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Natural Compounds in Guava (*Psidium guajava*) Leaves: A Phytochemical Study

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Abstract

This study investigates the chemical composition and hepatoprotective potential of ferrous nanoparticles (FeNPs) synthesized from *Psidium guajava* (Linn.) leaf extract, focusing on their effects on liver function in male Wistar rats. Fresh leaves of *P. guajava* were collected from the Government Reservation Area (G.R.A), Wukari, Taraba State, Nigeria, washed, air-dried, and pulverized for analysis. The resulting leaf powder was subjected to phytochemical screening, vitamin profiling, and mineral composition analysis, while the synthesized FeNPs were characterized using UV–visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and gas chromatography–mass spectrometry (GC–MS). GC–MS analysis revealed a rich profile of bioactive compounds, with 2-hexyl-1-octanol identified as the most abundant constituent. Vitamin profiling showed a high concentration of folate (65.10%), moderate levels of thiamine (32.90%), and trace amounts of vitamin E (1.40%) and vitamin K (0.60%). Mineral analysis indicated potassium (9.40 ± 0.89 ppm) as the predominant element among the five minerals detected. UV–visible spectroscopy confirmed the successful synthesis of FeNPs, evidenced by a characteristic absorption peak at 360 nm, while

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FTIR analysis identified functional groups such as hydroxyl (O–H), alkane (C–H), and alkene (C=C), suggesting the presence of phytochemicals capable of reducing and stabilizing the nanoparticles. Overall, the findings demonstrate that *Psidium guajava* leaf extract is a rich source of vitamins, minerals, and phytochemicals that effectively mediate the green synthesis of ferrous nanoparticles, and that the presence of bioactive compounds and functional groups supports the potential of these FeNPs for biomedical applications, particularly in liver function modulation. This study provides a foundational basis for further exploration of the therapeutic efficacy and safety of guava leaf-derived nanoparticles in hepatoprotection and other health-related interventions.

Keywords: Natural Compounds; Guava; *Psidium guajava*; Leaves; GC–MS; Phytochemicals

INTRODUCTION

Guava leaves is rich source of minerals, such as calcium, potassium, sulfur, sodium, iron, boron, magnesium, manganese, and vitamins C and B. The higher concentrations of Mg, Na, S, Mn, and B in GLs makes them a highly suitable choice for human nutrition and as an animal feed to prevent micronutrient deficiency (Adrian *et al*., 2015). Thomas *et al.* (2017) reported the concentration of minerals such as Ca, P, K, Fe, and Mg as 1660, 360, 1602, 13.50, and 440 mg per 100g of guava leaf dry weight (DW), respectively. The concentration of vitamins C and B was 103.0 and 14.80 mg per 100g DW, respectively. Consumption of Ca and P-rich GLs reduces the risk of deficiency-related diseases like hypocalcemia, hypophosphatemia, and osteoporosis. The study also reported that the concentration of Ca, P, Mg, Fe, and vitamin B in GLs was higher than that in Guava fruit. The higher vitamin C content in GLs may help in improving the immune system and maintain the health of blood vessels, whereas vitamin B plays an important role in improving blood circulation, nerve relaxation, and cognitive function stimulation.

Chemical Composition of Guava Leaves

Polysaccharides

Polysaccharides are macromolecules that are ubiquitously present in nature. They are made of long polymeric chains, which are composed of monosaccharide units. These polysaccharides demonstrate various physicochemical, biological, and pharmacological

properties, such as antioxidant, anti-inflammatory, antidiabetic, immunomodulatory, and antitumor activities (Luo *et al.*, 2019). Guava leaf polysaccharides (GLPs) can be isolated using ultrasound-assisted extraction (UAE) (time: 20 min, power: 404 W, temperature: 62 ˚C). These GLPs contain about 9.13% uronic acid and 64.42% total sugars, out of which 2.24% are reducing sugars. GLPs are soluble in water, while insoluble in organic solvents like ethanol, diethyl ether, ethyl acetate, acetone, and chloroform. Extracted GLP with a concentration of 100 μg/mL exhibits good antioxidant capacity with 56.38% and 51.73% 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical- and 2,20 -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical caution-scavenging capacity, respectively (Luo *et al.*, 2018). GLPs can be categorized into two groups: unsulfured and sulfated GLPs. Sulfated GLP contains about 18.58% sulfate content. Sulfated GLP exhibited good antioxidant activity in terms of DPPH, hydroxyl, and alkyl radical-scavenging activity (0.10, 0.02, and 0.17 IC50, mg/mL, respectively). Studies showed that guava leaves extracts (GLE) effectively reduced the oxidative stress and toxicity caused by hydrogen peroxide in mammalian cell lines (Vero cells) (Kim *et al.*, 2016). GLPs are also found to be beneficial in treating diabetes mellitus symptoms. Acarbose (an antidiabetic drug) is commonly used for the treatment of type 2 diabetes (Luo *et al.*, 2018). It acts as an inhibitor of glycoside hydrolases like α-glucosidase and α-amylase and thus prevents rapid release of glucose from complex carbohydrates (Zhang *et al.*, 2018). This activity causes some of the incompletely digested complex carbohydrates to remain in the intestine and be transported to the colon. The intestinal microflora digests these complex carbohydrate fractions, causing gastrointestinal problems like diarrhea and flatulence. A study reported that GLP inhibited α-glucosidase more efficiently than acarbose without significantly blocking the α-amylase activity (Zhang *et al.*, 2018). Moreover, it also caused a substantial drop in fasting blood sugar, total cholesterol, total triglycerides, glycated serum protein, creatinine, and malonaldehyde in diabetic mice without causing any major side effect (Luo *et al.*, 2019). Therefore, GLP can be used as a replacement of acarbose for managing diabetes mellitus and also as an antioxidant additive in foods.

Proteins

Guava leaves contain 9.73% protein on a dry weight basis (Rahman *et al.*, 2013). Proteins are large biomolecules composed of amino acids and act as building blocks of cells. Proteins play a major role in growth and maintenance, enzyme regulation, and cell signaling, and also as biocatalysts (Albert *et al.*, 2002). Recently, plant-based nutrients have

gained potential because of the high demand for nutritionally rich food, particularly protein. A great effort is now being made to find highly sustainable nutritionally rich food sources (Lonnie *et al.*, 2018). Thomas *et al.*, (2017) reported 16.8 mg protein/100g and 8 mg amino acids/100g in guava leaves as estimated according to Lowry's and ninhydrin methods, respectively. Jassal and Kaushal, (2019) reported that guava leaves can be utilized as a novel and sustainable dietary source as they are a rich source of proteins, carbohydrates, and dietary fibers.



Figure 2.1: *Psidium guajava* with flowers (Patil and Rane, 2020).

Phytochemical Profile

GLs are a rich source of essential oils. The major constituent of GL essential oil includes 1,8-cineole and trans-caryophyllene (Lee *et al.*, 2012). Chen *et al.* (2007) identified 50 compounds in GL essential oil using gas chromatography (GC) and gas chromatography/mass spectrometry (GC–MS), where they found β-caryophyllene, α-pinene, and 1,8-cineole to be the major ones. GL essential oil from the Philippines was found to contain a different profile, with limonene, α-pinene, β-caryophyllene, and longicyclene as major compounds (Sacchetti *et al.*, 2005). Ecuadorian GL essential oil contained a higher content of monoterpenes (limonene and α-pinene) whereas Tunisian guava leaf oil displayed a higher content of veridiflorol and trans-caryophyllene (Smith and Oliveros-Belardo 1977; Khadhri *et al.*, 2014). Soliman *et al.*, (2016) reported a larger number of monoterpenes, contrary to the other studies, where sesquiterpenes constituted the major compound in GL essential oil. El-Ahmady *et al.*, (2013) reported 4 α-selin-7(11)-enol, α-

selinene, β-caryophyllene, and β-caryophyllene oxide as the major constituents of GL essential oil. In another study, sixty-four different compounds were determined in essential oil extracted from GLs by gas chromatography–mass spectrometry (GC–MS). Among them, caryophyllene (24.97%) was found to be predominantly present, which acts as an antioxidant, anticancer, anti-inflammatory, and antimicrobial agent (Jassal and Kaushal 2019). This study reported the concentration of non-oxygenated sesquiterpenes, oxygenated sesquiterpenes, and monoterpenes as 73.67, 12.94, and 8.55%, respectively.

Phenolic Compounds

GLs are widely popular as a traditional source of medicine in Asian countries due to their antihyperglycemic effect. As mentioned in the previous sections, they contain superior quality bioactive polysaccharides, proteins, lipids, essential oils, vitamins, and minerals. The various secondary metabolites present in GLs include phenolic acids, flavonoids, triterpenoids, sesquiterpenes, glycosides, alkaloids, and saponins. Phenolic compounds (PCs) serve as key bioactive compounds which provide antioxidants and hypoglycemic properties to GLs. Generally, these PCs play a major role in managing various metabolic and physiological activities in the human body. About seventy-two different phenolic compounds have been determined in GLs using high-performance liquid chromatography– diode array detector–quadrupole time-of-flight tandem mass spectrometry (Díaz-de-Cerio *et al*., 2016). Generally, five quercetin glycosides are present in GLs. The presence of two new benzophenone galloyl glycosides (guavinosides A and B) and one quercetin galloyl glycoside (guavinoside C) was also reported (Matsuzak *et al*., 2010). Seventeen types of triterpenoids, thirty types of flavonoids, and nineteen types of sesquiterpenoids in GLs have also been reported (Jiang *et al*., 2020). Moreover, diphenylmethane (Shu *et al*., 2010) sesquiterpenoid-diphenylmethane meroterpenoids (psiguadials A and B) (Shao *et al*., 2012) and psiguanins A–D (1–4) (Shao *et al*., 2012) were also found in GLs. Epidemiological studies have established the roles of polyphenolic compounds against chronic diseases, such as diabetes, cancer, and neurodegenerative and cardiovascular diseases (Rasouli *et at*., 2017). Phenolic compounds modulate numerous physiological processes like cell proliferation, enzymatic activity, cellular redox potential, and signal transduction pathways to fight against chronic pathologies (Luca *et al*., 2020).

Biological Activities of Guava Leaf Extracts

The compound from guava leaf extracts contained multidirectional biological activities, including antioxidants, hypoglycemic, anticancer, antibacterial, and antitumor effects. The useful bioactivities of GL extract are presented in the following subsections.

Anticancer/Antitumor Activity

Cancer is a complex health disorder which is identified by the development of cell proliferation or a decrease, causing apoptosis (Toyokuni, 2016). It can be caused by several exogenous and endogenous factors involved in the excessive production of reactive oxygen species (ROS). This can result in single- or double-strand breaks in DNA or RNA, base mutations, chromosomal breaking and reorganization, DNA cross-linkage, nucleic acid degradation, damage to cell membrane integrity due to lipid peroxidation, and tumor formation (Gonzalez *et al.*, 2018). GLs are a good source of triterpenoids, sesquiterpenes, tannins, psiguadials, volatile oils, flavonoids, benzophenone glycosides, and miscellaneous quinones (Jiang *et al.*, 2020). Psiguadial D and psiguadial C act as inhibitors of human hepatoma cells (HepG2) and protein tyrosine phosphatase 1B (PTP1B). Terpenoids and flavonoids present in GLs exhibit antitumor effects by regulating the immune system, suppression of signal transfer and tumor cell adhesion, and an impediment to tumor angiogenesis and cell proliferation (Biswas *et al.*, 2019). Studies suggest that these leaves exhibit a potent inhibitory effect against cancer cell lines like MDAMB-231 and Michigan Cancer Foundation-7 (MCF-7) for breast cancer, Henrietta Lacks (HeLa) for cervical cancer, KB for nasopharyngeal cancer, LNCaP, DU 145, and prostate cancer-3 (PC-3) for prostate cancer, and colorectal 320 double minutes (COLO320DM) for colon cancer (Correa *et al.*, 2016).

The growth of colorectal tumors chiefly relies on angiogenesis, a process by which new blood vessels develop from pre-existing ones. Prolonged angiogenesis is vital for the progression of tumors towards malignancy since the blood vessels efficiently supply the developing tumor cells with vital metabolites and oxygen and it also functions as an efficient means for cellular waste disposal. A study was conducted to investigate the anticancer and antiangiogenic potential of GL extracts against angiogenesis-dependent colorectal cancer (Lok *et al.*, 2020). Guava leaf extracts rich in vitamin E, flavonoids (apigenin), and β-caryophyllene demonstrated strong antiproliferative activity against human colon carcinoma cell lines Caco-2, HT-29, and SW480.

Antidiabetic Activity

Diabetes is a major chronic disease and about 10% of the world's population suffer from blood glucose metabolic disorder, mainly characterized by a hyperglycemic condition. This situation is either characterized by insufficient secretion of insulin from β-cells of pancreatic islets (type 1 diabetes) or the inability of cells to react in response to the secreted insulin (type 2 diabetes). (Mazumdar *et al.*, 2015; Punia and Kumar, 2021). The International Diabetes Federation (IDF) stated that 451 million people were affected by diabetes mellitus, resulting in 5 million deaths, in 2017 and the global prevalence of diabetes is projected to hit 693 million cases by 2045 (Cho *et al.*, 2018). The prolonged condition of hyperglycemia leads to increased production of ROS and dyslipidemia, causing severe cellular damage and complications (Hu *et al.*, 2018).

GLs have been widely used as ethnomedicine for diabetes management (Luo *et al.*, 2019). Flavonoids and polysaccharides of GLs have been reported for their antidiabetic potential in several studies. Guaijaverin and avicularin flavonoids of GL extract were associated with significant improvement in the function of β-cells of pancreatic islets and hepatocyte morphology in diabetic mice (Zhu *et al.*, 2020). Guaijaverin suppressed the activity of the blood glucose homeostasis enzyme dipeptidyl-peptidase IV (Eidenberger *et al.*, 2013), while avicularin inhibited intracellular lipid aggregation by impeding glucose uptake through GLUT-4 in vitro and revealed no distinct toxicity for 3T3-L1 adipose cells (Fujimori and Shibano, 2013). Luo *et al.* (2020) extracted GL polysaccharides (GLPs) and further tested the antidiabetic effects on streptozotocin-induced diabetic mice in combination with a high-fat diet. The authors revealed that GLP was associated with a significant reduction in total cholesterol, triglycerides, glycated serum protein, creatinine, fasting blood glucose, and malonaldehyde content, and increased total superoxide dismutase and total antioxidant capacity enzyme activity in vivo. Suboptimal glycemic regulation may lead to elevated postprandial glucose concentrations. Nair *et al.* (2013) suggested that the inhibitors of α-amylase and α-glucosidase enzyme can decline postprandial glucose absorption and are therefore possible targets for diabetes management. The polysaccharides were isolated from GLs by ultrasound-assisted extraction and the antiglycation activity of extracted polysaccharides was studied (Luo *et al.*, 2018). The authors found that GLP showed strong inhibition of α-glucosidase, with a 99.54% inhibition rate at a 100 μg/mL concentration, and less inhibition of α-amylase, with

a 14.06% inhibition rate at a 1mg/mL dose concentration. The findings suggest that bioactive compounds from GLs can be effective in reducing the risk of diabetes.

Antioxidant Activity

Oxygen is an important element for aerobes since it acts as a terminal electron acceptor during the respiration process, which is the key source of energy production. However, free radicals produced during metabolic processes are responsible for numerous ailments in the human body, namely, inflammatory diseases, ischemic diseases, neurological disorders, hemochromatosis, emphysema, acquired immunodeficiency syndrome, and many others (Stefanis *et al.*, 1997). The presence of phenolic compounds, such as gallic acid, pyrocatechol, taxifolin, ellagic acid, ferulic acid, and several others, is responsible for the antioxidant roles of GLs (Chen and Yen, 2007; Farag *et al.*, 2020). High-performance liquid chromatography analysis of GL extracts revealed the presence of seven major flavonoids: quercetin, hesperetin, kaempferol, quercitrin, rutin, catchin, and apigenin, while other bioactive compounds, such as kaempfertin, isoquinoline, and corilaginoline alkaloids, were also identified (Taha *et al.*, 2019). These compounds are the major compounds responsible for the antioxidant properties of GLs.

The significance of antioxidant compounds from GLs in minimizing the harmful effects of free radicals has been shown by numerous studies. Essential oils extracted from GLs were found to function as moderate antioxidants with an IC_{50} value of ~460.37 ± 1.33 μg/mL, as demonstrated by a DPPH assay (Lee *et al.*, 2012). The reduction of linoleic acid oxidation and the scavenging effect on peroxyl radicals were revealed by other such analyses on GL extract. The study also showed that there was a linear association between the antioxidant's potency, the ability to scavenge free radicals, and the phenolic content of GL extract (Chen and Yen, 2007). The protective effect of GL polysaccharide was studied in zebrafish. The authors revealed that GL polysaccharides exerted a protective effect against oxidative stress induced by hydrogen peroxide by inhibiting the formation of reactive oxygen species (ROS), reducing lipid peroxidation and cell death (Kim *et al.*, 2016). In another study, it was revealed that GL extracts at 4000 ppm or higher can prevent the oxidation of fresh pork sausages, suggesting its application as a functional food ingredient (Tran *et al.*, 2020). To release insoluble bound polyphenol components, GLs were co-fermented with yeast and bacterial strains and it was observed that fermentation enhanced the antioxidant ability of soluble guava leaf polyphenols (Wang *et al.*, 2017). In an advanced

study, silver nanoparticles were synthesized by utilizing crude polysaccharides of GLs and showed high DPPH radical- and ABTS radical cation-scavenging activity (Wang *et al.*, 2017). It is evident from the findings that GL extracts can be a useful antioxidant material in the food preservation and cosmetic industries.

Antidiarrhea Activity

Currently, diarrhea is one of the prominent root causes of mortality among children in the age group of 0–5 years. Attempts have been made to discover new drugs with minimal side effects on the other organs of the body. In developing nations, attention has been devoted to identifying novel phytochemicals derived from medicinal plants to develop new drugs with minimal side effects (kim, 2005). Most pharmaceutical industries are engaged in the innovation of different drugs which have therapeutic potential to combat this disease. A number of therapeutic treatments are available for treating diarrhea in the form of synthetic drugs which cause many side effects in the human body, such as constipation, intestinal obstruction, induction of bronchospasm, and vomiting (Palombo, 2006; Mehra *et al.*, 2013). To combat these side effects, focus should be directed to investigate and isolate potent bioactive compounds from medicinal plants. GLs are considered to possess antidiarrheal properties, as reported by many researchers. Mazumdar *et al.* (2015) reported the antidiarrheal potential of ethanolic isolates of GLs in Wistar rats. The authors reported that a dosage level of extracts at a concentration of 750 and 500 mg/kg had antidiarrheal potential in castor oil-fed rats. Besides this, Ojewole *et al.* (2008) reported similar activity using aqueous extracts of GLs in rodents. They reported that GL extracts at doses of 52–410 mg/kg when administrated orally were found to combat diarrhea and also resulted in reduced intestinal transit and dilatory removal of unwanted gastric products. Further, loperamide (13 mg/kg) reduced the occurrence of defecation, along with the severity of diarrhea in the same animal models. The GL extracts reduced diarrheal symptoms, such as secretion of interstitial fluid and wetness of fecal droppings in a dose-dependent manner. GLs at different concentrations in rabbits showed concentration-dependent pulsing and pendulum retrenchments in the duodenum.

Antimicrobial Activity

The evolution of novel disease-causing strains and resistance of microbes to classical antibiotics are currently serious concerns. The incidence of systemic microbial infections such as septicemia, urinary tract infections, meningitis, pneumonia, and gastritis

affects the entire human body and contributes significantly to global mortality. Food-borne diseases are mostly caused by pathogens including *Staphylococcus, Shigella, Salmonella, Bacillus, Escherichia coli, Clostridium,* and *Pseudomonas* (Ullah *et al*., 2020). Plant-derived bioactive compounds are promising sources of antimicrobials. These compounds act by the inhibition of microbial cell wall development, disruption, and lysis, hampering biofilm formation, repression of DNA replication and transcription, impeding adenosine triphosphate (ATP) production, suppression of bacterial toxins, and the generation of reactive oxygen species (ROS) (Mickymaray, 2019). GLs, owing to the presence of different organic and inorganic antioxidants and anti-inflammatory compounds, are known to possess antimicrobial properties (Naseer *et al*., 2018). GL essential oils display strong antimicrobial properties against *Pseudomonas aeruginosa, Escherichia coli, Streptococcus faecalis, Staphylococcus aureus*, and *Bacillus* subtilis (Soliman *et al*., 2016).

Guava Leaves (Gls) as a Functional Food Ingredient

Recent articles have shown that plant byproducts, such as fruit or vegetable pomace, seeds, husk/bran/seed coat, peel, and leaves, are important sources of bioactive compounds and can be utilized as functional food ingredients (Punia and Kumar, 2021; Kumar *et al*., 2019; Punia *et al*., 2020). Numerous reports suggest the beneficial effects of the inclusion of GL extract in food as a functional food ingredient, because of the presence of a myriad of compounds like rutin, naringenin, gallic acid, catechin, epicatechin, kaempferol, isoflavonoids, vitamins, citric acid, and flavonoids such as quercetin and guaijaverin, which are well known for their antimicrobial, antioxidant, and anti-inflammatory actions (Shaheena *et al*., 2019). A study on the hypoglycemic effects of GL extract, due to the presence of its phenolic compounds, were shown to improve vascular dysfunction in mice with diet-induced obesity (Díaz-de-Cerio *et al*., 2017). Recently, GL extract has been used in the preparation of jelly with pectin and was subjected to mass spectrometry analysis, which verified the presence of quercetin, gallocatechin, esculin, 3-sinapoylquinic acid, ellagic acid, gallic acid, and citric acid that are responsible for antioxidants and antimicrobial properties.

Additionally, the addition of GL did not cause any change in the texture properties of the jelly (Kumar *et al*., 2021). The potentiality of GL as a functional immunostimulant ingredient in fortified foods, owing to the presence of a high level of antioxidant and phenolic compounds, was also studied in detail (Laily *et al*., 2015). Another study on the

evaluation of food–drug interactions of guava leaf tea (GLT), which is a functional food and beverage that is commercially available in Japan, showed no possibility of interactions between GLT and medicines, indicating the safety of GLT in terms of food–drug interactions. Borderline diabetics, who are at high risk of the development of diabetes, take GLT to suppress a rapid increase in blood sugar level after meals. GLT consists of carbohydrate and dietary polyphenols which bind to digestive enzymes and are known to contribute to health through poor absorption of dietary sugar or lipids (Kaneko *et al.*, 2013). Furthermore, a recent report that studied herbal tea also stated that guava tea showed no interaction with medicine (Matsuda *et al.*, 2007).

MATERIALS AND METHODS

Materials

Glassware and Facilities

Mortar and pestle, digital analytical weighing balance (ohaus: pa-1000), beakers, whatman number 1 filter paper, conical flask, spatula, measuring cylinder, aluminum foil, cotton wool, sample bottles, separating funnel, plastic funnels, thermostatic water cabinet (Model:HH-W420), Spectrophotometer(UV-Visible double beam light), micro pipette, liston classic centrifuge (C2204), sykam HPLC (S3250 UV/visible detector). Micropipettes, Cuvettes and test tube.

Reagents and Chemicals

Ferric chloride ($FeCl_3$), ethanol, water, sodium carbonate, trichloroacetic acid (TCA), normal saline. The rest of the chemicals were of analytical grade; Reagents used for the assays were products of Bioscience commercial kits and Spectrum commercial assay kits.

Collection and Preparation of Plant Materials

The fresh leaf of *Psidium guajava* were collected from Government reservation Area (G.R.A) Wukari, Taraba State. The guava leaf collected was washed several times with water and rinsed with distilled water for the removal of impurities. The washed leaf obtained were air dried until they become crispy, after which they were pulverized in a clean and dry mortar and pestle.

Methods

Vitamins Profile of Guava Leaf Sample

The following vitamin levels (vitamins K, B_1, B_9, A and E) were determined using high performance liquid chromatography (HPLC).

Principle: Stationary Phase: A column packed with small, porous particles (stationary phase) retains the sample components based on their interactions with the stationary phase.

Mobile Phase: A liquid solvent (mobile phase) is forced through the column under high pressure, carrying the sample components.

Separation: Components of the sample interact differently with the stationary phase, causing them to move through the column at different rates and separate from each other.

Detection: As the separated components exit the column, they are detected and quantified by a suitable detector, producing a chromatogram (Aryal, 2024).

Methodology: The extract was diluted with the same solvent used for extraction.

Two (2ml) of guava leaf ethanol extract was diluted and filtered with 0.45um syringe filter into the vial. The mobile phase is composed of the following solvents (water, methanol, ethanol and buffer).

The vials were labelled appropriately and placed in the auto sampler of the HPLC for analysis.

The wavelength was set at 230nm to run for 10 minutes using the Reprosil 100, C8, 5um, 4,6 x 150mm column. The sequence was run and observed the retention time and peaks in the chromatogram and then the chromatogram was printed.

Determination of Minerals Composition

Atomic Absorption Spectroscopy (AA S) was used for the determination of minerals in guava leaf sample.

Principle

The principle of AAS is based on the fact that atoms or ions of a specific element can absorb light at unique, characteristic wavelengths. When a sample containing a particular element is exposed to light at that element's specific wavelength, the atoms or ions of that element absorb the light, causing electrons to transition from a ground state to excited states. The amount of light absorbed is directly proportional to the concentration of the absorbing atoms or ions in the sample.

Procedure

Atomization: The sample is atomized, typically using heated graphite tube (Electrothermal AAS) to convert the metal compounds in the sample into free gaseous atoms.

Light Source: Light from a radiation source, usually a hollow-cathode lamp specific for the element being analyzed. This was passed through the cloud of free atoms. This lamp emits light at the precise wavelength that the target element's atoms absorb.

Absorption: The free atoms of the target element in the sample absorbs some of the light from the lamp.

Monochromator: A monochromator is used to isolate the specific wavelength of light absorbed by the analyte from other wavelengths.

Detection: A detector measures the intensity of the light beam after it has passed through the atomized sample. The decrease in light intensity is measured as absorbance.

Concentration determination: The measured absorbance is directly related to the concentration of the element in the sample, following Beer's law. The concentration is typically determined by comparing the sample's absorbance to a calibration curve generated using solutions of known concentrations of the element.

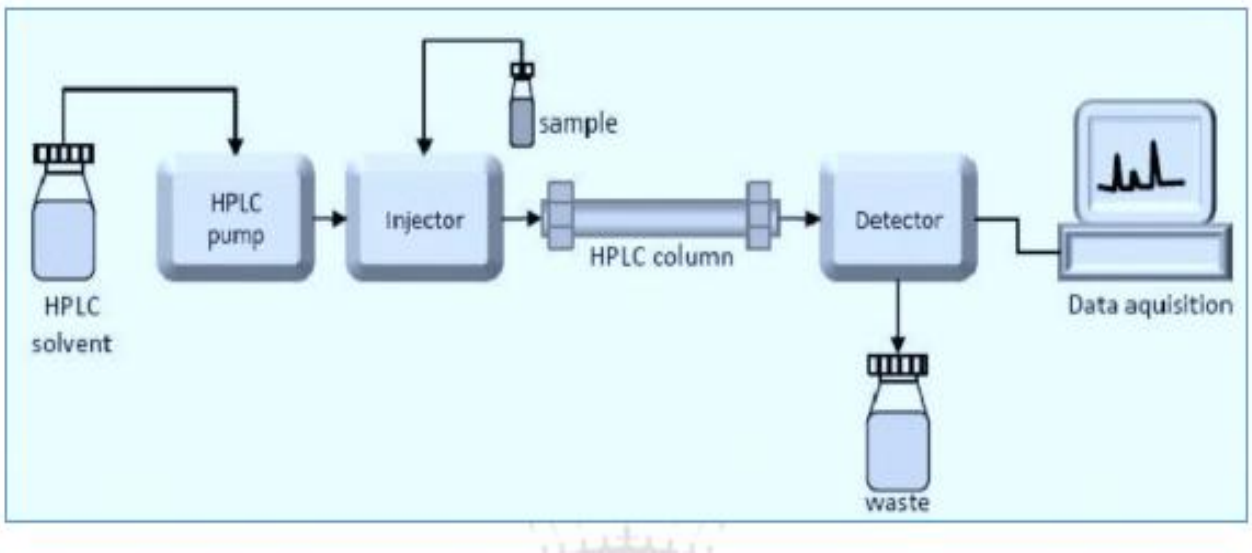


Figure1: HPLC Instrumentation Flow chart (Sonia and Nappinnai, 2016).

Determination of Phytochemicals Using Gas Chromatography-Mass Spectrometry (GC-MS)

The phytochemicals were determined using gas chromatography-mass spectrometry (GC-MS).

Principle: Gas Chromatography (GC): The sample is vaporized and injected into a GC column (Turner, 2024). The compounds in the sample are separated based on their volatility and interaction with the column's stationary phase (Aryal, 2024). The carrier gas (often helium) transports the separate compounds through the column (Aryal, 2024).

Mass Spectrometry (MS): As the separated compounds exit the GC column, they enter the mass spectrometer (Turner, 2024). Here, they are ionized, usually by electron impact, and fragmented into ions. The mass spectrometer measures the mass-to-charge ratio (m/z) of these ions, producing a mass spectrum for each compound (Turner, 2024).

Procedure: The phytochemical analysis of the extract of P. guajava was carried out using GC-MS. Thermo GC-trace standard non-polar column (Dimension: 30 meters, film: 0.25 µm) was used for the analysis. The injector temperature was set at 260 °C and 1 µl of sample injected into the instrument. The oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min-1; and 300 °C, where it was held for 6 min. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 50-650 (m/z) and the ionization voltage was 70eV. The spectrum of the components was compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

Preparation of Guava Leaf Extract

The extract was prepared with slight modification on the method reported by (Saranyaadevi *et al., 2*014). Exactly, 50g of the *Psidium guajava* leaf were weighed and transferred into 250mL beaker containing 500mL distilled water and was heated at 60ºC, stirred for 30mins, after that, it was covered with cotton wool and aluminum foil and allowed to stay for 24 hours.

RESULTS

Vitamin Profile of Ethanol Leaf Extract of P.gujava Using HPLC

The following vitamins (vitamins A, K, B_1, B_9 and E) were determined using high performance liquid chromatography (HPLC) from ethanol leaf extract of P*sidium guajava* which showed high percentage soluble Folate (Vitamin B_9) (65.10%)**,** moderate levels of Thiamine (Vitamin B_1) (32.90%), vitamin E (1.40%) and vitamin K (0.60%) present in small amount, with no detectable present of Vitamin A (0.0%).

Table 1: Vitamins Profile using HPLC in Ethanol Leaf Extract of *P. gujava*

Sn	Retention time (min)	Response	Amount (mg)	Amount (%)	Compound Name
1	0.372	34.67	0.00	0.00	Vitamin A
2	1.812	14.38	0.36	0.60	Vitamin K
3	2.508	31.34	21.27	32.90	Vitamin B_1
4	2.893	170.27	42.04	65.10	Folate B_9
5	5.742	44.10	0.92	1.40	Vitamin E
	Total		64.59	100	

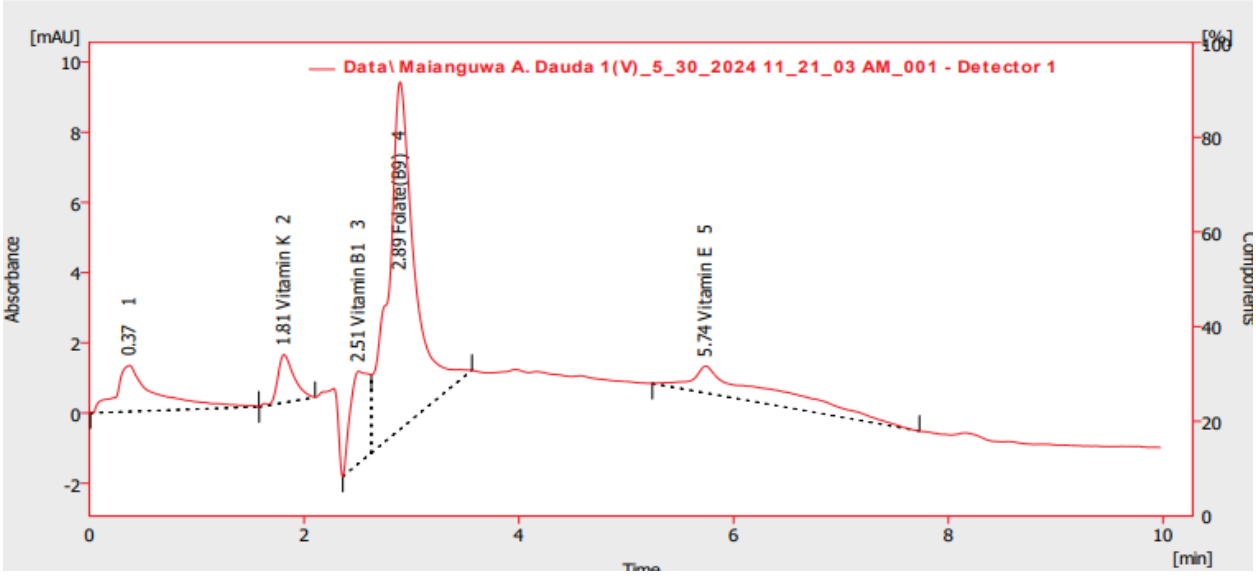


Figure 2: Chromatogram of Vitamins Profile of Ethanol Leaf Extract of Psidium guajava Using HPLC

Minerals Composition of Guava leaf

Determination of minerals (Fe, Zn, Cu, Mg, Ca, P, K, Mn and Na) were carried out using the method of the Atomic absorption spectrophotometer (AAS), which showed high concentration in potassium (K) (9.40 ± 0.89 ppm) and iron (Fe) (5.80 ± 0.57 ppm), moderate levels of phosphorus (P) (4.13 ± 0.08ppm), Sodium (Na) (2.85 ± 0.18 ppm), Calcium (Ca) (2.46 ± 0.06 ppm), with low concentration in zinc (Zn) (0.45 ± 0.07 ppm), manganese (Mn) (0.23 ± 0.01 ppm), copper (Cu) (0.14 ± 0.01 ppm) and lowest concentration in magnesium (Mg) (0.08 ± 0.01).

Table 2: Mineral Composition of Guava Leaf

Parameters	Minerals concentration of Guava leaves powder (mean ± SD) (ppm)
Fe	5.80 ± 0.57
Zn	0.45 ± 0.07
Cu	0.14 ± 0.01
Mg	0.08 ± 0.01
Ca	2.46 ± 0.06
P	4.13 ± 0.08
K	9.40 ± 0.89
Mn	0.23 ± 0.01
Na	2.85 ± 0.18

Phytochemical Analysis Using GC-MS

The GC-MS analysis results of ethanol extract of *Psidium guajava* revealed 2-Hexyl-1-octanol (100%), 2-Ethyl-1-dodecanol (19.68%), Ether, 6-methylheptyl-vinyl (3.11%), 2-Hexyl-1-octanol (6.08%), 5-Hydroxy-2-methylthiopyrimidine (6.43%), 1H-indene-1-methanol-α-methyl-acetate (2.5%), (1S,15S)-Bicyclo-[13.1.0]hexadecan-2-one (1.27%), Unknown (1.54%), 1-Hexadecanol (19.12%), Hydroxylamine, O-decyl (5.13%), 1-phenyl-3-methylpenta-1,2,4-triene (1.4%), 1-Formyl-2,2,6-trimethyl-3-cis-(3-methylbut-2-enyl)-5-cyclohexane (4.4%), Unknown (1.53%), Cyclopropane,1-(3,7-dimethyl-1-octenyl)- (1.39%), 2H-Naphtho[2,3-b]furan-5,10-diene-3,4-dihydro-3,4-dihydroxy-2-methyl-,[2s-(2α,3α,4α)] (6.72%), Nonadecane (2.1%), Phenol 2,4-bis(1,1-dimethylethyl) (15.56%), 1,3-Benzodioxide, 4-methoxy-6-(2-propenyl) (2.47%), Cetene (23.77%), Unknown (2.73%), 1-Dodecanol, 3,7,11-trimethyl (1.6%), 10-Heneicosene (2.42%), 2,4-Dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-C)triazine (1.05%), 1-Octadecene (20.31%), Tetracosane (1.97%), 2-

Ethyl-1-dodecanol (1.9%), 3,7,11,15-Tetramethyl-2-hexadecan-1-ol (2.09%), 2,6,10,14-Tetramethylpentadecan-2-ol (1.16%), Pentadecanal (2.23%), Hexadecanoic acid, methyl ester (8.52%), 1,2-Benzenedicarboxylic acid, butyl-2-ethylhexyl ester (24.52%), 10-Heneicosene (c,t) (23.42%), 9,15-Octadecadienoic acid, methyl ester, (Z,Z) (6.1%), cis,cis,cis-7,10,13-Hexadecatrienal (10.3%), Phytol (16.34%), Octadecanoic acid, 11-methyl-, methyl ester (2.12%), 1,22-Docosanediol (3.71%), Linoleic acid ethyl ester (5.6%), cis,cis,cis-7,10,13-Hexadecatrienal (11.51%), and 2-Undecanol (1.38%).

The key high-abundance phytoconstituents (≥15% area) include: **2-Hexyl-1-octanol** (100%), 1-**Hexadecanol** (19.12%), **Phenol 2,4-bis(1,1-dimethylethyl)** (15.56%), **Cetene** (23.77%), **1-Octadecene** (20.31%), **1,2-Benzenedicarboxylic acid, butyl-2-ethylhexyl ester** (24.52%), **10-Heneicosene (c,t)** (23.42%), **Phytol** (16.34%).

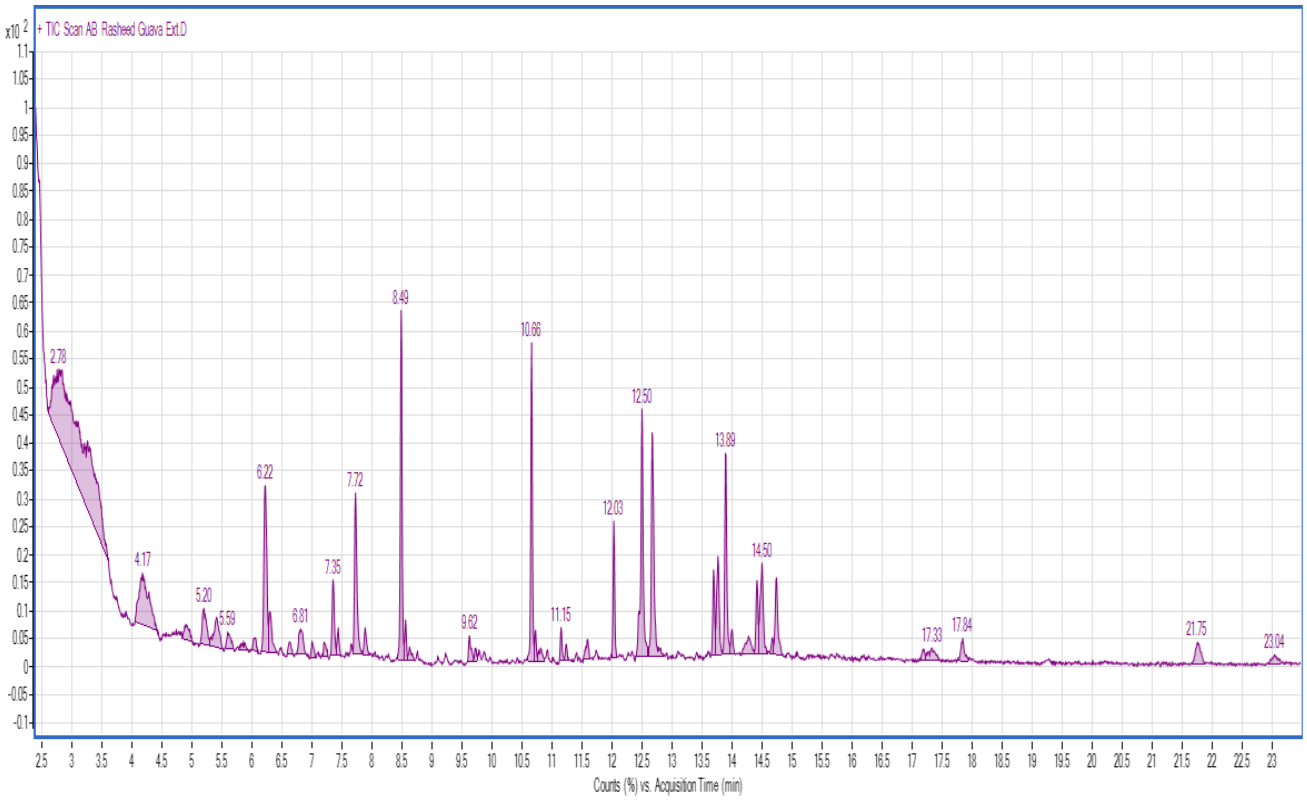


Figure 3: GC-MS Chromatogram for Guava leaf extract

Table 3: GC-MS Phytoconstituents of Ethanol Extract of *Psidium guajava* Leaf

S/N	Compound	Retention Time	Area%	Chemical formula
1	2-Hexyl-1- octanol	2.78	100	$C_{14}H_{30}O$
2	2-Ethyl-1-dodecanol	4.17	19.68	$C14H_{30}$
3	Ether, 6-methylheptyl-vinyl	4.91	3.11	C10H20O
4	2-Hexyl-1-octanol	5.20	6.08	$C_{14}H_{30}O$
5	5-Hydroxy-2-mehylthiopyrimidine	5.41	6.43	$C_5H_6N_2OS$
6	1H-indene-1-methanol-α-methyl-acetate	5.59	2.50	$C_{13}H_{14}O_2$
7	(1S,15S)-Bicyclo-[13.1.0] hexadocan-2-one	5.86	1.27	$C_{16}H_{28}O$
8	**Unknown**	**6.05**	**1.54**	**-**
9	1-Hexadecanol	6.22	19.12	$C_{16}H_{34}O$
10	Hydroxylamine, O-decyl	6.30	5.13	$C_{10}H_{23}NO$
11	1-phenyk-3-methylpenta-1,2,4,-triene	6.63	1.40	$C_{12}H_{12}$
12	1-Formyl-2,2,6-trimethyl-3-cis-(3-methylbut-2-enyl)-5-cyclohexane	6.81	4.40	$C_{15}H_{24}O$
13	**Unknown**	**7.00**	**1.53**	**-**
14	Cyclopropane,1-(3,7-dimethyl-1-octenyl)-	7.20	1.39	$C_{13}H_{24}O$
15	2H-Naptho[2,3-b]furan-5,10-diene-3,4-dihydro-3,4-dihydroxy-2methyl-,[2s-(2α,3α,4α)]	7.35	6.72	$C_{14}H_{12}O_5$
16	Nonadecane	7.44	2.10	$C_{19}H_{40}$
17	Phenol 2,4-bis(1,1-dimethylethyl)	7.72	15.56	$C_{14}H_{22}O$
18	1,3-Benzodioxide, 4-methoxyl-6-(2-popenyl)	7.89	2.47	$C_{11}H_{12}O_3$
19	Cetene	8.49	23.77	$C_{16}H_{32}$
20	**Unknown**	**8.56**	**2.73**	**-**
21	1-Dodecanol, 3,7,11-trimethyl	8.63	1.6	$C_{15}H_{32}O$
22	10-Heneicosene	9.62	2.42	$C_{21}H_{42}$
23	2,4-Dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-C)triazine	9.73	1.05	$C_6H_7N_5O$
24	1-octadecene	10.66	20.31	$C_{18}H_{36}$
25	Tetracosane	10.73	1.97	$C_{24}H_{50}$
26	2-Ethyl-1-dodecanol	10.81	1.90	$C_{14}H_{30}O$
27	3,7,11,15-Tetramethyl-2-hexadecan-1-ol	11.15	2.09	$C_{20}H_{40}O$
28	2,6,10,14- Tetamethylpentdecan-2-ol	11.24	1.16	$C_{19}H_{40}O$
29	Pentadecanal	11.59	2.23	$C_{15}H_{30}O$
30	Hexadecanoic acid, methyl ester	12.03	8.52	$C_{17}H_{34}O_2$

S/N	Compound	Retention Time	Area%	Chemical formula
31	1,2-Benzenedicarboxylic acid, buty-2-ethyl hexyl ester	12.50	24.52	$C_{20}H_{30}O_4$
32	10-Heneicoene (c,t)	12.67	23.42	$C_{21}H_{42}$
33	9,15-octadecadienoic acid, methy ester, (Z,Z)	13.69	6.10	$C_{19}H_{34}O_2$
34	Cis,Cis, Cis,-7,10,13-Hexadecatrienal	13.77	10.30	$C_{16}H_{26}O$
35	Phytol	13.89	16.34	$C_{20}H_{40}O$
36	Octadecanoic acid, 11-methyl--methyl ester	14.00	2.12	$C_{20}H_{40}O_2$
37	1,22-Docosanediol	14.27	3.71	$C_{22}H_{46}O_2$
38	Linoleic acid ethyl ester	14.42	5.60	$C_{20}H_{36}O_2$
39	Cis,Cis, Cis,-7,10,13-Hexadecatrienal	14.50	11.51	$C_{16}H_{26}O$
40	2-undecanol	14.67	1.38	$C_{11}H_{24}O$

DISCUSSION

Vitamin Profile of Psidium guajava Using HPLC

The result in Table 4.1 shows that five different vitamins were detected. Vitamins are essential organic micronutrients that are needed by the body in small amounts to function properly. They are essential for normal growth, development, metabolism, immune function and overall health. This finding indicates that Vitamin B_9 (65.10%) and Vitamin B_1 (32.90%) are the most abundant vitamins in the ethanol extract. However, vitamin B-complex are for energy production, nerve function and red blood cell formation. This suggests that guava leaves are a significant source of these essential vitamins, which are crucial for various physiological activities and maintain good health. This is in correlation with a study conducted by Kumar *et al.* (2021) who reported that guava leaves are the rich source of minerals and vitamins such as calcium, potassium, sulfur, sodium, iron, magnesium, manganese, and vitamins B-complex and C respectively. Vitamin B also plays an important role in improving blood circulation, nerve relaxation, and cognitive function stimulation.

Thomas *et al.* (2017) also reported that guava leaves have the following concentration of vitamins C and B which were 103.0 and 14.80 mg per 100g on dry weight bases, respectively. The ethanol leaf extract of guava leaves exhibits a notable vitamin profile, particularly rich in folic acid and thiamine. Thiamine plays a role in cellular energy metabolism pathways such as pentose synthesis cycle and the tricarboxylic acid cycle.

Thiamine is involved in alpha-keto acid oxidative decarboxylation, which includes the oxidative conversion of pyruvate to acetyl-CoA. Transketolase, the enzyme that catalyses the exchange processes of two carbon pieces in the oxidation of glucose via hexose monophosphate, used it as a cofactor (Kochetov and Solovjeva, 2014). Lack of vitamin B_1 lead to beriberi, a disorder that affects the heart and nervous system, cyanosis, peripheral neuropathy, tachycardia, peripheral paralysis of the lower extremities, and eventually death due to heart failure (Spinazzi, *et al.*, 2010). Pyruvate cannot enter the tricarboxylic acid cycle without thiamine pyrophosphate, resulting in cardiac failure due to a lack of energy for the heart muscle. Folic acid is a methyl group donor that is involved in DNA synthesis, especially in cells that need to expand more, tetrahydro folic acid is its active form. Megaloblastosis of intestinal cells and macrocytic anaemia are symptoms of folic acid insufficiency (Vora *et al.*, 2022). Folate deficiency is linked to hiperhomocysteinemia, or excess homocysteine, which is linked to an increased risk of coronary vascular disease and stroke. Lack of this vitamin B_9 in pregnant women can cause neural tube problems in babies, such as spine bifida and anencephaly (Vora *et al.*, 2022), consistent with existing literature that emphasizes the nutritional benefits of guava leaves. This profile supports the use of guava leaves in dietary applications and their potential role in health promotion.

Minerals Composition of Psidium guajava Leaf Extract

Mineral analysis of *Psidium guajava* leaf was carried out using Atomic Absorption Spectrophotometry (AAS). In Table 4.2, the results indicated that the leaf contains various essential minerals in varying concentrations. The order of abundance was: K > Fe >P > Na > Ca > Zn > Mn > Cu > Mg.

The mineral analysis of the *Psidium guajava* leaf powder showed high concentration in potassium and iron, 9.40 ± 0.89 ppm and 5.80 ± 0.57 ppm respectively. Potassium is an important mineral that plays a role in heart health, muscle function, and nerve transmission. Kumar *et al.* (2021) reported that guava leaves are the rich source of minerals, such as calcium, potassium, sulfur, sodium, iron, boron, magnesium, manganese, and vitamins C and B. Adrian *et al.* (2015) also reported that higher concentrations of Mg, Na, S, Mn, and B in guava leaves makes them a highly suitable choice for human nutrition and also as an animal feed to prevent micronutrient deficiency. Consumption of Ca- and P-rich guava leaves reduces the risk of deficiency-related diseases such as hypocalcemia, hypophosphatemia, and osteoporosis, as reported by Kumar *et al.* (2021). Since guava

leaves are a rich source of minerals, they play critical roles in various physiological and biochemical processes. The presence of these minerals, (Fe, Zn, Cu, Mg, Ca, P, K, Mn and Na) makes guava leaves not just useful in traditional medicine but also of interest in nutrition and pharmacology.

GC-MS analysis of Psidium gujava Leaf Extract

As shown in Figure 4.1, the GC-MS spectrum indicated the presence of various components with varying retention times. Individual compounds were identified by comparing their mass-spectral database with National Institute of Standards and Technology (NIST 11) libraries, ensuring the registered spectra with more than 80% of similarity Index (SI): and by comparing them with the values published in the literature. The GC-MS analysis of the ethanol extract of *Psidium guajava* (guava) leaves elicited a diverse array of phytochemicals, with notable compounds including: 2-Hexyl-1-octanol - a fatty alcohol that may contribute to the extract's bioactivity. 2-Ethyl-1-dodecanol - another fatty alcohol, which is known for its potential applications in cosmetics and pharmaceuticals. 1-Hexadecanol - a long-chain fatty alcohol that can have antimicrobial properties. Phenol 2, 4-bis (1, 1-dimethylethyl) - a phenolic compound that could exhibit antioxidant activity. Cetene - a hydrocarbon that can be involved in various biological activities. 1-octadecene - an alkene that may have applications in the synthesis of other compounds. 1,2-benzenedicarboxylic acid, buty-2-ethyl hexyl ester - a compound that can act as a plasticizer and has potential health implications. 9, 15-octadecadienoic acid, methyl ester (Z,Z) - a fatty acid methyl ester, known for their anti-inflammatory properties. Phytol - a diterpene alcohol that has been studied for its antioxidant and anti-inflammatory effects. The findings from the GC-MS analysis of guava leaves align with previous studies that had identified various bioactive compounds in *Psidium guajava*. For instance, a study by Ibrahim *et al.* (2021) reported the presence of similar fatty acids and alcohols, highlighting the plant's potential as a source of natural antioxidants and antimicrobial agents. Additionally, the presence of phytol has been noted in other studies, where it was associated with various therapeutic properties, including anti-inflammatory and antioxidant effects (Ishak *et al.,* 2024). Moreover, the identification of 1, 2-benzenedicarboxylic acid derivatives in guava extracts is consistent with the findings in other plant studies, where such compounds were linked to beneficial health effects, including potential anticancer properties (Kumar *et al.,* 2021). The diversity of compounds found in the ethanol extract of guava leaves suggests a rich source of phytochemicals that could be explored for their medicinal applications. The

GC-MS analysis of the ethanol extract of *Psidium guajava* leaves has identified several compounds with potential health benefits. The results are consistent with existing literature, reinforcing the idea that guava leaves are a valuable source of bioactive compounds that warrant further investigation for their therapeutic properties.

CONCLUSION

The HPLC analysis from ethanol leaf extract of *Psidium guajava* leaf revealed the sample contains a significant quantity of folate (VitaminB_9) and Vitamin B_1, indicating a potential source of essential B-complex vitamins. The presence of trace amounts of Vitamins K and E also contributes to the sample's nutritional value. Mineral analysis of *Psidium guajava* leaf was performed using Atomic Absorption Spectrophotometry (AAS). The results indicated that the leaf contains various essential minerals in varying concentrations. The order of abundance was K > Na > Ca > Fe > P > Zn > Mn > Cu > Mg.

Potassium (K) was the most abundant mineral, suggesting a strong presence of electrolytes beneficial for cardiovascular and muscular health. Sodium (Na) and Calcium (Ca) followed with moderately high concentrations, essential for fluid balance, nerve function, and bone health. Iron (Fe) and Phosphorus (P) were present in moderate quantities, important for blood formation and energy metabolism. Zinc (Zn), Manganese (Mn), and Copper (Cu) were found in relatively low concentrations, but still contribute to enzymatic and antioxidant activities. Magnesium (Mg) was found to have the lowest amount of mineral concentration, despite its known role in neuromuscular functions and metabolic processes.

The result of phytochemical analysis showed that the ethanol extract of *P.guajava* has 40 phytoconstituents, significant constituents included 2-Hexyl-1-octanol, a fatty alcohol with potential antimicrobial properties; cetene (1-Hexadecene) and 1-octadecene, long-chain alkenes noted for their industrial applications; and 1,2-Benzenedicarboxylic acid, buty-2-ethyl hexyl ester, a common plasticizer with implications for environmental health. The identification of these compounds underscores the medicinal merit of *Psidium guajava*, suggesting its utility in treating various ailments, including diabetes, cancer, and cardiovascular diseases.

The chemical composition of *psidium guajava* (linn) leaf revealed appreciable levels of minerals composition with the highest in potassium and iron, while vitamins profile elicited highest level for Folate (Vitamin B9) (65.10%)**,** moderate levels of Thiamine (Vitamin B_1) (32.90%). The leaf also exhibited high percent of the following phytoconstituents: 2-Hexyl-1-octanol, 2-Ethyl-1-dodecanol, 1-Hexadecanol, Phenol 2, 4-bis (1, 1-dimethylethyl): Cetene, 1-octadecene, 1, 2-Benzenedicarboxylic acid, buty-2-ethyl hexyl ester, 9, 15-octadecadienoic acid, methyl ester, (Z, Z), Phytol among others.

Conflict of Interest

The authors affirm that there are no conflicts of interest associated with this publication.

Authors' Declaration

The authors confirm that the research presented in this article is entirely original. They accept full responsibility for any claims or issues arising from the content herein.

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