignified tissues of stem bark and root. The leaf is also known to be metabolically active organs, often accumulating higher levels of bioactive compounds like flavonoids and terpenoids as synthesis of secondary metabolites begins at the leaf, then distributed to the other parts of the plant (Kiplimo et al;2011) .

On the other hand, the lower yields obtained from the stem bark and root extracts may result from the presence of structural polysaccharides such as lignin, cellulose, and

hemicellulose, which are less soluble in both ethanol and acetone. Acetone, while moderately polar, is generally less effective than ethanol in penetrating plant matrices and solubilizing a broad spectrum of phytochemicals (Do *et al.*, 2014).

These observations underscore the importance of selecting appropriate solvents and plant parts during phytochemical investigations. The superior yield from the ethanol leaf extract not only reflects the efficiency of ethanol as a solvent but also points to the potential of tender leaf of *Guizotia scabra* as a rich source of bioactive compounds for therapeutic development. It also supports harvesting of leaves rather than stem or roots for plant conservation policies.

5.2 Phytochemical constituents in the tender leaf, stem bark and root extracts of *Guizotia scabra*.

The phytochemical analysis of the root, stem, and leaf extracts of *Guizotia scabra* revealed the presence of anthraquinones, alkaloids, terpenoids, flavonoids, steroids, saponins, and quinones. Interestingly, tannins and phenols were absent in all tested parts of the plant. These findings align with prior research highlighting the diverse medicinal properties of phytochemicals in plants (Aryal *et al.*, 2019; Borges *et al.*, 2020). In Kenya, local studies such as those by Kigen *et al.* (2019) and Kareru *et al.* (2021) emphasize the role of indigenous plants, including *Guizotia scabra*, in traditional medicine. However, there are differing views among scholars, especially regarding the variations in phytochemical content due to environmental factors, extraction methods, and plant genetics as well as the age of the plant (Singh *et al.*, 2020).

The detection of anthraquinones in all plant parts is notable given their reputation for antimicrobial, antibacterial, antioxidant, and anticancer effects (Ahmed *et al.*, 2021). Researchers like Kigen *et al.* (2019) have reported the use of plants containing anthraquinones for managing skin infections and digestive disorders. However, Okello *et al.* (2022) raise concerns about the variability in the bioavailability of anthraquinones, suggesting that their therapeutic potential may not always be consistent across plant species or preparations.

Alkaloids, also detected in all extracts, are widely recognized for their analgesic, anti-inflammatory, and antimalarial properties (Kumar *et al.*, 2020). Locally, traditional healers in Kenya have relied on alkaloid rich plants for treating fever and pain (Gachathi & Ayuko, 2020). Despite their efficacy, there is some caution about the potential toxicity of alkaloids in higher doses (Yadav *et al.*, 2021), underscoring the importance of safety evaluations for *Guizotia scabra* extracts.

The presence of terpenoids, which are known for their anti-inflammatory, antibacterial, antioxidant, and antiviral effects, adds to the plant's therapeutic potential (Yadav *et al.*, 2021). In Kenya, terpenoid rich plants have historically been used to boost immunity and treat respiratory ailments (Kareru *et al.*, 2021). However, Singh *et al.* (2020) argue that standardizing terpenoid doses in herbal remedies remains a challenge, which could limit their clinical application if not addressed.

Flavonoids, detected across all plant parts, are particularly valued for their antioxidant and anti-inflammatory benefits (Pandey & Rizvi, 2020). Researchers, such as Muregi *et al.* (2020), have documented the use of flavonoid rich plants in managing oxidative stress-

related conditions, including hypertension. However, Patel *et al.* (2021) caution that the bioavailability of flavonoids may sometimes be limited, reducing their effectiveness unless advanced extraction techniques are applied.

Steroids and saponins present in the extracts play key roles in immune modulation and cholesterol regulation (Patel *et al.*, 2021). Studies by Gakuya *et al.* (2020) in Kenya have emphasized their antimicrobial and anti-inflammatory applications in traditional medicine. That said, Kareru *et al.* (2021) point out that the mere presence of these compounds does not always guarantee significant therapeutic effects, especially in crude herbal preparations.

The absence of tannins and phenols in the plant parts tested is an intriguing finding, as these compounds are often associated with strong antioxidant activity (Singh *et al.*, 2020). This could reflect the specific environmental conditions like stresses causing defensive mechanism, varying ecological zones, climatic conditions in which *Guizotia scabra* grew or the methods used for phytochemical extraction. A study, such as those by Kigen *et al.* (2019), have reported variations in tannin and phenol content across plant species, suggesting that their absence in *Guizotia scabra* may not be unusual but warrants further investigation.

Quinones were detected in all plant parts and are widely recognized for their antibacterial and anticancer properties (Gupta *et al.*, 2022). Locally, quinone containing plants have been used in Kenya to treat infections and other ailments, as noted by Muregi *et al.* (2020). However, Guest *et al.* (2021) caution that quinone concentration and effectiveness can be heavily influenced by soil composition and other environmental factors.

The phytochemical analysis of *Guizotia scabra* extracts confirmed the presence of flavonoids, alkaloids, saponins, and quinones, all of which have been extensively reported for their antibacterial properties (Sharma *et al.*, 2021). Flavonoids, particularly myricetin and quercetin determined under HPLC-UV Analysis of *Guizoia scabra* extracts are known to exhibit antibacterial activity by disrupting bacterial cell membranes and inhibiting enzymatic activity (Kumar *et al.*, 2022). Alkaloids also contribute to antibacterial effects by interfering with DNA replication and protein synthesis in bacterial cells (Nguyen *et al.*, 2023). The presence of saponins further enhances antibacterial efficacy through their ability to form complexes with sterols in bacterial membranes, leading to increased permeability and cell lysis (Chen *et al.*, 2022).

Overall, the results (Table 4.3) demonstrate that *Guizotia scabra* contains a diverse range of secondary metabolites that may contribute to its therapeutic potential. The presence of these bioactive compounds further suggests that the extracts may possess antibacterial properties, supporting their possible use in dental caries control.

5.3 Analysis of phytochemicals in *Guizotia scabra* extracts by Thin-layer chromatography

Thin-layer chromatography (TLC) analysis of *Guizotia scabra* leaf, stem bark, and root extracts revealed five distinct spots with varying R_f values under different solvent systems (H:E and H:A). Leaf extracts generally showed more spots than stem bark and root, suggesting greater phytochemical richness and compound diversity. The spot colors (yellow-green, brown-yellow, orange, light purple, red-brown) indicated the presence of diverse phytochemical classes, consistent with findings from ethanol and acetone TLC

profiles (Dubale *et al.*, 2023). Variation in R_f values reflects differences in compound polarity and solubility, highlighting the role of solvent systems in separation. As shown in (Table 4.3, Table 4.4, Table 4.5), supporting the need for targeted solvent systems to optimize compound isolation (Singh & Pandey, 2020).

These results confirm the diverse distribution of multiple bioactive constituents across plant parts and align with earlier studies that demonstrated TLC as a reliable method for detecting antibacterial phytochemicals in medicinal plants (Patil *et al.*, 2021).

5.4 High performance liquid chromatography analysis of selected phytochemicals in extracts of *Guizotia scabra*.

High-performance liquid chromatography (HPLC) quantitative analysis of *Guizotia scabra* extracts identified the presence of individual key flavonoids, particularly myricetin and quercetin, as evidenced by retention times (R_t) consistent with reference standards. Quantitative analysis revealed variations in their distribution across leaf, stem bark, and root extracts, indicating that different plant parts may possess distinct phytochemical richness and potential therapeutic roles (Table 4.6).

Myricetin, identified in the extracts, is known to inhibit *Streptococcus mutans*, the principal cariogenic bacterium, by reducing biofilm formation and acid production (Zhou *et al.*, 2021). Similarly, quercetin demonstrated strong antibacterial and anti-inflammatory activity, underscoring its potential as a natural candidate for dental caries prevention and oral health promotion (Gao *et al.*, 2022). The chromatograms further revealed additional peaks corresponding to other phenolic and aromatic compounds, highlighting the chemical diversity of *Guizotia scabra*.

The differences in peak intensities and R_t values across plant parts suggest variations in concentration and distribution of these bioactive compounds (Table 4.7). Such diversity not only supports the results of phytochemical screening and TLC profiling but also emphasizes the therapeutic versatility of the plant. Overall, HPLC analysis provides strong evidence for the presence of flavonoids with antibacterial potential, reinforcing the traditional use of *Guizotia scabra* in managing oral infection.

5.5 FTIR analysis of functional group absorption frequency of phytochemical present in plant extracts of *Guizotia scabra*.

FTIR spectroscopy was employed to characterize the phytochemical constituents of *Guizotia scabra* leaf, stem bark, and root extracts. The spectra revealed both shared and distinct functional groups across plant parts, highlighting their biochemical diversity. In the 3700–3200 cm^{-1} region, strong absorption bands were detected in the leaf and bark (3441.01 cm^{-1}), corresponding to hydrogen-bonded O–H and N–H groups. These are characteristic of flavonoids, tannins, and alkaloids, with quercetin and myricetin serving as representative compounds. The leaf spectrum also showed a unique strong band at 3759.26 cm^{-1} , weakly expressed in bark but absent in root, which may be linked to additional hydroxyl or amine groups in alkaloid structures, further emphasizing leaf phytochemical richness. The 1750–1500 cm^{-1} region exhibited strong peaks at 1643.35 cm^{-1} in all plant parts, attributed to C=O stretching in carbonyl groups. This suggests the presence of terpenoids, flavonoids, and glycosides, consistent with HPLC identification of myricetin (O–H, C=O, and aromatic ring systems). This region is also indicative of structural compounds like lignin in stem bark and

glycosylated flavonoids in roots. Absorptions in the 2920 cm^{-1} region correspond to aliphatic C–H stretching, pointing to lipids, terpenoids, and steroids. This was evident across all extracts, though more prominent in the stem, consistent with its structural support role. The 1396 cm^{-1} region showed bending vibrations of C–H bonds, often associated with alkanes and detection of tannins and phenolic acids particularly in the leaf and stem extracts. The $1250\text{--}1030\text{ cm}^{-1}$ region indicated C–N and C–O stretching vibrations, strongly represented in leaf and root extracts.

These are characteristic of alkaloids, glycosides, polysaccharides, and anthraquinones, linking well with phytochemical qualitative test results as discussed and structures drawn in chapter two. The band at 1031.92 cm^{-1} further supports ether and amine vibrations, suggesting anthraquinones and flavonoid glycosides, with myricetin contributing in this region.

A weak band around 2000 cm^{-1} suggests the presence of cyano ($\text{--C}\equiv\text{N}$) groups, which may be linked to terpenoids, flavonoids, and alkaloid derivatives. Meanwhile, bending vibrations at $538\text{--}515\text{ cm}^{-1}$ are consistent with metal–ligand complexes or benzene derivatives (Smith, 2011), suggesting possible coordination of secondary metabolites with mineral elements.

Table 5.1: Phytochemicals Identified from FTIR Spectra

Plant extracts	Major /Minor Peaks (cm ⁻¹)	Possible Functional Groups	Phytochemicals Indicated
Leaf	3759,3441,	O-H,N-H group,	Flavonoids(Quercetin), Alkaloids, glycosides, terpenoids, Myrecitin Carbohydrates Alkaloids
	2929,	C-H stretching	
	1643, 1396,	C=C/C=O stretching,	
	1252, 1031, 515	C-N stretching, C-O benzene derivative	
Stem	3759, 2920, 2360, 1988-2000,1624,1386,	N/ O-H stretching, aliphatic C-H, aromatic C=C, C-O cyano (-C≡N) stretching	Polysaccharides, lignin, flavonoids, alkaloids, terpenoids,
	538-471,1024,	Metal ligand/Benzene derivatives	
Root	3400, 2920-2850, 1630-1650,1380-1400,	Hydroxyl (H-bonded), aliphatic C-H, aromatic C=C, C-O stretching	Carbohydrates (cellulose, starch, glycosides), terpenoids, lignin, alkaloids Anthroquinones,alkaloids,Myrecitin Benzene derivative
	1029, 542	Ether/amine/C-O Metal-ligand	

Comparatively, the leaf extract was enriched in phenols and flavonoids (strong broad O-H peaks, additional 3759.26 cm⁻¹ band), consistent with its role in antioxidant defence. The stem extract showed a broader range of peaks including strong C-O and aliphatic stretches, reflecting higher contributions of polysaccharides, lignin, and structural flavonoids. The root extract emphasized carbohydrate-related peaks (1030 cm⁻¹, 2920 cm⁻¹), supporting its role in storage, with moderate contributions from glycosides, terpenoids, and lignin.

Overall, FTIR results showed that *Guizotia scabra* may contain a wide array of phytochemical groups of flavonoids (quercetin, myricetin), terpenoids, tannins, glycosides, lignin, and alkaloids, with distinct distributions across plant parts.

These findings collaborated TLC and HPLC analyses further establishing the leaf as the richest source of antioxidant flavonoids alkaloids and other bioactive compounds.

5.6 Antibacterial activity of the tender leaf, stem bark and root extracts of *Guizotia scabra* and commercial toothpastes against *streptococci* bacteria using bioassay

The antibacterial activity of *Guizotia scabra* extracts was evaluated against *Streptococcus mutans* and *Streptococcus sobrinus*, (gram positive) (Bekele, T., Abebe, G., & Alemu, D. (2022) major pathogens implicated in dental caries and plaque formation.

5.6.1 Sensitivity Patterns of *streptococci* bacteria strains to Ethanol and acetone Extract as Measured by Zone of Inhibition

The results (Table 4.11 and 4.12) demonstrated significant antibacterial effects of both ethanol and acetone extracts, with the leaf ethanol extract showing the highest inhibition. This suggests that leaves contain higher concentrations of bioactive compounds compared to roots and stem bark.

Streptococcus mutans is a primary cariogenic bacterium, and its susceptibility to plant-derived phytochemicals has been widely reported (Akinmoladun *et al.*, 2016; Adeyemo *et al.*, 2019). In this study, the leaf extract of *Guizotia scabra* exhibited the largest zone of inhibition (5.0 mm, 62.5%), attributable to phytochemicals such as flavonoids, alkaloids, and saponins (Chah *et al.*, 2015; Olajuyigbe & Afolayan, 2017). These compounds are known to interfere with bacterial membranes and enzymatic activities (Ncube *et al.*, 2012). The root extract (3.0 mm, 37.5%) exhibited moderate inhibition, likely due to its lower phytochemical content, while stem extracts were the least effective. These findings align

with previous reports linking phenolic-rich extracts with *S. mutans* inhibition (Zhou *et al.*, 2014).

Commercial toothpastes (Brand 1 and herbal Brand 2) displayed stronger antibacterial effects than *Guizotia scabra* extracts, with Brand 2 showing the highest inhibition (87.5%). This can be attributed to fluoride and other optimized antibacterial agents, which are well-documented for disrupting bacterial metabolism and suppressing acid production (Marinho *et al.*, 2013). Nevertheless, some studies such as Ochieng *et al.* (2015) reported weaker activity of *Guizotia scabra* compared to other medicinal plants like *Azadirachta indica* and *Acacia nilotica* suggesting that extraction method, plant age, and environmental factors influence its antibacterial potency.

Similarly, *S. sobrinus* another cariogenic bacterium often coexisting with *S. mutans*, was moderately inhibited by *Guizotia scabra*. The leaf extract produced the highest inhibition (4.0 mm, 50%), while the root (2.5 mm, 31.25%) and stem (1.5 mm, 18.75%) extracts exhibited weaker activity. These results support the notion that secondary metabolites such as tannins, flavonoids, and terpenoids were more concentrated in leaf (Adeyemo *et al.*, 2019). However, variability exists, for instance, Kiplimo *et al.* (2020) found that *S. sobrinus* was more resistant to extracts of other Kenyan plants, suggesting strain differences and phytochemical variation may affect outcomes.

While commercial toothpastes consistently outperformed *Guizotia scabra* extracts, the plant still showed promising antibacterial activity. Its ethanol leaf extract, in particular, demonstrated comparable inhibition to some toothpaste formulations, especially against *S. mutans*. This reinforces its potential as a natural, sustainable alternative in oral care,

especially in low-resource settings where access to commercial toothpaste may be limited (Akinmoladun *et al.*, 2016). Nonetheless, concerns about fluoride sensitivity in certain populations (Sadeghi *et al.*, 2020) further highlight the relevance of exploring natural alternatives.

5.6.2 Comparative antibacterial activity of ethanol, acetone Extracts of *Guizotia scabra* and Commercial Toothpastes

The antibacterial activity of *Guizotia scabra* extracts varied between ethanol and acetone solvents. Ethanol extracts, particularly from the leaves, showed the largest inhibition zones against cariogenic bacteria. This can be attributed to ethanol's broad ability to extract diverse bioactive phytochemicals such as flavonoids, phenolic acids, tannins, and alkaloids, which are associated with antimicrobial effects (Sankaranarayanan *et al.*, 2018). These compounds disrupt bacterial cell membranes and inhibit enzyme functions critical to bacterial survival (Patel *et al.*, 2020).

In contrast, acetone though also polar, extracted a narrower range of phytochemicals, favouring less polar constituents with weaker antimicrobial activity (Sharma *et al.*, 2020), which explains the smaller inhibition zones observed in root and stem extracts. When compared to commercial toothpastes, the latter performed better overall due to the presence of fluoride and other optimized active ingredients such as triclosan and essential oils. Fluoride, in particular inhibits bacterial enzymes and supports tooth remineralization, contributing to its antibacterial effect (Lima *et al.*, 2021).

TLC showed the presence of compounds of varying polarity, while FTIR revealed functional groups such as hydroxyl (-OH), carbonyl (C=O), Cyano ($-C\equiv N$), (C-O, C-

H,(C–N stretching) and aromatic rings, consistent with flavonoids, terpenoids and alkaloids identified in qualitative standard laboratory tests of phytochemical. The bioassay results demonstrated that leaf ethanol extracts were especially effective, supporting phytochemical evidence that leaves contain higher levels of antioxidant and antibacterial flavonoids. While toothpastes exhibited stronger inhibition overall, *Guizotia scabra* extracts nonetheless showed promising antibacterial potential. HPLC-UV further resolved individual compounds, with peaks corresponding to flavonoids and other metabolites detected in standard qualitative laboratory tests.

Collectively, TLC, HPLC-UV, FTIR, and bioassays confirmed that the phytochemical richness of *Guizotia scabra*, especially in flavonoids and alkanoids underpins its antibacterial bioactivity. These findings reinforce its potential as a natural alternative or complement to synthetic agents for managing dental caries.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study investigated the phytochemical composition and antibacterial activity of ethanol and acetone extracts of *Guizotia scabra* tender leaf, stem bark, and root. Standard laboratory test procedures confirmed the presence of key phytochemicals such as alkaloids, flavonoids, anthraquinones, terpenoids, steroids, saponins, and quinones, while tannins and phenols were absent. Of the plant parts, tender leaves exhibited the richest phytochemical profile, which corresponds to their stronger antibacterial activity. This finding supports the traditional use of *Guizotia scabra* leaf extract in managing dental infections and highlights its therapeutic potential.

Advanced analytical techniques such as HPLC and FTIR validated and expanded the phytochemical findings. TLC revealed varied R_f values corresponding to different compounds, while HPLC identified peaks suggestive of flavonoids such as quercetin and myricetin, with higher concentrations in leaf extracts. FTIR spectra further confirmed functional groups such as hydroxyl, carbonyl and amine groups consistent with bioactive phytochemicals. Together these techniques provided comprehensive evidence of phytochemical richness in *Guizotia scabra* extracts.

Antibacterial assays demonstrated that *Guizotia scabra* extracts particularly ethanol leaf extracts exhibited notable activity against *Streptococcus mutans* and *Streptococcus sobrinus*, the primary bacteria responsible for dental caries. The root and stem bark extracts showed moderate activity, while acetone extracts generally performed weaker compared

to ethanol extracts. When compared to commercial toothpaste products the extracts demonstrated lower antibacterial activity, as commercial formulations contain fluoride and synthetic agents that enhance antibacterial potency. Nonetheless, the results validate the traditional use of *Guizotia scabra* leaf extract and underscore its promise as a natural oral healthcare agent.

Overall, the findings confirm that the bioactive potential of *Guizotia scabra* leaf extract is strongly linked to its phytochemical composition. The study contributes to the scientific validation of indigenous knowledge and demonstrated the potential of this plant as a natural antibacterial agent in dental care applications.

6.2 Recommendations

To build upon the phytochemical analysis results, future studies should undertake comprehensive isolation and structural elucidation of individual bioactive compounds from *Guizotia scabra* using LC-MS, UHPLC, UV-VIS and NMR spectroscopy. Advanced chromatographic and spectroscopic techniques should be employed to standardize and quantify these compounds. This will enable the identification and characterization of phytochemicals responsible for medicinal activity and support the development of quality controlled herbal formulations for oral care, while also adding to the scientific validation of traditionally used plant-based remedies.

Further phytochemical analysis is recommended to refine and expand the current understanding of *Guizotia scabra's* bioactive profile. Also determine MIC and MBC values as well as biofilm inhibition zones. Such studies would facilitate the standardization of extracts, improve reproducibility and enable formulation of effective and consistent

products. This will also help ensure that active compounds are present in sufficient concentrations for therapeutic efficacy in oral healthcare applications.

It is recommended that in vivo studies and clinical trials be conducted to assess the antibacterial efficacy of *Guizotia scabra* extracts in real-life oral healthcare settings. These trials should evaluate the plant's effectiveness in managing dental issues such as plaque, cavities, and gum inflammation. Additionally, studies should explore synergistic effects with other herbal or conventional agents to enhance potency. Evaluate toxicological effects and safety as well as determine appropriate dosage and delivery methods (e.g. mouthwash or toothpaste) for practical application of the leaf extract.

Although *Guizotia scabra* extracts showed promising antibacterial properties, they were outperformed by commercial toothpastes. It is therefore recommended to explore the integration of *Guizotia scabra* leaf extract compounds into improved herbal formulations (mouthwash, toothpaste) that may include fluoride or other approved active agents. Public education campaigns should also be launched to promote awareness and acceptance of plant-based oral care alternatives. This approach may help diversify dental product choices, especially in regions with limited access to affordable conventional products.

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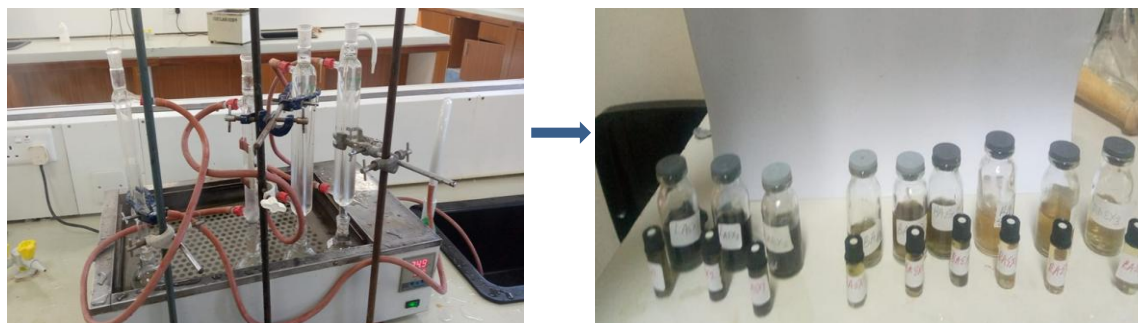
APPENDICES

Appendix I: Crushed *Guizotia scabra* plant parts (Source: Author, 2024)



(1) Crushed stem bark sample, (2) Crushed leaf samples and (3) Crushed root sample

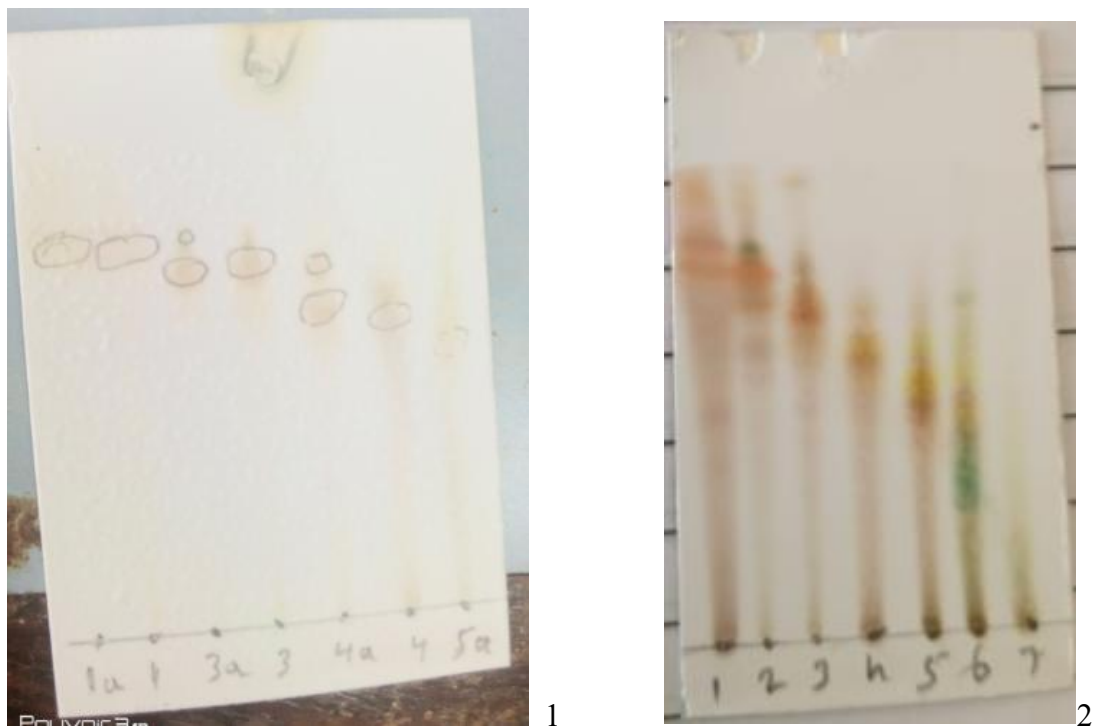
Appendix II: Extraction Process and the Extracts



a) Extraction set-up

b) Extracts stored in air-tight containers.

(Source: Author, 2024)

Appendix III: TLC Plates of extracts

(1) TLC Plate of Isolated crude extracts and (2): TLC Plate purified compounds

Source: Author (2024)

Appendix IV: Pooled fractions of plant extracts in vials (Source: Author, 2024)

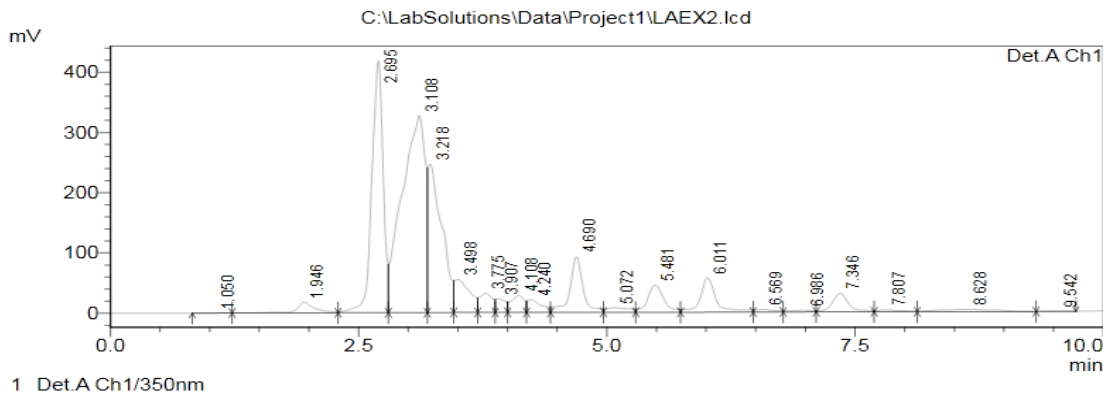
POOL A: Ethanol based extracts



POOL B: Acetone based extracts; **Source: Author (2024)**

Appendix V: HPLC chromatogram and Rt of *Myricetin* and *Quercetin* in *Guizoa scabra* tender leaf, stem bark and root samples

<Chromatogram>



Calibration Curve

ID# : 1
 Name : Myricetin
 Quantitative Method : External Standard
 Function : $f(x)=4.26201e-005*x-0.0908617$

Detector A Ch1 350nm

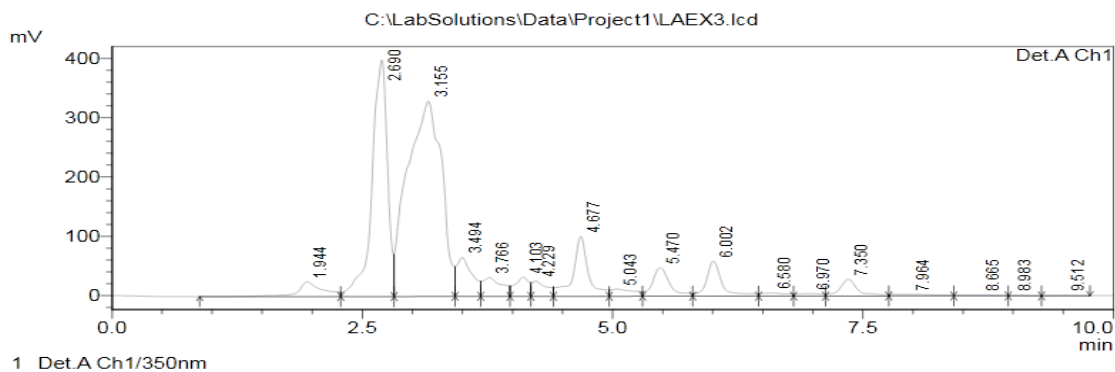
Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.050	1132	86	0.007	0.006
2	1.946	221833	17803	1.423	1.256
3	2.695	3337074	419095	21.404	29.576
4	3.108	5340165	327290	34.252	23.097
5	3.218	2402905	246529	15.412	17.398
6	3.498	592428	54761	3.800	3.865
7	3.775	288402	32135	1.850	2.268
8	3.907	153996	22842	0.988	1.612
9	4.108	263041	28701	1.687	2.025
10	4.240	211505	21180	1.357	1.495
11	4.690	855970	92376	5.490	6.519
12	5.072	123384	7411	0.791	0.523
13	5.481	506553	45066	3.249	3.180
14	6.011	624055	56856	4.003	4.012
15	6.569	56278	4066	0.361	0.287
16	6.986	46062	2503	0.295	0.177
17	7.346	354902	30929	2.276	2.183
18	7.807	53478	3216	0.343	0.227
19	8.628	151790	3684	0.974	0.260
20	9.542	5675	476	0.036	0.034
Total		15590626	1417004	100.000	100.000

Quantitative Results

Detector A					
ID#	Name	Ret. Time	Area	Height	Conc.
1	Myricetin	5.072	123384	7411	5.168
2	Quercetin	6.986	46062	2503	1.034

4 LAEX2 (leaf extract sample 1)

<Chromatogram>



Calibration Curve

ID# : 1
 Name : Myricetin
 Quantitative Method : External Standard

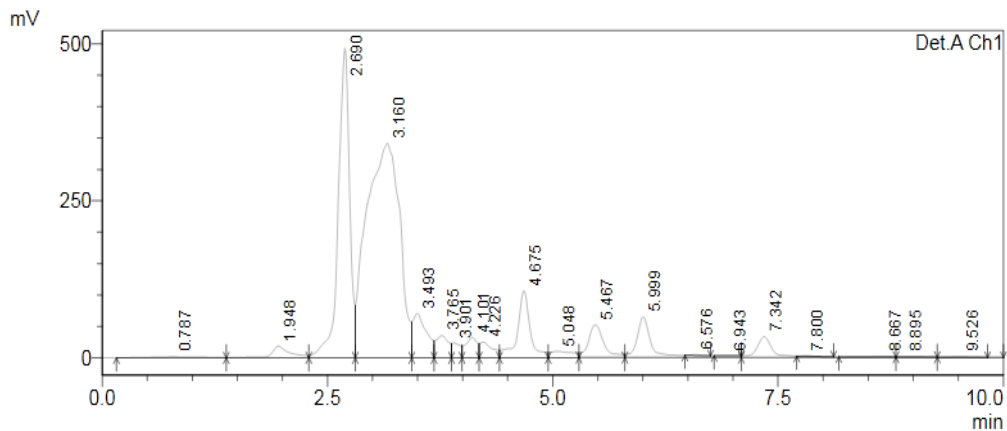
PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.944	429116	25120	2.471	2.144
2	2.690	4319881	399063	24.878	34.058
3	3.155	7781970	329398	44.816	28.113
4	3.494	706046	65681	4.066	5.606
5	3.766	421524	31580	2.428	2.695
6	4.103	312015	32269	1.797	2.754
7	4.229	263166	25499	1.516	2.176
8	4.677	1073054	100759	6.180	8.599
9	5.043	196605	11840	1.132	1.010
10	5.470	570950	47978	3.288	4.095
11	6.002	632109	59033	3.640	5.038
12	6.580	88755	5195	0.511	0.443
13	6.970	68946	3979	0.397	0.340
14	7.350	351939	28204	2.027	2.407
15	7.964	78838	2657	0.454	0.227
16	8.665	43961	1548	0.253	0.132
17	8.983	13982	1102	0.081	0.094
18	9.512	11366	793	0.065	0.068
Total		17364223	1171698	100.000	100.000

Quantitative Results

ID#	Name	Ret. Time	Area	Height	Conc.
1	Myricetin	5.043	196605	11840	8.288
2	Quercetin	6.970	68946	3979	1.602

LAEX3 (leaf extract sample 2)



1 Det.A Ch1/350nm

Calibration Curve

ID# : 1
 Name : Myricetin
 Quantitative Method : External Standard
 Function : $f(x)=4.26201e-005*x-0.0908617$
 Rr1=0.9866812 Rr2=0.9735398
 MeanRF:4.15125e-005 RFSD:3.13481e-006 RFRSD:7.55149

Detector A Ch1 350nm

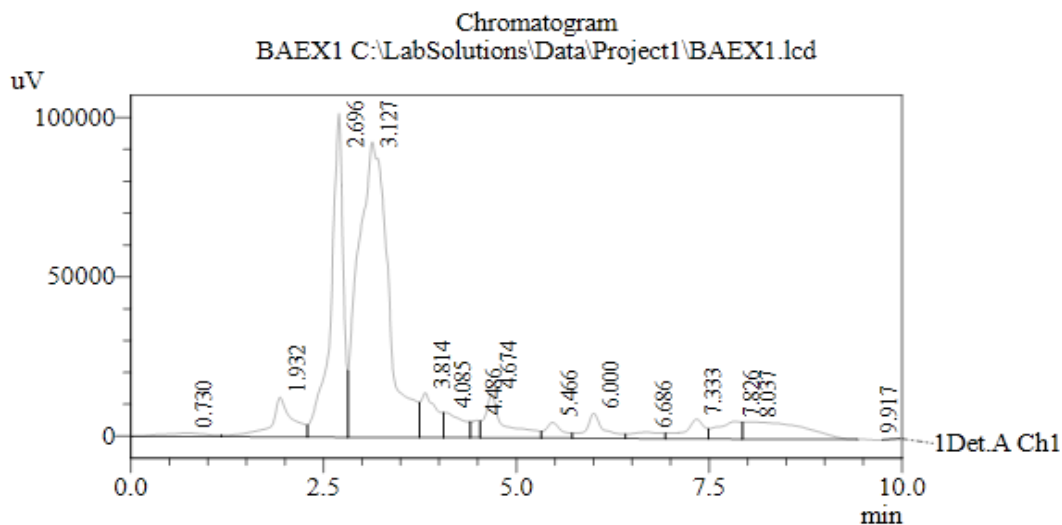
Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.787	57587	1596	0.315	0.121
2	1.948	257134	18694	1.404	1.423
3	2.690	4323555	493357	23.614	37.547
4	3.160	8674449	341285	47.377	25.973
5	3.493	742813	70572	4.057	5.371
6	3.765	338315	35232	1.848	2.681
7	3.901	149751	23450	0.818	1.785
8	4.101	294253	32111	1.607	2.444
9	4.226	242286	25003	1.323	1.903
10	4.675	1041250	106468	5.687	8.103
11	5.048	179221	10125	0.979	0.771
12	5.467	624088	52501	3.409	3.996
13	5.999	803921	64856	4.391	4.936
14	6.576	10517	1372	0.057	0.104
15	6.943	1698	165	0.009	0.013
16	7.342	502704	33269	2.746	2.532
17	7.800	7111	810	0.039	0.062
18	8.667	21925	1034	0.120	0.079
19	8.895	17987	901	0.098	0.069
20	9.526	18854	1178	0.103	0.090
Total		18309419	1313978	100.000	100.000

Quantitative Results

Detector A					
ID#	Name	Ret. Time	Area	Height	Conc.
1	Myricetin	5.048	179221	10125	7.548
2	Quercetin	6.943	1698	165	-0.068

5 BAEX 1(leaf extract sample 3)

Source: Author (2024)



1 Det.A Ch1 / 350nm

PeakTable

Detector A Ch1 350nm

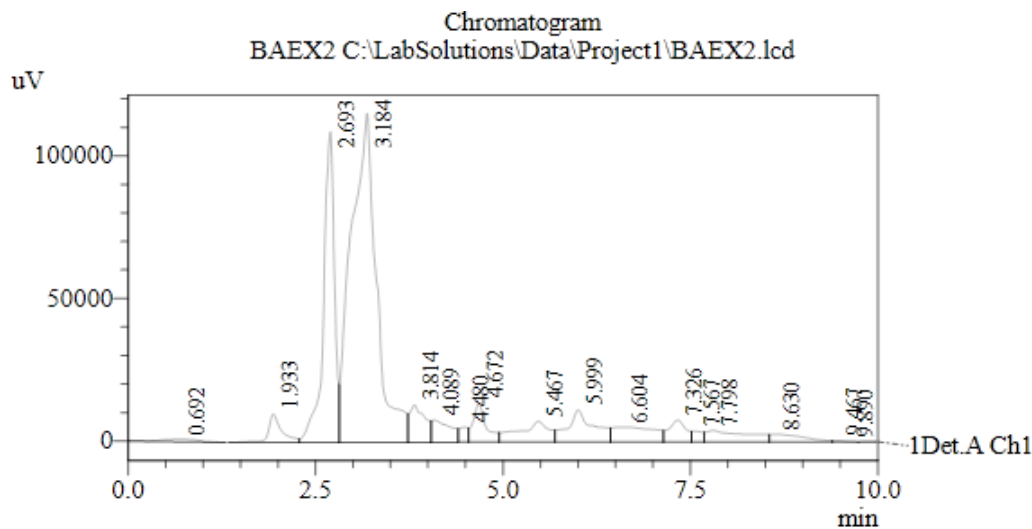
Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.730	42207	1058	0.798	0.377
2	1.932	239245	12368	4.523	4.412
3	2.696	1136983	101559	21.493	36.231
4	3.127	2513859	92686	47.521	33.066
5	3.814	200220	14012	3.785	4.999
6	4.085	128743	7867	2.434	2.807
7	4.486	41872	5393	0.792	1.924
8	4.674	230169	13757	4.351	4.908
9	5.466	70022	4865	1.324	1.736
10	6.000	121526	7815	2.297	2.788
11	6.686	53189	1943	1.005	0.693
12	7.333	106714	6121	2.017	2.183
13	7.826	116682	5418	2.206	1.933
14	8.037	287220	5306	5.430	1.893
15	9.917	1310	143	0.025	0.051
Total		5289960	280308	100.000	100.000

Quantitative Results

Detector A

ID#	Name	Ret. Time	Area	Height	Conc.
1	Myricetin	0.000	0	0	0.000
2	Quercetin	7.333	106714	6121	2.539

6 BAEX2 (stem bark extract sample 1)



1 Det.A Ch1 / 350nm

PeakTable

Detector A Ch1 350nm

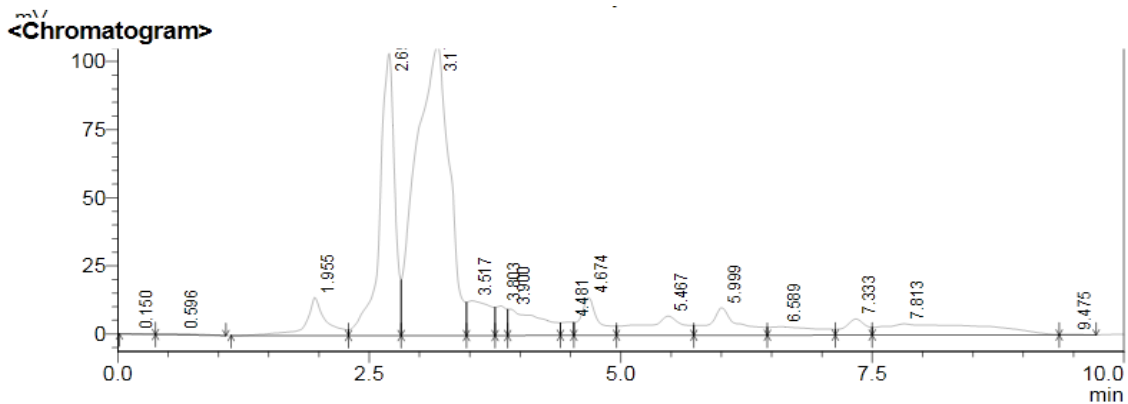
Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.692	30586	925	0.547	0.292
2	1.933	118841	9921	2.126	3.127
3	2.693	1108521	108929	19.827	34.332
4	3.184	2718476	115215	48.623	36.313
5	3.814	185753	13084	3.322	4.124
6	4.089	133032	7693	2.379	2.425
7	4.480	42877	5206	0.767	1.641
8	4.672	165689	13764	2.964	4.338
9	5.467	202732	7246	3.626	2.284
10	5.999	272604	11301	4.876	3.562
11	6.604	204181	5283	3.652	1.665
12	7.326	122531	7706	2.192	2.429
13	7.567	36054	3718	0.645	1.172
14	7.798	158992	4045	2.844	1.275
15	8.630	83475	2647	1.493	0.834
16	9.467	5388	440	0.096	0.139
17	9.890	1163	157	0.021	0.050
Total		5590896	317279	100.000	100.000

Quantitative Results

Detector A

ID#	Name	Ret. Time	Area	Height	Conc.
1	Myricetin	0.000	0	0	0.000
2	Quercetin	7.326	122531	7706	2.932

7 BAEX3 (stem bark extract sample 2)



Calibration Curve

ID# : 1
 Name : Myricetin
 Quantitative Method : External Standard
 Function : $f(x)=4.26201e-005*x-0.0908617$
 Rr1=0.9866812 Rr2=0.9735398

PeakTable

Detector A Ch1 350nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.150	3105	129	0.058	0.042
2	0.596	12645	463	0.238	0.151
3	1.955	193644	14018	3.639	4.565
4	2.693	1081529	103625	20.322	33.746
5	3.175	2427009	106419	45.603	34.656
6	3.517	199273	12736	3.744	4.148
7	3.803	77518	10871	1.457	3.540
8	3.900	213367	9647	4.009	3.142
9	4.481	38367	4916	0.721	1.601
10	4.674	169401	13837	3.183	4.506
11	5.467	198231	6993	3.725	2.277
12	5.999	207063	10075	3.891	3.281
13	6.589	104789	3041	1.969	0.990
14	7.333	85030	5935	1.598	1.933
15	7.813	307346	4086	5.775	1.331
16	9.475	3670	280	0.069	0.091
Total		5321988	307071	100.000	100.000

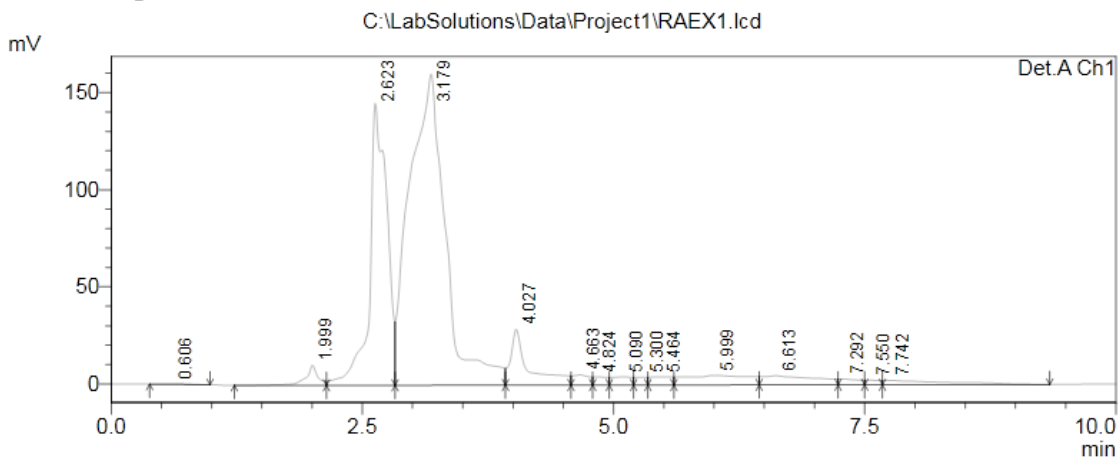
Quantitative Results

Detector A

ID#	Name	Ret. Time	Area	Height	Conc.
1	Myricetin	0.000	0	0	0.000
2	Quercetin	7.333	85030	5935	2.001

BAEX3 (stem bark extract sample 3)

<Chromatogram>



Calibration Curve

ID# : 1
 Name : Myricetin
 Quantitative Method : External Standard
 Function : $f(x)=4.26201e-005*x-0.0908617$
 Rr1=0.9866812 Rr2=0.9735398
 MeanRF:4.15125e-005 RFSD:3.13481e-006 RFRSD:7.55149

PeakTable

Detector A Ch1 350nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.606	4927	232	0.070	0.061
2	1.999	87827	10360	1.256	2.703
3	2.623	1751156	145148	25.033	37.871
4	3.179	3972095	160243	56.782	41.809
5	4.027	358685	28717	5.127	7.493
6	4.663	61387	5202	0.878	1.357
7	4.824	40174	4117	0.574	1.074
8	5.090	57873	4146	0.827	1.082
9	5.300	33394	3962	0.477	1.034
10	5.464	63339	4208	0.905	1.098
11	5.999	221097	4951	3.161	1.292
12	6.613	176339	4653	2.521	1.214
13	7.292	41655	2889	0.595	0.754
14	7.550	23396	2270	0.334	0.592
15	7.742	101980	2174	1.458	0.567
Total		6995323	383273	100.000	100.000

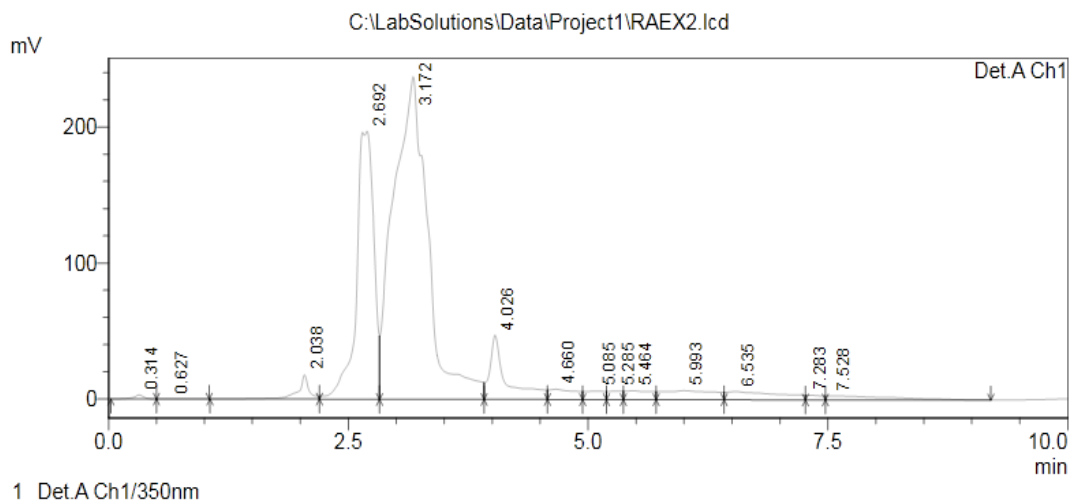
Quantitative Results

Detector A

ID#	Name	Ret. Time	Area	Height	Conc.
1	Myricetin	5.090	57873	4146	2.376
2	Quercetin	7.292	41655	2889	0.924

8 RAEX2 (root extract sample 1)

<Chromatogram>



Calibration Curve

ID# : 1
 Name : Myricetin
 Quantitative Method : External Standard
 Function : $f(x) = 4.26201e-005 * x - 0.0908617$
 Rr1=0.9866812 Rr2=0.9735398
 M... ..

PeakTable

Detector A Ch1 350nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.314	23550	3041	0.230	0.555
2	0.627	3491	172	0.034	0.031
3	2.038	144588	17932	1.414	3.270
4	2.692	2508041	196962	24.520	35.919
5	3.172	5890339	237454	57.587	43.303
6	4.026	565778	47206	5.531	8.609
7	4.660	146645	7567	1.434	1.380
8	5.085	91565	6295	0.895	1.148
9	5.285	64632	6271	0.632	1.144
10	5.464	124484	6408	1.217	1.169
11	5.993	252359	6614	2.467	1.206
12	6.535	233863	6006	2.286	1.095
13	7.283	40032	3463	0.391	0.631
14	7.528	139145	2967	1.360	0.541
Total		10228513	548355	100.000	100.000

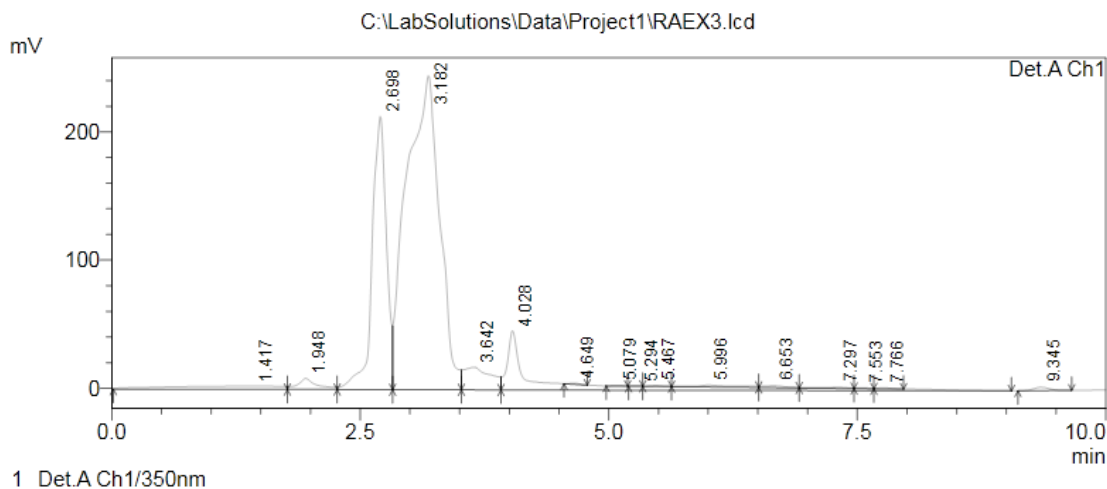
Quantitative Results

Detector A

ID#	Name	Ret. Time	Area	Height	Conc.
1	Myricetin	5.085	91565	6295	3.812
2	Quercetin	7.283	40032	3463	0.884

9 RAEX3 (root extract sample 2)

<Chromatogram>



Calibration Curve

ID# : 1
 Name : Myricetin
 Quantitative Method : External Standard
 Function : $f(x)=4.26201e-005*x-0.0908617$
 Rr1=0.9866812 Rr2=0.9735398
 MeanRF:4.15125e-005 RFSD:3.13481e-006 RFRSD:7.55149

PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.417	200689	2433	2.090	0.448
2	1.948	115539	8681	1.203	1.598
3	2.698	2145138	213088	22.339	39.224
4	3.182	5635561	245118	58.687	45.120
5	3.642	335812	17614	3.497	3.242
6	4.028	1025278	46250	10.677	8.514
7	4.649	5851	836	0.061	0.154
8	5.079	2244	360	0.023	0.066
9	5.294	1447	244	0.015	0.045
10	5.467	8337	749	0.087	0.138
11	5.996	44676	1451	0.465	0.267
12	6.653	23818	1599	0.248	0.294
13	7.297	16516	1017	0.172	0.187
14	7.553	4738	454	0.049	0.084
15	7.766	6703	660	0.070	0.122
16	9.345	30455	2704	0.317	0.498
Total		9602801	543258	100.000	100.000

Quantitative Results

ID#	Name	Ret. Time	Area	Height	Conc.
1	Myricetin	5.079	2244	360	0.005
2	Quercetin	7.297	16516	1017	0.300

RAEX3 (root extract sample 3)

Appendix VI: Plant Extracts zone of Inhibition. (Source: Author, 2024)

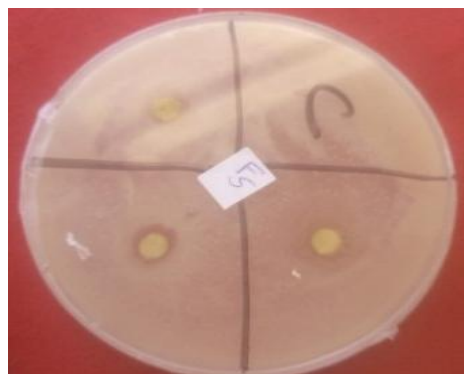
Leaf Ethanol Extract inhibition zone



Leaf Acetone Extract inhibition zone



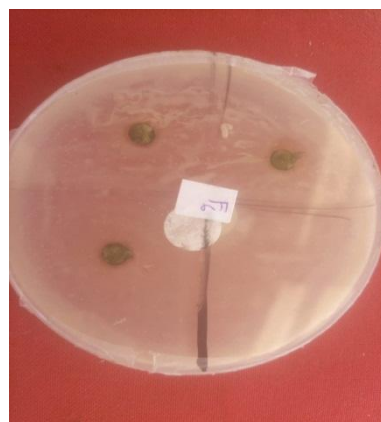
Stem bark Ethanol inhibition zone



stem bark Acetone Extract inhibition zone



Root Ethanol extract inhibition zone



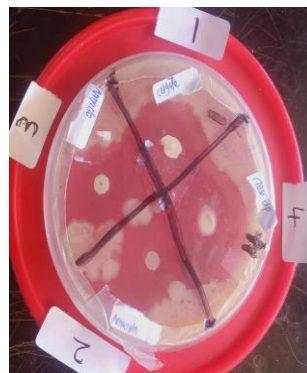
Root Acetone extract inhibition zone

Appendix VII: Commercial toothpastes zone of Inhibition (Source: Author, 2024)

Start set-up



Brand 1



Brand 2(herbal)

Appendix VIII: Similarity Report



University of Eldoret
Certificate of Plagiarism Check for Thesis



Author Name	ANJELINE JEPCHUMBA PAUL SSCI/CHEM/ M/007/18
Course of Study	Type here...
Name of Guide	Type here...
Department	Type here...
Acceptable Maximum Limit	Type here... <input type="text"/>
Submitted By	titustoo@uoeld.ac.ke
Paper Title	PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF Guizotia scabra LEAF, STEM BARK AND ROOT EXTRACTS FOR DENTAL CARIES CONTROL
Similarity	5%
Paper ID	4607064
Total Pages	136
Submission Date	2025-11-01 15:13:30

Signature of Student



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Signature of Guide

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Director of Post Graduate Studies

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