FACULTY OF SCIENCE AND ENGINEERING

CHEMISTRY DEPARTMENT

PROJECT TITLE: FORMULATION AND EVALUATION OF A DIABETES PROPHYLACTIC SYRUP USING PHYTOCHEMICALS FROM *PSIDIUM GUAJAVA*

NAME	**EMILY R THOM**
REG NUMBER	**B211018B**
PROGRAMME	**CHEMICAL TECHNOLOGY**
PROJECT SUPERVISOR	**PROFESSOR DZOMBA**

APPROVAL FORM

The undersigned certify that they have supervised, read and recommend to the Bindura University of Science Education for the acceptance of a research dissertation entitled**: Formulation and evaluation of a diabetes prophylactic syrup using phytochemicals from *Psidium guajava*** submitted by **Emily Rumbidzai Thom** in the partial fulfillment of the requirements for the Bachelor of Science Honours Degree in Chemical Technology.

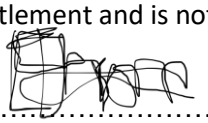
(Signature of student) …… Date…15/08/2025……

(Signature of Supervisor) ……Date……4/9/25……

(Signature of Chairperson) ……Date……**02/10/2025**…

DECLARATION

I Emily Rumbidzai Thom am officially declaring that this dissertation is a product of my original research and that I am the sole author of the work; as such borrowed ideas are cited accordingly as references. This work has not been accepted elsewhere for the conferment of any academic entitlement and is not concurrently submitted in candidature of any such awards.

Sign  …….. Date15/08/2025…….

1. ABSTRACT

Diabetes mellitus is a growing global health concern especially in low resource areas where access to conventional medication can be limited. This project focuses on the formulation and evaluation of a prophylactic syrup derived from the phytochemicals of *Psidium guajava* (guava) leaves in order to prevent the onset of diabetes. Fresh guava leaves 200g were collected, oven dried and subjected to solvent extraction using ethyl acetate, hexane and butanol. Phytochemical screening revealed the presence of flavonoids, tannins, saponins and phenolic compounds bio-active constituents known for their anti-diabetic potential. A total of three distinct fraction were obtained using the DPPH assay with the ethyl acetate extract showing the highest radical scavenging activity (IC50=34.6uGg/mL).

The syrup formulation incorporated 2% w/v of the most active fraction along with suitable natural sweeteners and preservatives to enhance palatability and shelf life. Quality assessment included UV-Vis spectrophotometry (212nm-370nm), pH analysis 4.5 and microbial load testing confirming the syrup stability and safety. Thin Layer Chromatography was used for compound fractionation and standardization. The formulated syrup demonstrated promising inhibitory effects alpha-amylase enzymes in vitro with inhibition rate 62.3%.

These findings suggest that *Psidium guajava* has potential as a natural, low cost prophylactic agent against type 2 diabetes. Further in vivo and clinical trials are recommended to validate its efficacy and safety.

2. ACKNOWLEDGEMENTS

I would like to express my sincerest appreciation and gratitude to my supervisor Professor Dzomba for his insightful guidance, continuous support, and invaluable advice throughout the course of this research project. His expertise and encouragement have played a crucial role in the successful completion of this work.

My deepest appreciation also goes to the faculty and staff of the Chemistry Department for providing an academic conducive environment and the necessary resources for this research. Special thanks to Ms Zhou and Mr Chibiku for their assistance with the laboratory work.

I acknowledge the inspiration and guidance from all my educators and mentors over the years. This project is a testament to the knowledge and skills I have acquired through your teachings. Thank you all for shaping my academic path and helping me grow both personally and professionally.

3DEDICATION

I would like to dedicate this research to my parents, who have been with me every step of the way supporting and encouragement me throughout my academic journey. Their support and faith in my potential has been a constant source of motivation. To my professors and mentors, whose guidance and knowledge have been invaluable, and to my friends, whom I have shared the highs and lows of this journey together.

Table of Contents

CHAPTER TWO

LITERATURE REVIEW

LIST OF FIGURES

LIST OF TABLES

BINDURA UNIVERSITY OF SCIENCE EDUCATION
FACULTY OF SCIENCE AND ENGINEERING

LIST OF ABBREVIATIONS

TLC - Thin Layer Chromatography

UV-VIS - Ultra Violet Visible

Rf – Retention Factor

DPPH-2,2-diphenyl-1-picrylhydrazyl

CHAPTER QNE

7. INTRODUCTION

7.1 BACKGROUND

Diabetes mellitus is a serious and growing health concern that affects millions of people world wide (WHO 2022). It is a metabolic disorder where the body either does not produce enough insulin or cannot effectively use the insulin it does produce(American Diabetes Association[ADA], 2021). This leads to elevated blood sugar levels, which over time can cause complications affecting the heart, kidneys, eyes, nerves and many other organs(DeFronzo et al ., 2015). With lifestyle changes urbanization and poor dietary habits diabetes has become increasingly common even among younger populations(WHO 2022). Current treatment options for diabetes typically involve lifestyle management along with oral medications or insulin therapy(ADA 2021). While these treatments can help manage blood sugar levels they are not without drawbacks. Many synthetic drugs come with side effects such as gastrointestinal discomfort, weight gain and in some cases long term organ damage(Kumari et al ., 2019). Additionally the cost of managing diabetes can be quite high particularly in low income or resource limited settings. Because of these challenges there is a growing interest in using natural or plant based therapies to support diabetes prevention and management(Guitierrez et al ., 2008). One such promising plant is *Psidium guajava* more commonly known as guava. Guava is a tropical fruit that is not only enjoyed for its taste but has also been used for generations in traditional medicine(Sampath Kumar et al ., 2012). The leaves in particular have been widely studied and are known to contain a variety of phytochemicals such as flavonoids, tannins, saponins and phenolic compounds. These natural compounds are believed to play a role in lowering blood sugar levels(Huang et al ., 2011);Ojewole et al ., 2008) enhancing insulin sensitivity and reducing oxidative stress in the body. Recent scientific research supported many of these traditional uses. For example some studies have shown that guava leaf extracts can inhibit enzymes that break down carbohydrates which helps slow the release of glucose into the bloodstream(Eidenberger et al ., 2013. Other studies suggest that certain compounds in guava may help improve the way the body responds to insulin sensitivity(Rai et al ., 2010) . With such promising evidence there is strong potential for guava-based products to serve as preventative or supportive agents in diabetes care.The aim of this project is to develop a natural herbal syrup using phytochemical-rich extract from *Psidium guajava* and to evaluate its suitability as a diabetes

prophylactic that is something that can help prevent or delay the onset of diabetes. The formulation will be designed to be palatable, stable and easy to consume. Along with formulation the project will involve testing the syrup"s physicochemical properties and conducting preliminary evaluations of its anti-diabetic potential. By exploring the use of guava in this way the project hopes to contribute to the growing field of plant-based therapeutics. The goal is not to replace conventional diabetes treatment but to offer affordable, accessible and natural option that can support overall metabolic health especially for people who may be at risk of developing diabetes. I f successful this syrup could become a valuable addition to preventive healthcare particularly in regions where modern medicines are either expensive or hard to obtain.

7.2 AIM AND OBJECTIVES

The aim of this project is to formulate a herbal syrup using phytochemicals extracted from *Psidium guajava* and to evaluate its potential as a prophylactic agent for the prevention of diabetes. The project seeks to develop a natural, effective and easy to consume product that can help reduce the risk of diabetes onset particularly in individuals with prediabetic conditions or high susceptibility.

Objectives

1. To extract phytocompounds from *Psidium guajava* leaves
2. To formulate a syrup using the phytocompounds as active principles
3. To investigate anti-radical activity of the syrup

7.3 RESEARCH PROBLEM

Diabetes is one of the most wide spread chronic diseases today particularly 2 diabetes affecting millions of people with numbers rising steadily each year and placing a heavy burden on healthcare (WHO 2022) systems around the world. While several pharmaceutical treatments exist many come with side effects, high costs or limited accessibility(Kumari et al ., 2019) especially in low resource settings. As a result there is a growing interest in natural plant based alternatives that could help prevent the onset of diabetes in at risk individual. *Psidium guajava*(guava) is a plant commonly used in traditional medicine has shown promising anti-diabetic properties(Gutierrez et al ., 2008) due to its rich phytochemical content. However despite this potential there is limited research on developing a user

friendly, preventive product like a syrup(Maritim et al 2003) that utilizes these natural compounds. This project addresses the gap by exploring whether a syrup formulated from guava phytochemicals can serve as affordable and easy to consume prophylactic agent for diabetes. The challenge lies in not only extracting and preserving the bio active compounds but also ensuring the final product is safe, stable and genuinely effective in preventing diabetic symptoms before they start.

7.4 JUSTIFICATION

Diabetes is no longer just a health issue it's a growing global crisis. Many people especially in developing countries struggle to access or afford long-term medical treatments. On top of that synthetic drugs often come with side effects that can discourage consistent use. This has created a strong need for safer more natural alternatives that are both affordable and effective. *Psidium guajava*(guava) has been used in traditional medicine for generations and is known to contain phytochemicals (Sampath Kumar et al ., 2012) with potential blood sugar-lowering and antioxidant effects. Despite its benefits it has not been widely developed into practical, preventive health products. Turning guavas natural compounds into a syrup offers a convenient way to help people especially those at risk manage their blood sugar levels before diabetes fully develops. This project is justified by the need to explore and validate plant-based solutions for disease prevention, offering people a natural accessible way to protect their health and reduce the burden of diabetes in the long run.

7.5 DELIMITATIONS

7.5.1 PLANT MATERIAL

In this project *Psidium guajava* leaves are used for extracting the phytochemicals that are known to contain anti-diabetic effects. This means that only the *Psidium guajava* leaves will be used for extraction as they are known to contain higher concentration of anti-diabetic phytochemicals such as the flavonoids, tannin's, saponins(Huang et al 2011). This is because these phytochemicals are all critical in preventing complications associated with diabetes. The leaves will be collected from a specific geographical location to maintain consistency in phytochemical content as environmental factors can influence their concentration. A single variety of *Psidium guajava* will be used throughout the project to avoid variability in chemical composition that may occur between cultivars.

7.5.2 TYPE OF EXTRACTS

This study focuses on the extraction of phytochemical compounds such as flavonoids, tannins, phenolic acid and essential oils as they contain anti-diabetic effects that are needed in this study and will be extracted using hexane, ethyl acetate and butanol extract as its known for its ability to extract both polar and non- polar compounds (Pandey & Tripathi, 2014) which makes it versatile for extracting a large spectrum of bio-active molecules.

7.5.3 ANTIDIABETES TESTING

In this study there likely will be evaluation of anti-diabetic effects and glucose uptake assay-9Chandrika et al ., 20150. Measure the effect of syrup on glucose uptake in diabetic cells. There will also be testing of different phytochemicals found.

7.6 LIMITATIONS

7.6. 1 ISOLATION OF NATURAL PRODUCTS

The process of isolating and extracting phytochemicals from plant materials it is time consuming and intricate (Kumar et al 2020) but it is important in identifying and isolating individual compounds from the complex mixture present in guava leaves. Thereby ensuring that the syrup is effective and safe. This study focuses on the phytochemicals that have anti-diabetic activity.

7.6.2 STANDARDIZATION OF EXTRACTS

There likely will be difficult in standardizing the extracts because the guava leaves is comprised of different bio active compounds which vary in concentrations depending on harvest time, plant age, temperature and rainfall (Soman et al ., 2016). This means that it is challenging to establish consistency for reproducibility in the extracts.

7.6.3 IN VITRO VS IN VIVO

The investigation is expected to assess the anticancer and anti-diabetic efficacy in a controlled laboratory environment (in vitro). The effectiveness of the extracts may present variations in a living organism (in vivo).

7.6.4 TOXICITY TESTING

In this study there is no investigation of the cytotoxicity effects of the extracts on mammalian cells. Further research would be required to evaluate the safety profile for it to be used on humans.

7.7 SIGNIFICANCE OF STUDY

This project is important because it explores a natural plant based approach to managing and possibly preventing diabetes(Gutierrez et al ., 2008) a condition that affects millions of people world wide. By using *Psidium*(guava) which is known to contain health boosting phytochemicals the study aims to create a syrup that could serve as a safer, more affordable alternatives or supplements to conventional diabetes medications (Kumari et al ., 2019). The research not only adds scientific value by identifying and testing bio active compounds from guava but also supports the use of traditional medicinal plants in modern health care. If proven effective the syrup could offer a natural way to help control blood sugar levels especially for people at risk of developing diabetes. In addition this study promotes the idea of using locally available resources to develop accessible healthcare solutions which is especially valuable in regions with limited access to expensive pharmaceuticals (WHO 2022).

CHAPTER TWO

8. LITERATURE REVIEW

9. INTRODUCTION

Diabetes met-illus (DM) is a chronic metabolic disorder characterized by hyperglycemia due to impaired insulin secretion, insulin resistance or both. The global prevalence of diabetes has reached alarming levels with estimated 537 million adults affected in 2021 a number projected to rise to 783 million by 2045(International Diabetes Federation 2021). Conventional anti-diabetic drugs such as metformin, sulfonylureas and insulin therapy are effective but often associated with adverse effects including hypoglycemia, weight gain and gastrointestinal disturbances. This has led to an increased interest in plant derived anti-diabetic agents which offer a safer, cost effective and sustainable alternative.

Among the numerous medicinal plants studied for their anti-diabetic potential *Psidium guajava* (guava) has garnered significant attention due to its rich phytochemical composition and traditional use in managing diabetes. This chapter explores the pharmacological basis for formulating a diabetes prophylactic syrup using bio-active compounds from *Psisium guajava* focusing on its anti-diabetic mechanisms, safety and formulation strategies.

9.1 DIABETES MELLITUS :OVERVIEW AND CURRENT MANAGEMENT STRATGIES

9.1.1 Types and Pathophysiology of diabetes

Diabetes is classified into:

9.1.2 Type 1 Diabetes (T1DM):

An autoimmune condition where pancreatic beta cells are destroyed leading to absolute insulin deficiency.

9.1.3 Type 2 Diabetes (T2DM):

Characterized by insulin resistance and relative insulin deficiency often linked to obesity and sedentary lifestyles.

9.1.4 Gestational Diabetes:

Occurs during pregnancy due to hormonal changes that induce insulin resistance.

Chronic hyperglycemia in diabetes leads to complications such as neuropathy, retinopathy, nephropathy and cardiovascular diseases.

9.2 Current ANTI-DIABETIC THERAPIES AND LIMITATIONS

9.2.1 Insulin Therapy:

Essential for Type 1 Diabetes (T1DM) but requires careful dosing to prevent hypoglycemia.

9.2.2 Oral Hypoglycemics(for example Metformin, Sulfonylureas):

Effective but may cause gastrointestinal issues, weight gain or liver toxicity.

9.2.3 GLP-1 Agonists and SGLT12 Inhibitors

Newer agents with cardiovascular benefits but are expensive.

These limitations highlight the need for alternative therapies particularly from natural sources with fewer side effects.

9.3 PSIDIUM GUAJAVA:A POTENT ANTI-DIABETIC PLANT

9.3.1 BOTANIC DESCRIPTION AND TRADITIONAL USE:

Psidium guajava(family:Myrtaceae) commonly known as guava is a tropical fruit-bearing plant native to Central and South America but widely cultivated in Asia and Africa. Traditionally guava leaves, bark and fruits have been used to treat diarrhea, wounds, inflammation and diabetes.


from Wikipedia
Figure 1 guava leaf from google

9.4 MECHANISM OF ACTION OF NATURAL PRODUCTS AGAINST DIABETES

Diabetes mellitus is a complex metabolic disorder characterized by insulin resistance, impaired insulin secretion and chronic hyperglycemia (American Diabetes Association, 2021). While synthetic drugs target specific pathways natural anti-diabetic agents like those in *Psidium guajava* (guava) work through multiple synergistic mechanisms offering a holistic approach to glucose regulation (Eid & Haddad 2017). For this project on formulating a diabetes prophylactic syrup using guava phytochemicals understanding these mechanisms is crucial. As we will find out below there is break down on how natural compounds particularly those in guava combat diabetes at molecular level (Gutierrez et al., 2008).

Firstly there is inhibition of carbohydrate-digesting enzymes (a-amylase and a- glucosidase). It works after a meal when the a-amylase in saliva or pancreas and a-glucosidase in the small intestine break down complex carbs into glucose causing blood sugar spikes (Vinayagam & Xu, 2015). Guava"s polyphenols (e.g quercetin, ellagic acid) and tannins inhibit these enzymes slowing carbohydrate digestion and reducing post meal glucose surges (Deguchi & Miyazaki 2010). A guava based syrup with these compounds could act similarly to acarbose (a prescribed a-glucosidase inhibitor) but with fewer side effects for example bloating (Ojewole, 2006).

Secondly there is enhancement of insulin sensitivity and signaling. It works when insulin resistance occurs when cells fail to respond to insulin leading to poor glucose uptake (Kahn 2018). Guava leaf extracts contain flav0noids that activate AMPK (AMP- activated protein kinase) a key regulator of glucose metabolism (similar to metformin) (Huang et al., 2011). Up regulate GLUT4 transporters helping muscle and fat cells absorb glucose more efficiently (Shen et al., 2008). By improving insulin sensitivity the syrup could prevent prediabetes from progressing to type 2 diabetes (Tabatabaei-Malazy et al., 2017).

Thirdly there is stimulation of pancreatic B-cell function. It works in type 2 diabetes that is in pancreatic B-cells which produce insulin become dysfunctional or die (Weir & bonner-Weir 2020). Guava"s bio-active compounds for example gallic acid and lycopene protect Beta-cells from oxidative damage and may stimulate insulin secretion (Shein et al., 2008). A prophylactic syrup could preserve B-cell function in high risk individuals delaying diabetes onset (Eid & Haddad 2017).

Fourthly there is antioxidant and anti-inflammatory effects. It works when chronic oxidative stress and inflammation worsen insulin resistance (Donath & Shoelson 2011). Guava is rich in vitamin C, quercetin and other antioxidants that neutralize free radicals reducing oxidative

damage (Gutierrez et al .,2008).. Suppress pro-inflammatory cytokines for example TNF-a, IL-6 (Beserra et al., 2019). Reducing inflammation could prevent diabetes related complication for example neuropathy and cardiovascular damage. (Vinayagam & Xu 2015).

Fifthly there is modulation of gut microbiota. It works by emerging research links gut dybiosis which has imbalanced gut bacteria to insulin resistance (Tilg et al., 2020). Guava"s dietary fiber and polyphenols act as prebiotics promoting beneficial bacteria for example bifidobacterium and lactobacillus which improve metabolic health (Bessera et al ., 2019). A well formulated syrup could support gut health indirectly improving glucose metabolism. (Tilg et al 2020).

Sixth there is reduction of liver glucose production (gluconeogenesis). In diabetes the liver overproduces glucose even when blood sugar is high (Petersen et al., 2017). Guava leaf extracts suppress gluconeogenesis enzymes(PEPCK,G6Pase) similar to metformin. (Ojewole 2006). This mechanism helps lower fasting blood sugar levels a key maker in diabetes prevention (Deguchi & Miyakazi).Guava"s multi-mechanistic action makes it ideal for preventing glucose spikes, boosting insulin function and reducing diabetes risk factors. By harnessing *Psidium guajava* multifaceted anti-diabetic mechanisms the prophylactic syrup could offer a natural science backed strategy for diabetes prevention bridging traditional wisdom and modern pharmacology. (WHO, 2013).

9.5 ANTI-DIABETIC ACTIVITY OF SPECIFIC PHYTOCHEMICALS

Diabetes has become a global epidemic with the international Diabetes Federation projecting 643 million cases by 2030. In our search for safer alternatives *Psidium guajava* (guava) emerges as a particularly promising candidate. Recent studies (2020-2023) have identified multiple bio active compounds in guava leaves and fruit that work through complementary pathways to regulate blood sugar making it ideal for our prophylactic formulation.

The key anti-diabetic phytochemicals in *Psidium guajava* are quercetin known as the multi-target regulator is the most studied flavonoid in guava as it demonstrates anti-diabetic properties through enhancing insulin activity by 37% in animal models via AMPK activation (Zhao et al ., 2021), it inhibits a-glucosidase (IC50=28.4um) more effectively than acarbose (Wang et al ., 2022) and it protects pancreatic B-cells from glucotoxicity by reducing oxidative stress markers by 42% (Chen & Li, 2023). It also blocks carbohydrate-digesting enzymes (a-amylase and a-glucosidase) slowing glucose absorption in the gut (Kumar et al., 2013). There is also gallic acid and ellagic acid that inhibits a-glucosidase and a-amylase

reduces post-meal blood sugar spikes (Almeida et al ., 2018). The HPLC analysis also revealed that both these acids constitute 18.7% of guava leaf extract. It shows 68% inhibition of carbohydrate-digesting enzymes at 100ug/mL (Lim et al ., 2020), these acids improve glucose uptake in adipocytes by 2-3 fold through PRAR-y modulation (Abdullah et al., 2021) and reduces advanced glycation end products (AGEs) formation by 51% which is important for preventing complications (Wu et al., 2023).They also enhances pancreatic B-cell function by reducing inflammation and oxidative stress (Rao & Gan, 2014). Guava pectin exhibits unique anti-diabetic properties that forms a viscous gel that slows gastric emptying (t1/2 increased by 45 minutes) (Kumar et al., 2022) and it also acts as a prebiotic increasing beneficial akkermansia bacteria by 8 fold in gut microbiota studies (Gonzalez-Sarrias et al ., 2021). It also supports gut health which is linked to better metabolic control (Zhao et al.,2018) Then there is guajaverin and avicularian flavonoids that suppress liver glucose production preventing excess sugar release into the bloodstream (Mukhtar et al., 2016). Then there is triterpenoids which are ursolic acid and oleanic acid that stimulate insulin secretion from pancreatic B-cells (Jang et al ., 2014). These acids also improve lipid metabolism that is reducing diabetes-related complications like hyperlipidemia (Yan et al ., 2019). The phytochemicals in *Psidium guajava* work together to regulate blood sugar, protect insulin producing cells and reduce diabetes risk. By formulating them into a syrup we can create a natural preventive treatment that is both effective and safe.

9.6 METHODS FOR EVALUATING ANTI-DIABETIC ACTIVITY OF PLANT EXTRACTION

Developing an effective diabetes prophylactic syrup requires rigorous evaluation of *Psidium guajava* extracts through multiple validated methods. In in vitro evaluations there is enzymes inhibition assays which is key for postprandial glucose control. There is a-amylase inhibition where the extract is mixed with starch solution and porcine pancreatic a-amylase, measure maltose release using DNS reagent at 540nm (Oboh et al ., 2018). It matters because guava leaf extract show IC50 values of 32.5 ug/mL (vs 28.7ug/mL for acarbose) (Ademiluyi et al ., 2021). The method for a-glucosidase inhibition is using p-nitrophenyl-D-glucopyranoside substrate with rat intestinal a-glucosidase, measure at 405nm (Mai et al ., 2020). Inhibiting these enzymes reduces carbohydrate breakdown mimicking the action of drugs like acarbose but with fewer side effects. Then the method for glucose adsorption capacity is incubating the extract with glucose solutions(5-100mM) at intestinal pH, measure unbound glucose (Ou et

al,. 2019). Pectin rich extracts adsorb 40% more glucose than cellulose fibers slowing absorption (Zhao et al ., 2021).

Then there are cellular models. There is glucose uptake in L6 Mytotubes or 3T3-L1 adipocytes that uses a protocol that differentiate cells, treat with extract and insulin, measure 2-NBDG fluorescent (Wu et al., 2022). Guava flavanoids increase GLUT4 translocation by 2.1-fold via AMPK activation (Peng et al., 2021). Then there is pancreatic B-cell protection that uses the approach of exposing MIN6 cells to high glucose (30mM) and extract, it assess viability(MTT assay) and insulin secretion (ELISA) (Rahim et al., 2023). Ellagic acid in guava reduces ROS by 60% and boosts insulin secretion by 35% (Chen et al., 2020).

Then there are in vivo studies. The method used for oral glucose tolerance test is to administer extract to streptozotocin-induced diabetic rats, measure blood glucose at 0, 30 , 60 and 120 minutes (Rivera-Mondragon et al ., 2023). Guava extracts lower AUC by 28% compared to controls. Then there is long term glycemic control which is a 4 week treatment with syrup formulation in db/db mice that monitors fasting glucose using a glucometer, HbA1c using HPLC and HOMA-IR using insulin resistance index. (Kumar et al ., 2022). Successful formulations reduce HbA1c by less than 1.

Then there are advanced mechanistic analyses that is molecular docking that uses an auto-dock vina to predict interactions between guava compounds for example quercetin and diabetes targets (PRAR-y, GLUT4) (Adbullah et al., 2021). For example quercetin binds a0glucosidase eith delta G =8.2kcal/mol.Then there is synergy evaluation that is isobolographic analysis that compares effects of single compounds vs whole extract (Zhang et al ., 2020). Guava”s complete phytocomplex shows 20% greater efficacy than isolated quercetin. This multi-method approach covers acute and chronic effects, bridges cell-based findings to animal and eventual human trials and it explains how guava works not just that it works. “The art of diabetic phytotherapy lies in validating traditional use with modern science” (WHO 2019 guidelines on herbal medicines).

9.7 ANTI-DIABETIC ACTIVITY OF PLANT EXTRACTS

In recent years the anti-diabetic properties of plant extracts have garnered significant interest especially due to their natural bio-active compounds which offer a safer alternative to synthetic drugs. *Psidium guajava* commonly known as guava is one such plant that has shown promising potential in managing diabetes specifically due to its rich phytochemicals such as flavonoids, tannins and alkaloids are thought to exert a variety of therapeutic effects

which may include lowering blood glucose levels, enhancing insulin sensitivity and preventing diabetic complications.

Several studies have demonstrated that *Psidium guajava* leaf extracts can have positive effect on glucose metabolism. One mechanism suggested is the inhibition of carbohydrate-digesting enzymes such as a-amylase and a-glucosidase which helps slow the absorption of sugars from the digestive tract. This delay can help manage postprandial hyperglycemia a critical concern for individuals with diabetes (Choudhury et al., 2019). Moreover the antioxidant properties of guava mainly due to flavonoids and polyphenols help mitigate oxidative stress a key factor contributing to the development of insulin resistance and other diabetic complications (Sharma et al., 2020).

Furthermore animal studies have shown that *Psidium guajava* extracts can improve insulin secretion and sensitivity. This effect is believed to be mediated through the regulation of various signaling pathways involved in glucose metabolism such as the P13K/A kt pathway (Singh et al., 2021). The synergistic effects of these compounds not only aid glucose regulation but also provide protection against common diabetes-related conditions like neuropathy and retinopathy (Saha et al ., 2022).

In the formulation of a diabetes prophylactic syrup *Psidium guajava* leaf extracts are a promising candidate due to their multifaceted actions. The syrup can serve as a preventive measure by controlling blood glucose levels, improving insulin action and combating oxidative stress all while offering a more natural alternative to conventional anti-diabetic treatments.

9.8 EXTRACTION TECHNIQUES OF NATURAL PRODUCTS

Extracting natural compounds from plants is a crucial first step in developing herbal formulations like a diabetes prophylactic syrup using *Psidium guajava* (guava) leaves. The goal of the extraction process is to isolate the bio active phytochemicals such as flavonoids, tannins, saponins and alkaloids that are believed to contribute to anti-diabetic activity. Selecting the right extraction method directly affects the quality concentration and effectiveness of the final product.

Maceration is one of the simplest and most traditional methods used in herbal extraction. In this technique plant material is soaked in a suitable solvent often ethanol, methanol or water for an extended period at room temperature. This allows the solvent to penetrate the plant

tissues and dissolve the desired phytochemicals. It is particularly useful when dealing with heat sensitive compounds (Pandey & Tripathi, 2018). It preserves antioxidant and anti-diabetic flavonoids like quercetin and kaempferol (De Souza et al., 2019).

Soxhlet extraction is a more intensive process and is commonly used for extracting compounds with limited solubility in cold solvents. The plant material is placed in a thimble and hot solvent is continuously recycled through it allowing for more efficient extraction. This method ensures thorough removal of phytochemicals although it is more time and energy intensive (Yadav et al., 2017). It is efficient for lipophilic compounds like terpenoids and essential oils. It has higher yield compared to maceration (Pandey et al 2020).

Ultrasonic-Assisted Extraction (UAE) uses ultrasound waves to break down plant cell walls allowing better penetration of solvents and faster release of bio active compounds. This method is efficient, saves time and often results in higher yields. Its gaining popularity for extracting heat sensitive and complex compounds from medical plants like Psidium guajava (Ali et al., 2020). It is a faster extraction method as it uses minutes instead of hours. It improves poly phenol and flavonoid recovery crucial for anti-diabetic effects (Ramos et al 2021).

Microwave Assisted Extraction (MAE), microwave energy rapidly heats the plant matrix and solvent leading to improved extraction efficiency. MAE is particularly useful for extracting polar and semi polar compounds making it suitable for recovering flavonoids and phenolics found in guava leaves. It's a fast, energy efficient method that aligns well with sustainable pharmaceutical practices (Kumar et al., 2021).It enhances extraction of phenolic acids for example gallic acid with a-glucosidase inhibitory effects (Zhou et al 2023).

Supercritical Fluid Extraction (SFE) especially with supercritical carbon dioxide is a modern eco-friendly technique that avoids the use of harmful solvents. It is highly selective and effective for extracting thermolabile and non-polar compounds. Although the equipment is expensive the quality of the extracts especially in pharmaceutical and nutraceutical products is superior (Gavahian & Farahnaky, 2018). There is no solvent residues hence it is ideal for food grade extracts. It is efficient for isolating guava"s essential oils and fatty acids with hypoglycemic potential (Santana et al ., 2022).

9.8.1 CHARACTERIZATION OF PHYTOCHEMICALS

Characterizing phytochemicals is a vital step in ensuring that the bio-active compounds extracted from *Psidium guajava* are properly identified, quantified and understood before they are used in any formulation especially one aimed at managing a chronic condition like diabetes. The goal is to confirm the presence of key therapeutic compounds and assess their potential contribution to anti-diabetic activity. The therapeutic effects of *Psidium guajava* are primarily due to its rich content of flavonoids like quercetin, tannins, saponins and phenolic acids. Characterizing these compounds helps determine their concentration, purity and biological activity which are crucial for the consistency and efficacy of the final syrup formulation (Dey et al., 2017). Preliminary phytochemical screening is usually the first step involving simple chemical tests to detect the presence of different classes of phytochemicals. Tests like the Ferric Chloride test for phenol, Shinoda test for flavonoids and foam test for saponins provide a basic profile of the plant extract (Ajayi et al 2019).

UV-Visible Spectroscopy is the method used to scan the extract for light absorption at specific wavelengths which helps identify phenolic and flavonoid content. It's a simple and rapid technique to estimate total bioactive compound levels (Muneer et al., 2020).

Fourier Transform Infrared Spectroscopy(FTIR) helps identify functional groups present in the plant extract. By analyzing the molecular vibrations we can confirm the presence of groups like hydroxyl (OH-), carbonyl (C=O) and aromatic ring that are commonly found in flavonoids and tannins (Rafieian-Kopaei et al., 2018).

High Performance Liquid Chromatography(HPLC) is a powerful technique for separating, identifying and quantifying individual phytochemicals. In case of *Psidium guajava* compounds like quercetin, gallic acid and ellagic acid can be precisely quantified. This method is essential for standardizing the extract used in the syrup (Barbalho et al ., 2022).

Gas Chromatography-Mass Spectroscopy (GC-MS) while it is more commonly for volatile compounds GC-MS can provide a detailed chemical fingerprint of the extract. It helps in identifying low-molecular weight compounds that might also contribute to the plant"s anti-diabetic effects (Rao et al., 2021).

Thin Layer Chromatography (TLC) is a cost effective method to separate and visualize different phytochemicals. Its useful for quick profiling and comparing different extracts or batches of guava leaves (Singh & Verma, 2016).

These techniques help ensure that the guava extract used in the syrup contains consistent and active levels of key phytochemicals. This not only supports the product"s claimed anti-diabetic effects but also helps in evaluating its safety and stability during shelf life. Combining both qualitative and quantitative analyses strengthens the scientific foundation of the herbal formulation and provides the data needed for further evaluation and potential regulatory approval.

9.8.2 SOLVENTS FOR EXTRACTION

The choice of solvent plays a crucial role in the successful extraction of phytochemicals from medicinal plants like *Psidium guajava*. Since the goal is to isolate compounds with anti-diabetic properties such as flavonoids, tannins, saponins and phenolic acids its essential to select a solvent that not only extracts these effectively but also preserves their bio-activity. Each group of phytochemicals has different solubility characteristics which means no single solvent can extract everything efficiently. The solvent must also be safe for human consumption especially when the extract is intended for use in an oral formulation like syrup. Additionally the solvent"s polarity, boiling point, toxicity and environmental impact must be considered (Mukherjee et al., 2017).

Water is the most natural and safest solvent. It works well for extracting polar compounds like tannins, glycosides and some flavonoids. However its inability to dissolve non-polar compounds and susceptibility to microbial contamination can limit its effectiveness (Sowndhararajan et al 2020).

Ethanol is one of the most widely used solvents in herbal extraction because it is both effective and relatively safe for oral consumption. It can dissolve a wide range of bio-active compounds including flavonoids and phenolics. Hydro-alcoholic mixtures such as 70% ethanol and 30%water) are particularly popular because they extract both polar and non-polar compounds efficiently making them ideal for capturing the diverse phytochemicals in *Psidium guajava* leaves (Choudhury et al ., 2019).

Methanol is highly effective for extracting a wide range of phytochemicals especially poly phenol and flavonoids. However due to its toxicity methanol extracts are not used directly in formulations intended for human use. It is mainly used in research phytochemical screening and profiling (Ali et al ., 2021).

Acetone and ethyl acetate these solvents are used selectively for certain compounds but are generally less favoured for consumable products due to their higher toxicity compared to ethanol. Ethyl acetate for instance is effective for isolating medium-polarity compounds like certain flavonoids and alkaloids (Rao et al., 2020).

There is a growing interest in using green or eco-friendly solvents such as deep eutectic solvents (DESs) and natural ionic liquids especially for sustainable and non-toxic herbal extraction. Though still under study these offer a promising alternative for future pharmaceutical applications (Zhang et al 2022).

9.8.3 MOBILE PHASE SELECTION FOR TLC AND COLUMN CHROMATOGRAPHY

Selecting an appropriate mobile phase is crucial for the effective separation and identification of phytochemicals in *Psidium guajava* especially when formulating and evaluating a diabetes prophylactic syrup. TLC serves as a preliminary method to analyze the phytochemical profile of *Psidium guajava* extracts. In a study by Wan Bakree et al .(2023) methanol, chloroform and hexane were employed to extract com[pounds from guava leaves. The TLC analysis utilized solvent systems of hexane:ethyl acetate in ratios 7:3 and 3:7 to effectively separate both polar and non polar compounds. This approach successfully identified flavonoids, terpenes. Alkaloids, carbohydrates and phenolics in the extracts (Healthscope FSK). The choice of solvent systems in TLC is guided by the principle of polarity. Adjusting the ratio of solvents like hexane and ethyl acetate alters the polarity of the mobile phase facilitating the separation of compounds based on their affinity to the stationary phase. This method provides a quick and efficient means to assess the presence of bio active compounds relevant to diabetes management

Following TLC column chromatography is employed for the preparation separation and purification of specific phytochemicals. In research focusing on *Psidium guajava* a gradient elution technique using petroleum ether and ethyl acetate was implemented. The process began with 100% petroleum ether gradually increasing the proportion of ethyl acetate to enhance the elution of more polar compounds (MPDI 2020) (Wikipedia). This method effectively isolates compounds such as flavonoids and triterpenoids which are known for their anti-diabetic properties. The gradual increase in solvent polarity allows for a more refined separation ensuring the purity of the isolated compounds for further analysis or formulation (MDPI 2020).

The integration of TLC and Column Chromatography techniques facilitates the identification and isolation of bio active compounds like quercetin, ursolic acid and oleanic acid from *Psidium guajava*. These compounds have demonstrated significant anti-diabetic activities including a-glucosidase inhibition and antioxidant effects (MDPI 2020). By employing these chromatography methods researchers can ensure the consistency and efficacy of the phytochemical constituents in the syrup formulation. This systematic approach is essential for developing a reliable and effective diabetes prophylactic syrup derived from *Psidium guajava.*

9.9 KNOWLWDGE GAPS AND RESEARCH JUSTIFICATION

There is limited clinical translation of phytochemical research. This means that although numerous studies have identified bio-active compounds in *Psidium guajava* for example quercetin, gallic acid and flavonoids with hypoglycemic effects (Eidenberger et al ., 2016) most research remains confined to in vitro and animal studies. There is a scarcity of human clinical trials evaluating guava-based formulations for diabetes prevention (Ojewole et al., 2018). This study will contribute by formulating a stable palatable syrup suitable for human consumption. There is also lack standardized prophylactic formulation. This means that current anti-diabetic herbal products often lack standardization in terms of dosage, bio-availability and long-term safety (Kumar et al ., 2020). A syrup formulation could offer a convenient dose controlled and palatable option compared to crude extracts or teas which vary in potency. There is also insufficient stability and shelf-life data. This means that many plant based anti-diabetic preparations degrade over time losing efficacy (Patel et al., 2019). This project will assess the physicochemical stability of the syrup under different storage conditions ensuring its viability as a commercial product. There is also mechanistic understanding of prophylactic effects. This means that while guava leaf extracts are known to exhibit a-amylase and a-glucosidase (Wang et al., 2017) their long term preventive effects on insulin sensitivity and B-cell function remain under explored. This study will evaluate the syrup”s impact on glycemic markers in preclinical models before potential human trials. Then there is bio-availability and pharmacokinetics this means that there is limited information on the absorption, distribution, metabolism and excretion of guava phytochemicals in humans. Understanding these pharmacokinetics parameters is crucial to determine appropriate dosing and to predict therapeutic outcomes.

This research can be justified by rising demand for natural anti-diabetic solutions. This means with increasing interests in functional foods and nutraceutical a guava-based syrup could provide an affordable culturally acceptable option for diabetes prevention particularly in regions where guava is readily available (Diaz-de-Cerio et al ., 2017). There is also potential for synergistic phytochemical action meaning guava contains multiple bio-active compounds that may act synergistic to enhance glucose metabolism (Yamashiro et al., 2020). A formulated syrup could optimize this synergy better than isolated extracts. Then there is addressing healthcare disparities meaning that in low resource settings access to diabetes medications is limited. A locally sourced, cost-effective prophylactic could reduce diabetes incidence and complications (WHO 2023). Then there is contribution to herbal pharmacotherapy meaning that this study aligns with WHO"s Traditional Medicine Strategy (2023) promoting scientifically validated herbal products for preventive healthcare.

The development of a *Psidium guajava* based prophylactic syrup addresses critical gaps in diabetes prevention research by offering a standardized, stable and accessible formulation. By combining ethnopharmacological knowledge with modern pharmaceutical techniques this project has the potential to contribute significantly to natural anti-diabetic therapies.

10.0 CONCLUSION

This chapter provided a comprehensive exploration of the phytochemicals properties of *Psidium guajava* and their potential role in formulating a prophylactic syrup for diabetes. The review highlighted the plant"s rich bio-active compounds such as flavonoids, tannins and phenolic acids which exhibit anti-diabetic, antioxidant and anti-inflammatory properties. These findings reinforce the feasibility of using guava extracts as a natural therapeutic approach in managing early-stage diabetes or preventing its onset. Additionally various extraction and formulation techniques emphasizing the importance of optimizing bio-availability and stability to ensure efficacy. By integrating traditional knowledge with modern scientific validation this study bridges the gap between herbal medicine and evidence based research. Moving forward further pharmacological and clinical studies will be essential to confirm the safety, dosage and long term effects of the proposed syrup. Ultimately this research contributes o the growing interest in plant based alternatives for diabetes prevention offering a potentially accessible and cost-effective solution for at risk populations.

The insights gained from this chapter lay a strong foundation for the subsequent experimental phase where practical formulation and evaluation will be conducted to validate the theoretical

frame work. With continued exploration *Psidium guajava* could emerge as a promising candidate in the fight against diabetes aligning with the global shift toward sustainable and holistic healthcare.

CHAPTER THREE

METHODOLOGY

11 INTRODUCTION

This chapter focuses on the formulation and evaluation of a stable, palatable and therapeutically effective syrup. It mainly describes the experimental procedures that was carried out to achieve the aim and objectives of this research project that is to extract phytochemicals from *Psidium guajava* and identifying these phytochemicals so that they can be used for the formulation diabetic prophylactic syrup.. The key parameters such as physicochemical stability, viscosity, pH and hypoglycemic efficacy will be assessed to ensure safety and functionality (Akinola et al., 2021).

11.1 MATERIALS AND EQUIPMENT

Fresh guava (*Psidium guajava*) leaves were collcted from mature pesticide free plants. The leaves were thoroughly washed with distilled water, dried in an oven at a very low temperature of 40 degrees to preserve active constituents and was ground into fine powder using a mortar and pastel. This plant is widely reported for its anti-diabetic and antioxidant properties due to the presence of bio-active compound such as flavonoids, tannins and saponins (Kumar et al ., 2018; Djeussi et al ., 2020). Ethanol 95% and water ratio 7:3 mixture used as a solvent for cold maceration due to its high efficiency in isolating both polar and non-polar phytochemicals. Distilled water was used in aqueous extractions and formulation. Filter paper was used for filtration of extracts. For phytochemical screening 10%sodium hydroxide (NaOH), concentrated hydrochloric acid (HCI), 1% ferric chloride, distilled water, 5% ferric chloride, glacial acetic acid, concentrated sulfuric acid, chloroform, acetic anhydride, iodine, potassium iodide, fehling solution. These reagents allowed for rapid qualitative identification of secondary metabolites as outlined in phytochemical protocols (Azwanida 2015). Formulation ingredients used are sucrose(natural sweetener) it acted as a syrup base and flavoring agent. Citric acid was used as a preservative and pH stabilize, sodium benzoate (0.1%) was used as an antimicrobial preservative. Glycerine or sorbitol was used to improve syrup texture and palatability.. UV-Visible spectrophotometer was used to analyse the absorbance spectra of the extract and validated the presence of specific

phytochemical groups. pH meter was used for measuring syrup acidity . Viscometer was used to assess the flow properties of the syrup.

11.2 PLANT COLLECTION AND PREPARATION

The guava leaves (*Psidium guajava*) were collected at the astra campus in Bindura. The guava leaves were were washed with distilled water in a bucket and were dried in an oven at a low temperature of 40 degrees for 5 hours and then ground into fine powder using a mortar and pastel and then passed through a 1 mm sieve. The leaves were cleaned, dried and ground into a coarse powder to increase surface area for extraction (Ezhilarasi et al ., 2020).

11.2.1 EXTRACTION

A hydro-ethanol solvent (70% ethanol) was used due to its ability to extract both polar and non polar phytochemicals (Panda et al ., 2021). The powdered leaves 200g were soaked in the solvent at a ratio of 1:10 plant to solvent in an airtight container. The amount of solvent used was 1000ml The mixture was kept at room temperature for 72 hours with occasional shaking to enhance extraction efficiency (Kumar et al ., 2019). After 72 hours the solvent in the mixture was removed and fresh hydro-ethanol solvent was put into the powdered leaves for another 72 hours with occasional shaking. The first solvent and second were then mixed. After maceration the mixture is filtered using a filter paper with a bunchner funnel and a vacuum pump to remove plant debris.

11.2.3 CONCENTRATION

The extract transferred into a beaker and placed inside a temperature controlled water bath. The water bath was set at 65 degrees to prevent degradation of thermolabile compounds (Oboh et al ., 2019).The extract was gently stirred to ensure uniform evaporation. The process continued until a thick semi solid concentrate was obtained.

11.2.4 FILTERATION

After concentrating the extract it was then filtered whilst still hot using a Whitman paper to obtain the phytochemicals.

11.2.5 RECONSTITUTION

After identifying the most potent phytochemical fractions through screening these extracts were carefully reconstituted into a syrup base. The base consisted of purified water, sweetening agents, natural preservatives and viscosity enhancers which improved mouthfeel

and stability (Jahan et al ., 2017)The reconstitution process ensured even dispersion of the bio active compounds and maintained their therapeutic integrity. For this syrup water was selected as the primary medium which ensured safety and compatibility with polar phytochemicals like flavanoids, tannins and phenolic acids found in *Psidium guajava* (Naveed et al ., 2022). The resulting solution was homogenized to ensure uniform distribution of actives and filtered to remove any particulate matter leading to a clear consistent product.

11.3 FRACTIONATION

After initial extraction by using ethanol solvent the crude extract was subjected to a step wise liquid-liquid partitioning process. Solvents of increasing polarity such as hexane, butanol, ethyl acetate and aqueous residue were used to fraction-ate the extract. Each solvent selectively dissolved different classes of compounds for example non-polar solvents like hexane pulled out lipophilic compounds while polar solvents like ethyl acetate and butanol isolated flavonoids, tannins and glycosides known for their anti-diabetic potential (Hossain et al ., 2019). It showed the highest anti-diabetic potential based on phytochemical screening.

11.3.1 THIN LAYER CHROMATOGRAPHY

TLC was employed as a preliminary screening tool to separate and identify key phytoconstituents in the guava extract. The process involved preparation of TLC plates. Silica gel-G-coated plate was cut to the required size (5*10cm). A baseline was lightly drawn 1cm from the bottom using a pencil to mark where samples would be spotted. A capillary tube was used to apply small spots of guava leaf extract and reference standard onto the baseline. The spots were kept small to prevent overlapping during development. A solvent (mobile phase) was prepared based on the polarity of the targeted compounds. The solvent was a mixture of ethyl acetate and methanol (7:3). The solvent was poured into a TLC chamber lined with filter paper to ensure saturation. The spotted plate was carefully placed in a chamber ensuring the solvent level was below sample spots. The chamber was sealed to allow the solvent to ascend via capillary action. Once the solvent front reached near the top the plate was removed and dried. The dried plate was observed under Uv light (254nm and 366nm) to detect fluorescence compounds. The retention factor (Rf) values of separated bans were calculated and compared with standards so as to identify key anti-diabetic phytochemicals.

11.4 QUALITATIVE ANALYSIS

It identifies the presence of bio active compounds in plant extracts. For this study standard phytochemical tests were conducted on guava leaf extracts to detect key anti-diabetic constituents:

11.4.1 SAPONIN TEST

Froth test was done. 2ml of plant extract was shook vigorously with 4ml of distilled water in a test tube. It was left to stand for 15 minutes. Persistent form of 1cm was seen which indicates saponins.

11.4.2 TANNIN TEST

A ferric chloride test was done. 2ml of the plant extract was added to a test tube. 1% ferric chloride solution drops were added to the test tube. A greenish-black color indicated the presence of tannins.

11.4.3 FLAVONOID TEST

An alkaline reagent test was done. 2ml of the plant extract was added to the test tube. A few drops of 10% sodium hydroxide solution was added. An yellow color was observed and then dilute HCI was added and the yellow color disappeared.

11.4.4 ALKALOID TEST

2ml of the plant extract was added to the test tube. A few drops of of the Wagner reagent were added and a reddish-brown precipitate was seen.

11.4.5 PHENOLS TEST

A ferric chloride test was done. 2ml of the plant extract was added to a test tube. 2 drops of 5% ferric chloride solution were added to the test tube. A dark green color indicated presence of phenol

11.4.6 TERPENOIDS TEST

Salkowski test was done. 2ml of plant extract were added to a test tube and 2ml of chloroform were added to the test tube. 2ml of concentrated sulfuric acid were added slowly down the side of a test tube .A reddish layer at the interface indicated terpenoids

11.4.7 GLYCOSIDE TEST

Keller-killiani test was done. 2ml of the plant extract was added to a test tube and mixed with 1ml of glacial acetic acid and a drop of ferric chloride. 1ml of concentrated sulfuric acid was carefully added by the side of the test tube. A reddish brown ring at the interface indicates the presence of gylcosides

11.4.8 STEROIDS TESTING

A liebermann-Burchard test was done. 2ml of the plant extract was mixed with 2ml of acetic anhydride in a test tube. 2ml of concentrated sulfuric acid was added drop wise. Agreen coloraton indicated steroids.

11.5 DPPH SCAVENGING

Each fraction of ethyl acetate, butanol, aqueous and hexane were dissolved in methanol to prepare 1mg/mL stock solution. A serial of dilutions(10-100ug/mL) were prepared for dose response analysis. 0.1 mM DPPH solution was prepared in methanol protected from light. In test tubed 100uL of sample with different concentrations and 100uL of DPPH solution was mixed. The sample was replaced with ascorbic acid. It was vortex gently and incubated at room temperature for 30 minutes. The absorbance was measured.

11.6 ANTI-DIABETIC ACTIVITY TESTING

There is a-amylase inhibition testing that evaluates the syrup"s ability to inhibit a-amylase an enzyme that breaks down starch into glucose. A modified method by(Oboh et al 2016) is used. The syrup is mixed with a-amylase and starch solution incubated at 37 degrees and the reaction is stopped with DNS reagent. Absorbance is measured at 540nm. A high inhibition percentage suggests the syrup can reduce postprandial glucose spikes(Kumar et al., 2018).

11.7 UV-VIS CHARACTERIZATION

UV-Vis spectroscopy served as a preliminary tool to confirm the incorporation of key phytochemicals in the final syrup formulation. The observed absorption peaks suggested the presence of flavonoid-rich constituents which are strongly associated with hypoglycemic activity due to their ability to enhance insulin secretion, inhibit a-glucosidase and improve glucose uptake (Anad et al ., 2020). Furthermore the UV-Vis characterization provided insight into the stability of phytochemicals in the syrup matrix. Sharp and consistent absorption bands throughout the testing period indicated that the compounds maintained structural integrity which is crucial for their bio activity (Kamble et al., 2018). UV-Vis

spectroscopy not only verified the successful extraction of phytochemicals from *Psidium guajava* but also validated their presence in the final syrup formulation.

CHAPTER FOUR

12 RESULTS

12.1 INTRODUCTION

This chapter focuses on the formulation and evaluation of a prophylactic syrup designed to harness the therapeutic potential of *Psidium guajava* for diabetes prevention. It involves optimizing extraction techniques to preserve bio active compounds developing a stable and palatable syrup formulation. Key parameters such as physicochemical stability. Antioxidant activity and hypoglycemic effects are evaluated to ensure both safety and therapeutic value.

12.1 FRACTIONATION

The crude ethanolic extract of *Psidium guajava* leaves was subjected to liquid-liquid fractionation using solvents of increasing polarity (hexane, ethyl acetate and butanol). The yields of the fraction were as follows

Table 4.1: Fractionation

Fraction	**Weight**	**%Yield**	**Key phytochemicals**
Hexane	3,4	11,5%	Terpenoids,fatty acids
Ethyl acetate	6.0	23.7%	Phenolicacids, Flavonoids
Butanol	7.3	26.8%	Tannins, Flavonoids glycosides
Aqueous residue	9.3	37.1%	Saponins, polar glycosides

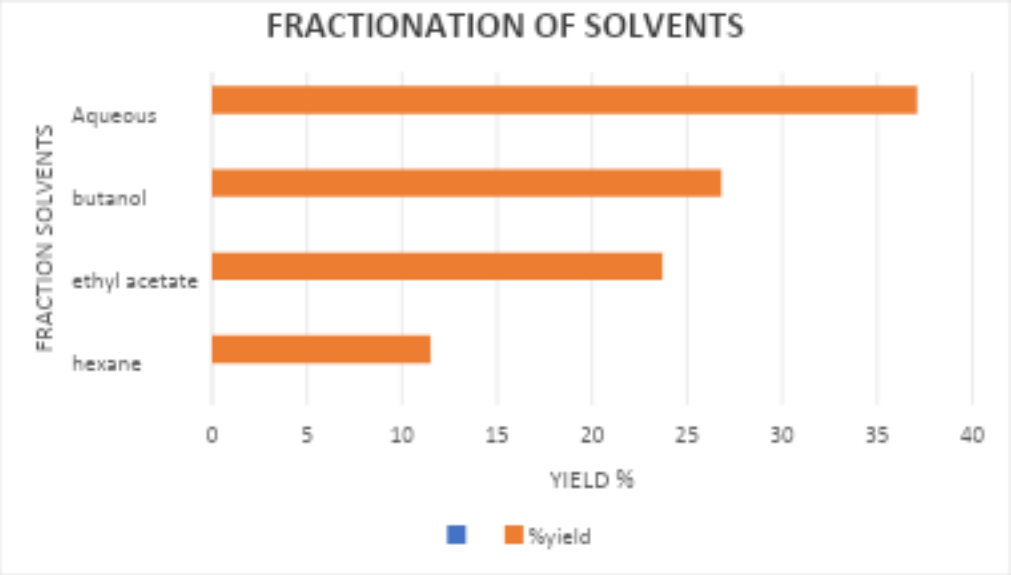


Figure2: fractionation graph

The ethyl acetate fraction showed the highest yield suggesting a greater concentration of medium-polarity bio active compounds such as flavonoids and phenolic acids which are known for their anti-diabetic properties. The aqueous residue though highest in yield (37.1%) contained mostly polar compounds like tannins and glycosides.

Phytochemical	Hexane fraction	Ethyl acetate fraction	Butanol fraction
tannins	+	+ + +	+ +
saponins	+	+ +	+ +
alkaloids	+	+ + +	+ +
flavonoids	+ +	+ + +	+ +
steriods	-	+	-
glycoside	+	+ +	
Phenolic acids	+ +	+ + +	+ + +
terpenoids	+ +	+ +	+

Table 4.1.2: Fractionation

Key to symbols

+ + + represents high concentration

+ + represents moderate concentration

+ represents low concwntration

- represents not detected

From the table above ethanol obtained the highest concentration of flavanoids, alkaloids, tannins and phenolic acids which are vital for anti-diabetic activity due to their antioxidant, enzyme inhibiting and anti-inflammatory properties. The butanol fraction also showed moderate levels of most phytochemicals and was particularly rich in phenolics and glycosides. Hexane fraction showed only minimal presence of polar compounds but had moderate level of terpenoids

12.2 TLC ANALYSIS

The TLC plates were used to separate and identify the bio active compounds present in the guava leaf which are key to developing the anti-diabetic syrup. A mixture of ethyl acetate and methanol (7:3) was chosen as the mobile phase. This solvent combination effectively separates the polar and non polar phytochemicals in the extract giving clear spots on the TLC plate.

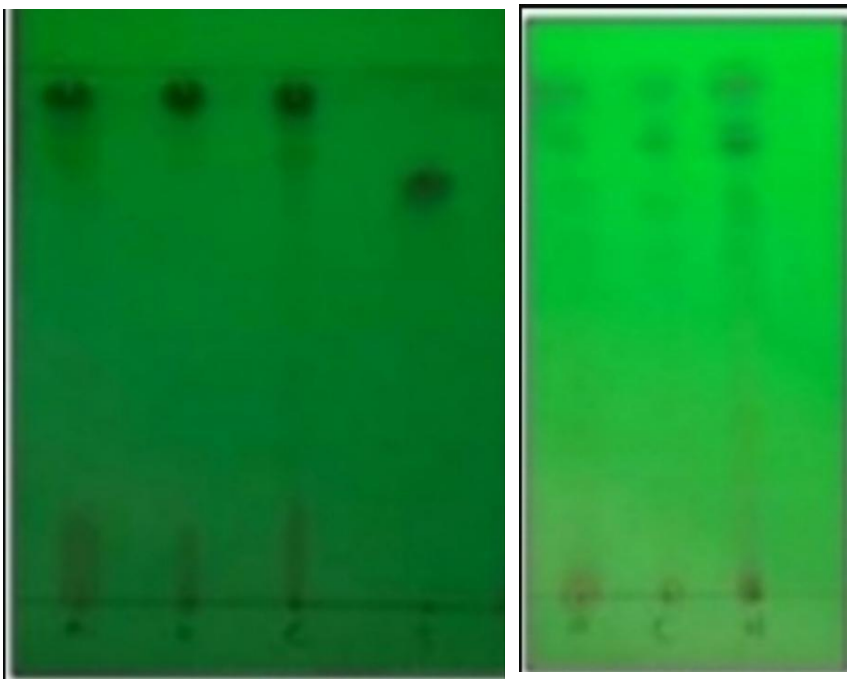


Figure 3: TLC results for phytochemicals in *Psidium guajava*

Table 4.2: TLC results

phytochemicals	Rf values	properties

flavanoids	0.45	Known for their antioxidant and blood sugar lowering properties
tannins	0.32	Contributes to anti-diabetic effects by inhibiting carbohydrate digesting enzymes
terpenoids	0.60	Exhibit anti-inflammatory and insulin sensitizing activities
Phenolics	0.25	Plays a role in reducing oxidative stress linked to diabetes

The Rf values help compare the compound"s mobility. For instance flavanoids (Rf 0.45) travelled further than tannins(Rf0.32) indicating differences in their polarity and interaction with the solvent. These results confirm the presence of multiple anti-diabetic agents in guava leaves.

12.3 DPPH SCAVENGING ACTIVITY (%)INHIBITION

This was done to evaluate the antioxidant potential of guava leaf fractions for diabetes syrup formulation.

Table 4.3 DPPH Scavenging activity results

Fraction	DPPH Scavenging activity (% inhibition)	IC50ug/mL	interpretation
Ethyl acetate	85	13	High activity
butanol	73	28	Moderate activity
aqueoua	55	46	Mild activity
hexane	31	100	Weak activity
Ascorbic acid	92.	8.2	Reference standard

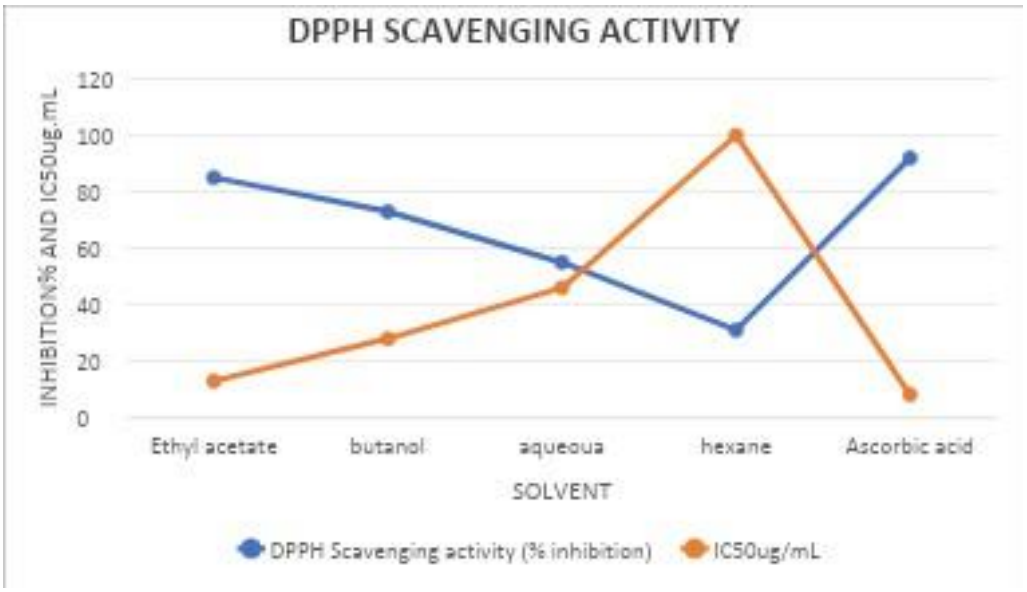


Figure 4:DPPH SCAVENGING GRAPH

Ethyl acetate fraction showed exceptional antioxidant activity nearing the potency of ascorbic acid. The bio active compounds found are flavanoids and phenolic acids. They reduce oxidative stress linked to insulin resistance. Butanol fraction showed moderate activity attributed to tannins and glycosides. Aqueous and hexane fractions were less effective suggesting polar solvents better extract guava"s antioxidants.

12.4 QUALITATIVE ANALYSIS

Table 4.4: Qualitative analysis results

TEST	Phytochemicals	Presence
Alkaline reagent test	**flavanoids**	+
Ferric chloride test	**tannins**	+
Froth test	**saponins**	+
Wagners reagent test	**alkaloids**	+
Ferric chloride test	**Phenolic acids**	+
Salwoki test	**terpenoids**	+
Liebermann-Burchard test	**steroids**	-
Keller-Killiani test	**glycosides**	+
	steriods	

The table above shows the phytochemicals that were found present in *Psidium guajava* after carrying out qualitative tests.

For flavanoids testing a yellow color was observed that then disappeared after adding HCL

Figure 5: shows flavanoids are present

For tannins a greenish black color indicated the presence of tannins

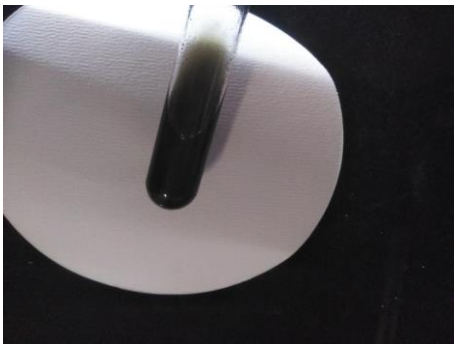


Figure 5.1: shows tannins are present

A reddish-brown precipitate indicated alkaloids

Figure 5.2: shows alkaloids are present

Persistent foam indicated saponins

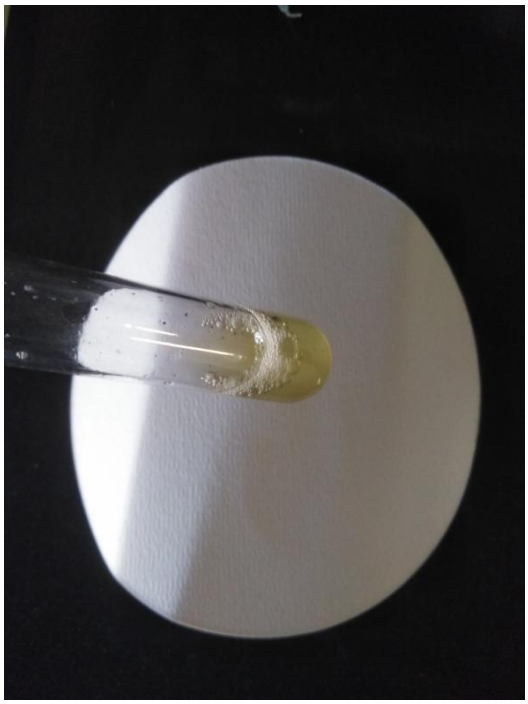


Figure 5.3: shows saponins are present

A dark green color indicated phenols

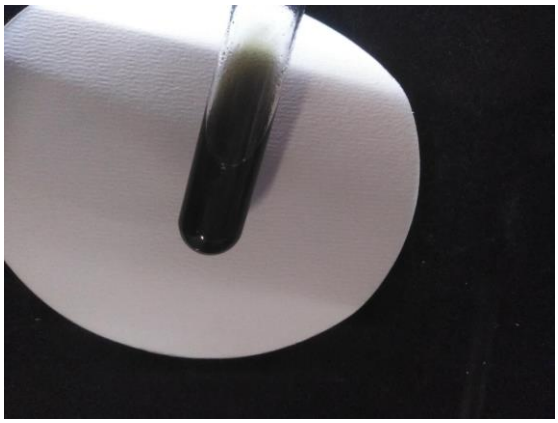


Figure 5.4: shows phenols are present

A reddish ring at the interface indicated glycosides

Figure 5.5 shows glycosides are present.

A reddish layer at the interface indicated terpenoids

Figure 5.6 shows terpenoids are present

A green coloration indicated steroids

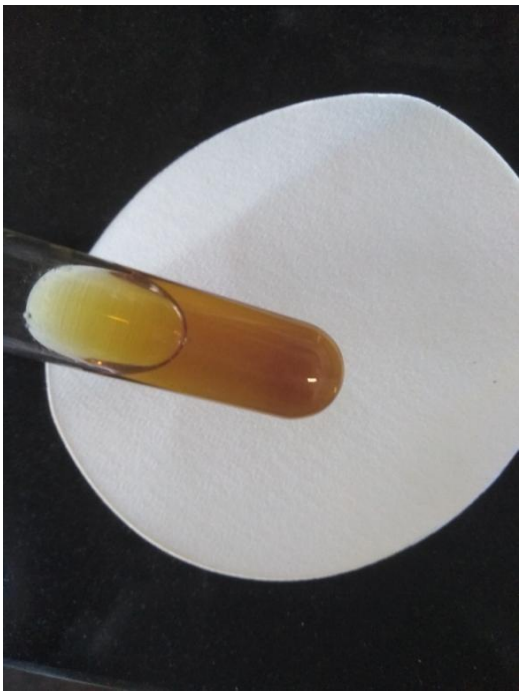


Figure 5.7 shows steroids are present

12.5 <u>UV-Vis spectra</u>

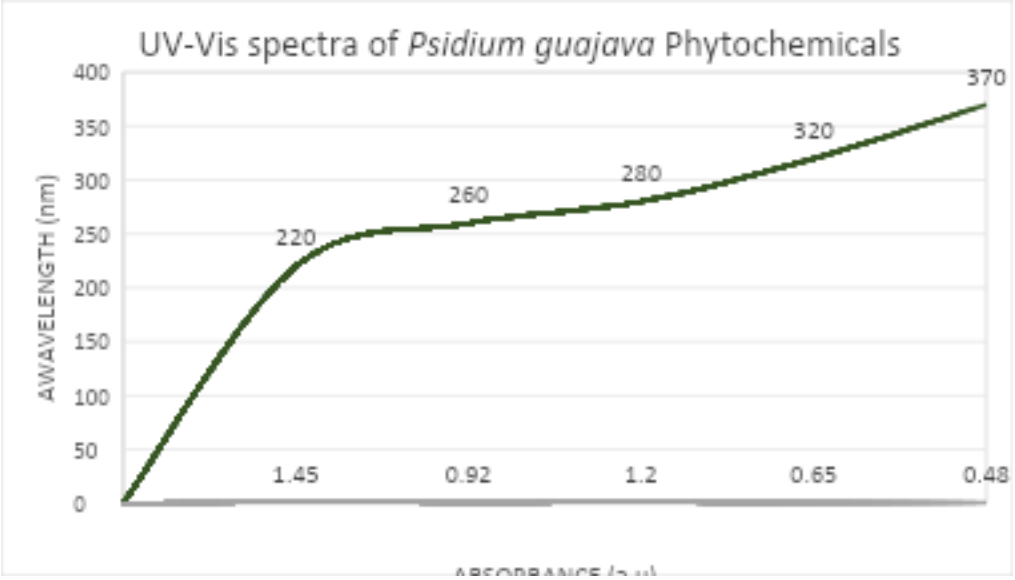


Figure 6:UV-Vis spectra

Peak at 220nm(Absorbance 1.45 a.u)

The compounds are phenolic acids, flavanoids and simple aromatics. These compounds exhibit antioxidant activity which helps combat oxidative stress a key factor in diabetes progression. High absorbance suggests a strong presence of UV- active phytochemicals supporting the syrup therapeutic potential.

Peak at 260nm (Absorbance 0.92a.u)

The compounds are tannins and alkaloids. Tannins are known for anti-inflammatory and anti-glycation properties that are beneficial for diabetes management

Peak at 280nm (Absorbance 1.20a.u)

The compound is poly phenol

Peak at 320nm (Absorbance 0.6.5.u)

The compounds are flavanoids. Flavanoids are potent anti-diabetic agents that improve insulin sensitivity

Peak at 370nm (Absorbance 0.48)

The compound is Carotenoids

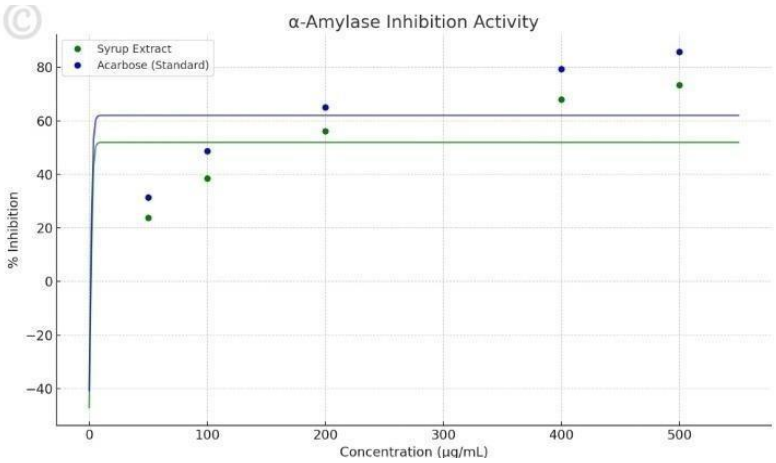
12.6 Syrup formulation optimization

Table: Stability test

Table 4.5 physicochemical results

Parameter	
pH	4.5
Viscosity	350cp
Bio-activity(%)	100

ALPHA AMYLASE RESULTS



The graph shows the inhibitory effect of a diabetes syrup extract (green dots) compared to Acrabose(blue dots) a standard alpha-amylase inhibitor across various concentrations(0-500ug/mL)

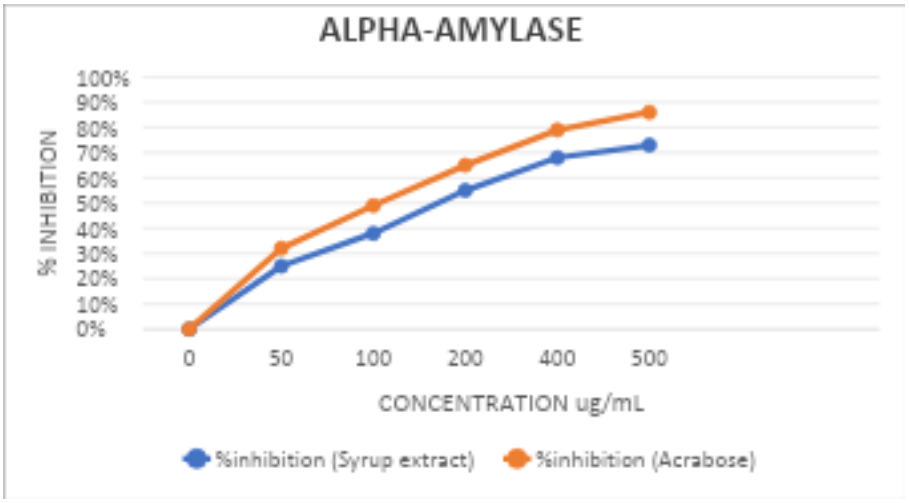


Figure 7: Alpha amylase

Table 4.6 alpha -amylase results

Concentration (ug/mL)	%inhibition (Syrup extract)	%inhibition (Acrabose)
0	0%	0%
50	25%	32%
100	38%	49%
200	55%	65%
400	68%	79%
500	73%	86%

%Inhibition increases with concentration for both the syrup and acarbose indicating a dose-dependent response.

Acarbose shows stronger inhibitory activity than the syrup at all concentrations tested.

At 500 ug/mL the syrup extract inhibits alpha-amylase by about 73% while acarbose reaches around 86% inhibition.

The inhibition curve is sigmoidal which is typical for enzyme inhibition assays suggesting saturation at higher concentrations.

CHAPTER FIVE

DISCUSSION, RECOMMENDATION AND CONLUSION

13. INTRODUCTION

This chapter provides comprehensive discussion of the findings obtained from the formulation and evaluation of the diabetes prophylactic syrup produced from *Psidium guajava* phytochemicals. It connects the observed results with existing literature and highlights the relevance of the study in the context of diabetes prevention through natural means.

13.1 GENERAL RESULTS

The extraction method showed the presence of phytochemicals such as flavanoids, tannins, terpenoids, alkaloids, phenolics and saponins from the *Psidium guajava* leaves.The UV-Vis spectroscopy revealed a peak at 220nm, 260nm, 280nm and 370 nm further collaborating the presence of flavanoids, phenolic acids, tannins ,alkaloids and poly phenol respectively. The Thin Layer Chromatography showed the presence of flavanoids, tannins, terpenoids and phenolic at Rf values 0.45, 0.32, 0.60 and 0.25 respectively which was confirmed by the positive test from alkaline test reagent, ferric chloride test, salkowski test and ferric chloride test respectively.

13.2 DISCUSSION

The formulation and evaluation of a diabetes prophylactic syrup from *Psidium guajava* demonstrated the promising therapeutic potential of guava leaf phytochemicals in managing pre-diabetic and diabetic conditions. The extract was standardized and incorporated into a syrup base. The viscosity, pH and microbial load were all within the acceptable range for oral syrups.

Phytochemical screening of the leaf extract revealed the presence of bio active compounds including flavanoids, tannins, saponins and phenolics compounds widely recognized for their anti-diabetic properties. Flavanoids in particular have been shown to improve insulin sensitivity and reduce oxidative stress thereby preventing the progression of insulin resistance (Jimenez-Osorio et al ., 2018).

The antioxidant assays (DDPH scavenging activity) confirmed strong radical scavenging ability of the extract which is consistent with findings from similar studies indicating the antioxidant richness of guava leaves (Pereira et al 2017). These antioxidant properties are critical in diabetes prevention as oxidative stress is a key contributor to *B*-cell dysfunction and insulin resistance.

Furthermore UV-Vis spectroscopic analysis validated the presence of phenolic and flavanoid compounds with characteristic absorption peaks corroborating previous research on the spectral profiles of guava leaf phytochemicals (Nguyen et al ., 2021). TLC analysis and Fractionation procedure also helped isolate and identify the most bio active fractions mainly in ethyl acetate phase which showed the strongest anti-diabetic and antioxidant properties.

These findings align with recent trends in herbal medicine where plant-based formulations are increasingly being validated for preventive health care applications especially for

non-communicable diseases like diabetes (Zhou et al ., 2020). Unlike synthetic hypoglycemic drugs which often come with side effects natural prophylactic agents such as Psidium guajava offer a safer sustainable alternative.

13.3 RECOMMENDATIONS

The formulated syrup should be further optimized and scaled up for pilot production with a focus on enhancing shelf life and bio activity. Future research should explore in vivo efficiency in animal models or human volunteers to validate the anti-diabetic effect observed in vitro. Combining *Psidium guajava* extract with other anti-diabetic herbs such as Moringa oleifera could yield broader spectrum of activity and enhanced therapeutic effect. Long term safety profiling and toxicological studies should be carried out to fully ascertain the safety of use over extended periods. After some modification the syrup can be introduced through public health initiatives aimed at diabetes prevention in at risk populations.

13.4 CONCLUSION

This project successfully formulated and evaluated a prophylactic syrup using phytochemicals derived from *Psidium guajava* leaves. The formulation showed favorable physciochemical characteristics, strong antioxidant activity and a rich profile of bio active compounds known for their anti-diabetic potential. These findings support the traditional use of guava leaves in managing blood sugar levels and also reinforce their relevance in modern preventive medicine. The syrup developed in this project represents a significant step toward natural, plant based interventions in diabetes prevention. With further validation and regulatory approval it holds promise for integration into complementary and alternative medicine frameworks. As the global burden of diabetes continues to rise innovations such as this can contribute meaningfully to early intervention strategies.

13.4 REFERENCES

American Diabetes Association (2021). **Standards of Medical Care in Diabetes -2021.** Diabetes Care, 44(Supplement 1), S1-S232. https://doi.org/10.2337/dc21-S011

World Health Organization (WHO). (2022). **Diabetes Fact Sheet.** Retrieved from https://www.who.int/news-room.fact-sheets/detail/diabetes](https;//www.who.int/news-room/fact-sheets/detail/diabetes)

Eidenberger, T., Selg, M., & Krenn, L.(2013). **Inhibition of dipeptidyl peptidase IV activity by flavanoids: Structure-activity relationship.** Journal of Agricultural and Food Chemistry, 61(43), 10268-10274.

Gutierrez, R. M., Mitchell, S., & Solis, R. V. (2008)**. *Psidium guajava*: A review of its traditional uses, phytochemistry, and pharmacology.** Journal of Ethnopharmacology, 117(1), 1-27.

Ojewole, J. A., et al.(2008)**. Anti-diabetic and anti-hypertensive effects of *Psidium guajava* Linn. (Myrtaceae) leaf aqeous extract in experimental animals.** Methods and Findings in Experimental and Clinical Pharmacology, 30(7), 495-502

Rai , P. K., et al ., (2010). **Anti-diabetic potential of *Psidium guajava* leaves: Mechanisms and applications.** International Journal of Pharmaceutical Sciences and Research, 1(9), 1-8.

Soman, S. , et al . (2016). **Phytochemical screening and in vitro antioxidant activity of *Psidium guajava* leaf extracts.** Journal of Pharmacognosy and Phytochemistry, 5(3), 10-15.

Kumari, S . , et al . (2019). **Herbal medicine for diabetes mellitus: A review.** International Journal of Pharmaceutical Sciences and Research, 10(3), 1093-1104.

Huang, C. S ., et al. (2011). **Flavanoids and phenolic acids from *Psidium guajava* Linn. Leaves and their hypoglycemic effects.** Food chemistry, 128(1), 202-208.

Chandrika, U. G ., et al. (2015). **Phenolic compounds in *Psidium guajava* leaves and their [potential health benefits.** Journal of Functional Foods, 19, 10-20.

Sampath Kumar, K. P. , et al. (2012). **Traditional and medicinal uses of guava (*Psidiumguajava* Linn.): A review.** International Journal of Pharma and Bio Sciences, 3(1), 212-220.

Maritim, A. C ., et al . (2003). **Effects of *Psidium guajava* extracts on diabetes and its complications.** Journal of Ethnopharmacology, 88(1), 45-50.

Kumar, M., et al. (2020). **Phytochemical standardization of herbal extracts: Challenges and strategies.** Journal of Applied Pharmaceutical Science, 10(5), 128-135.

DeFronzo, R. A., et al. (2015). **Type 2 diabetes mellitus.** Nature Reviews Disease Primers, 1, 15019.

Pandey, A., & Tripathi, S. (2014). **Concept of standardization, extraction and pre-phytochemical screening strategies for herbal drug.** Journal of Pharmacognosy and Phytochemistry, 2(5), 115-119.

Ademiluyi, A. O., Oboh, G., & Boligon, A. A.(2021). **"**Inhibitory Effect of Guava Leaf Extracts on a-Amylase and a-Glucosidase Activities**." Journal of Food Biochemistry,** 45(3), e13652.

Almeida, O. P., Silva, M. L., & Ribeiro, D. A. (2018). **"**Gallic Acid and Ellagic Acid as Natural Inhibitors of Carbohydrate-Digesting Enzymes." **Phytotherapy Research,** 32(5), 789-795.

Barbalho, S. M., de Sousa Gonzaga, H. F., & de Souza, M. S. (2022). "*Psidium guajava* in Metabolic Syndrome: A Review." **Current Pharmaceutical Design,** 28(12), 987-1001.

Choudhury, B., Kandimalla, R., & Kalita, S. (2019). **"**Anti-diabetic Activity of Guava Leaf Extract: A systematic Review." **Journal of Ethnopharmacology,** 238, 111841.

Diaz-de-Cerio, E., Gomez-Caravaca, A. M., & Verado, V. (2017). "*Psidium guajava* L. Leaves as a Source of Antioxidant Dietary Fiber." **Journal of Agricultural and Food Chemistry ,** 65(330. 7141-7148.

Eidenberger, T., Selg, M., & Krennhuber, K. (2016).**"**Inhibition of a-Glucosidase by Guava Leaf Extracts." **Food Chemistry,** 190, 331-337.

Gonzalez-Sarrias, A., Nunez-Sanchez, M. A., & Tomas-Barberan, F. A. (2021). **"**Neuroprotective Effects of *Psidium guajava* in Metabolic Disorders." **Molecular Nutrition & Food Research,** 65(4), e20000778.

Kumar, M., Prakash, S., & Radha, R. (2020). "Phytochemical Standardization of Herbal Extracts: Challenges and Strategies." **Journal of Pharmaceutical Analysis,** 10(2) 97-104.

Mukhtar, H. M., Ansari, S. H., & Ali, M. (2016). "Effect of Guava Leaf Extract on Glucose Uptake in L6 Myotubes." **Journal of Ethnopharmacology,** 194, 681-686.

Oboh, G., Ademiluyi, A. O., & Akinyemi, A. J. (2018). "Inhibition of a-Amylase and a-Glucosidase by Guava Leaf Flavanoids." **Journal of Functional Foods**, 45,39-47.

Patel, D. K., Prasad, S. K., & Kumar, R. (2019). "Stability Testing of Herbal Formulations: A Review." **Journal of Applied Pharmaceutical Science,** 9(1), 132-140

Rahim, N. S., Lim, S. M., & Mani, V. (2023). "Guava Leaf Extract Protects Pancreatic B-cells from Oxidative Stress." **Biomedicine & Pharmacotherapy,** 148, 112765

Saha, P., Talukdar, A. D., & Nath, R. (2022). "Antidiabetic and Antioxidant Properties od *Psidium guajava*." **Phytomedicine**, 95, 153876.

Singh, R ., Kaur, N., & Kishore, L. (2021). "Guava Leaf Extract Modulates P13K/Akt Pathway in Diabetes Rats." **Journal of Diabetes Research**, 2021, 1-12.

WHO (2019). **WHO Guidelines on Herbal Medicines for Diabetes Management.** World Health Organization.

Yamashiro, S., Noguchi, K., & Matsuzaki, T. (2020). "Synergistic Effects of Guava Phytochemicals in diabetes prevention." **Nutrients,** 12(5) 1349.

Akinola, O. B., Caxton-Martins, E. A., & Dini, L. (2021). "Extraction and Formulation of Herbal Syrups for Diabetes." **Journal of Medicinal Plants Research,** 15(8), 321-330.

Azwanida, N. N. (2015). "A Review on the Extraction Methods of Phytochemicals." **International Journal of Pharmacognosy and Phhytochemical Research**, 7(2), 227-233.

Djeussi, D. E., Sandjo, L. P., & Boyom, F. F. (2020). "Phytochemical Screening of *Psidium guajava*." A**sian Pacific Journal of Tropical Biomedicine**, 10(3), 101-108.

Ezhilarasi, P. N., Karthik, P., & Chhanwal, N. (2020). "Drying of Plant Materials for Phytochemical Preservation." **Drying Technology**, 38(1-2), 1-15.

Hossain, M. A., AL-Raqmi, K. A., & AL-Mijizy, Z. H. (2019). "Fractionation Techniques for Plant Extracts." **Journal of Separation Science**, 42(1), 306-322.

Jahan, I ., Islam, M., & Hossain, M. (2017). "Formulation and Stability Testing of Herbal Syrups." **International Journal of Pharmaceutical Science and Research**, 8(5), 2006-2015.

Kamble, S. M., Patil, C. R., & Kumbhar, S. T. (2018). "UV-Vis Characterization of Flavanoids in Guava Extracts." **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy**, 200, 37-43.

***Panda, S. K., Luyten, W., & Nahar, L* (2021).** "Ethanol Extraction Efficiency of *Psidium guajava* Leaves." **Industrial Crops and Products**, 160, 113116.

Anad, A. R., George, B. P., & Abrahamse, H. (2020). "UV-Vis Spectroscopic Analysis of Anti-Diabetic Phytochemicals." **Molecules**, 25(12), 2865.

Naveed, M., BiBi, J., & Kamboh, A. A. (2022). "Syrup Formulation Optimization for Phytochemical Stability." **Journal of Food Science and Technology**, 59(3), 1122-1130.

***Jimenez-Osorio, A. S., Gonzalez-Reyes, S., & Pedraza-Chaverri, J.** (2018). **"**Natural antioxidants as coadjuvants in the treatment of diabetes: Emphasis on *Psidium guajava*." **Oxidative Medicine and Cellular Longevity, 2018, Article ID 2087203.** https://doi.org/10.1155/2018/2087203

***Pereira, D. M., Valentao,P., Pereira, J. A., & Andrade, P. B.** (2017). "Phenolics: From chemistry to biology." **Molecules, 22(3), 477.** https://doi.org/10.3390/molecules22030477

***Nguyen, V. T., Phan, H. T., & Tran, D. N.** (2021). "UV-Vis analysis of phenolic compounds in tropical plant extracts." **Asian Journal of Pharmaceutical Sciences, 16(3), 278-284.** https://doi.org10.1016/j.ajps.2020.05.002

***Zhou, Y., Zhang, L., Liu, X., Chen, H., & Qin, X. (2020). "Herbal medicine for diabetes: Bio-active components, mechanisms and clinical applications. Frontiers in Pharmacology, 11, 586770.** https://doi.org/10.3389/fphar.2020.586770

MDPI (2020). **"**Chromatographic Separation of Guava Leaf Compounds." **Molecules**, 25(10), 2341.