Proximate, Phytochemicals and Gas Chromatography Flame Ionization Detection (GC-FID) Profiling of Ethanolic Extract of Guava (Psidium Guajava) Leaves and Its Antimicrobial Activities on Some Bacterial Isolates.

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ABSTRACT

This study examined the proximate, phytochemicals and antimicrobial properties of extract of *Psidium guajava* (guava) leaves against some bacterial isolates. Samples of guava leaves were collected, washed, dried, pulverized, and then subjected to ethanolic extraction and concentrated using rotary evaporator. The proximate, vitamins and phytochemicals were determined by standard methods. The bioactive compounds were further quantified using gas chromatography flame ionization detector (GC-FID) techniques. The minerals were determined using atomic absorption spectrophotometer (AAS). Using the diffusion technique with agar wells, some bacteria isolates were examined for their susceptibility to the extract. The proximate composition (%) showed that protein content was 18.73, ash (3.63), moisture (8.24), and carbohydrate (66.01). The physicochemical revealed that the temperature was 27.63ºC, pH (5.97), total solids (0.98), solids-non-fat (0.37), and specific gravity (0.73) with the titrable acidity content value as 0.32. The vitamin content (mg/100g) of the extract indicated that the value of vitamin B was 5.78, B_1 (4.77), B_6 (6.63), C (6.37), while the carotenoids was 5.67 mg/100g. The sodium (ppm), calcium (ppm), and phosphorous (mg/kg) content of the sample were 34.68, 6.06 and 7.79, respectively. Phytochemicals revealed moderate level of alkaloids, saponins etc., while glycosides was high. The GC-FID revealed the presence of twelve (12) bioactive constituents: lunamarin being the most abundant with a concentration of 33.6262 ppm, while kaempferol (0.3564 µg/ml) was the least. At 200 mg/ml, the extract showed higher activity and varying zones of inhibition (mm) at 17, 8, 20, and 9 on *E. coli*, *Shigella sp*., *Lactobacillus sp* and *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) of the sample against *E. coli* and *Lactobacillus sp*, was at 50, while *Shigella sp* and *Staphylococcus aureus* was at 200. This study revealed that guava leaves are rich sources of health promising nutrients and some bioactive compounds that are inhibitory against some pathogens.

Keywords: Psidium guajava, Extracts, Phytochemicals, Antibacterial activity.

INTRODUCTION

Traditional medicine practices have made use of phytomedicines since primeval period (WHO, 2023). The utilized medicinal plants are thought to be abundant sources of bioactive compounds that could aid in the development of new drugs (Yusuf *et al*., 2020; Atanasov *et al*., 2021). Bioactive chemicals found in all plant parts, including fruits, vegetables, nuts, oils, and whole grains, have beneficial metabolic and immunological effects that can be used to satisfy people's desire for a better, healthier lifestyle. These bioactive substances

reduce the risk of diseases like cancer, cardiovascular disease (CVD), neurological diseases, and type 2 diabetes by detoxifying free radicals and preventing oxidative cell damage (Shrinet *et al*., 2021; Zhang *et al*., 2022).

The use of medicinal plants as alternative source of medicine for human ailments have been bolstered by factors such as population growth, inadequate drug manufacturing and supply, the side effects of drugs, prohibitive treatment costs, the growing threat of antibiotic and synthetic drug resistance to infectious diseases. These plants are chemical goldmines that are widely recognized for their acceptance by both human and animal systems for use in conventional medical systems to treat a variety of illnesses (Romina, 2024). The Myrtaceae family, which are over 133 genera and 3,800 species, includes the guava (*Psidium guajava*). The guava is an evergreen shrub or small branching tree that grows to a height of 7 to 10 meters (Qa'dan *et al*., 2005). *Psidium guajava* L, also referred to as guava, is the most widely grown species of Psidium and is mostly grown in tropical and subtropical areas worldwide. It is an important herb in Nigeria, India, Indonesia, Pakistan, Bangladesh, and South America (Sharma *et al*., 2017).

The different parts of guava plant contain variety of phytochemicals with various pharmacological properties (Kafle *et al*., 2018). The broad spectral of phytochemicals in guava include; polysaccharides, vitamins, essential oils, minerals, enzymes, proteins, and triterpenoid acids (Begum *et al*., 2010). Joseph and Priyar (2011) reported that it is very rich in antioxidants and vitamins and also high in lutein. According to Adefagha and Obah (2011), their therapeutic use in prevention or fighting a number of diseases is the basis of their extensive use in traditional medicine. They equally posited that some of the phytochemicals are water soluble while others are not.

The leaf extracts of the guava plant have been studied for their medicinal applications and their benefits are attributable to their plethora of phytochemicals, such as quercetin, avicularin, apigenin, guaijaverin, kaempferol, hyperin, myricetin, gallic acid, catechin, epicatechin, chlorogenic acid, epigallocatechin gallate, and caffeic acid (Ashraf *et al*., 2016). The presence of phenolic compounds, such as gallic acid, pyrocatechol, taxifolin, ellagic acid, ferulic acid, and several others, are responsible for the antioxidant roles of guava leaves (Farag *et al*., 2020). Also, many of these extracts have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions. The plant has also been used for the controlling of life-changing conditions such as diabetes, cancer, hypertension, and obesity (Sofowora *et al*., 2013). Studies on animal models have also established the role of guava leaves isolates as potent antitumor, anticancer, and cytotoxic agents (Jiang *et al*., 2020).

The general techniques of medicinal plant extraction can be done by various conventional and non- conventional procedures including; maceration, infusion, percolation, digestion, decoction, Soxhlet extraction, aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction, supercritical fluid extraction, phytonic, column chromatography extraction among other method (Altemimi,*et al*., 2016). The technique of Soxhlet extraction is conventional method based on exhaustive extraction of organic compounds (analytes) in a Soxhlet system by an organic solvent, which is continuously refluxed through the sample contained in a porous thimble (Azmir *et al*., 2013. Extraction of phytochemicals are affected by such factors including; its origin, variations in the extraction technique, the time, temperature of extraction, solvent concentration and polarity, as well as quantity and secondary metabolite composition of an extract (Nawaz, et al., 2020). Other factors are the variations in extraction methods which are usually found by the length of the extraction period, the solvent used, pH, particle size, and the solvent-to-sample ratio (Ncube *et al*. (2008).

Proximate analysis of the bioactive compounds which is important in the determination of the major components of food such as; moisture, protein, fat, ash, crude fibre and total carbohydrate had been reported by many researchers (Amadi *et al*., 2017). Physicochemical compositions of bioactive compounds properties are also important in the elucidation of the physical and chemical characteristics that influence their ability to interact with the body and exert therapeutic effects. In conventional analysis, the gross components (protein, fat, carbohydrate, ash etc.), physical and chemical compositions of the food material rather than individual nutrients (amino acid, fatty acids, monosaccharides, mineral, etc.) are determined (Prohp *et al*., 2006).

Albeit, the continuous evolution of multidrug resistant pathogens is a global clinical concern (Nikaido, 2009).

In recent years there has been an increasing incidence of multiple drug resistance in human pathogenic microorganisms due to the indiscriminate uses of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases (Tsaku *et al*., 2017). Bacterial resistance to antibiotics presents a serious problem for clinicians and the therapeutic industry and great efforts are being made to reverse this trend. One of the remedies suggested was the widespread screening of medicinal plants from the traditional system of medicine that would proffer solutions for more effective agents for the treatment of infectious diseases (Abdallah *et al*., 2023). The antimicrobial activities of some plant species have been widely researched. For example, the crude extracts of cinnamon, garlic, basil, curry, ginger, sage, mustard, and other herbs exhibit antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria (Castro *et al*., 2008). Nzeako *et al.* (2006) reported that thyme oil extract could decrease the growth of *Candida albicans* and *Pseudomonas aeruginosa*. The search for new antibacterial agents by the screening of many plant families had been encouraged (Dipankar, 2011). Additionally, using antibiotics had been associated with adverse effects, therefore phytomedicine could be an alternative treatment method for bacterial infections which may decrease the incidence of such problems (Alekshun and Levy, 2007).

The Psidium guajava is a phyto therapeutic plant used in folk medicine and is believed to have active components that helps in treatment and management of various diseases (Pandey *et al*., 2017). Guava had exhibited remarkable antimicrobial activity against microorganisms such as Bacillus, E. coli, Staphylococcus, Pseudomonas, Clostridium, Shigella, Salmonella and yeast such as Candida species (Fugaban, 2016). This study therefore, investigates the proximate, phytochemical and antimicrobial properties of extract of Psidium *guajava* leaves against some clinical bacterial isolates

MATERIALS AND METHODS

Collection and Identification of Plant Samples

Fresh and healthy leaves of *P. guajava* L. were collected from Carmelite farm in Enugu State, Nigeria

Preparation of Plant Material

The leaves were washed in distilled water to remove debris and allowed to dry under shade for 7 days. The dried leaves were then pulverized into powder using blending machine. The powdered leaves were extracted using ethanol extraction (Soxhlet) method. In this method, five hundred (500) grams of the sample were weighed out, wrapped in filter paper and then put in the thimble of the Soxhlet apparatus compartment. Thereafter, the condenser was carefully and efficiently connected. An initial 500 ml volume of the solvent (ethanol) were added with the aid of a funnel by passing it through the thimble containing the sample to the round bottom flask system of the Soxhlet. The inlet and outlet of the condenser were connected to a hose for the recycling of the cold water during the extraction. Thereafter, the heat source was switched on about 5cm from the flask. Finally, the crude extracts were concentrated at 50 ^{o}C using a rotary evaporator, and the extract was stored in sterile screw-capped bottles and kept at 4 ^{o}C in a refrigerator for further use.

Determination of Physicochemical Properties and Proximate Parameters

The method described by A.O.A.C (2006) was adopted for the physicochemical compositions (pH, temperature, total solids, solid non-soluble fat, titratable acidity, and specific gravity) and proximate parameters (ash, moisture, fat, fiber and protein). Carbohydrate was estimated by difference; % carbohydrate = 100 - % (protein + moisture + fat + fiber + ash).

Determination of Vitamin Composition

The vitamin content was analyzed by a modification of A.O.A.C (1990). The sample was subjected to the laboratory atmospheric condition on the bench after removing the sample from the storage chamber at 4^{0}C. The sample was pressed and completely homogenized in the mortar carefully with pestle to avoid forming balls. The homogenized sample (0.10g) was weighed into a beaker after extraction; the extract was concentrated to 1. 0 ml and for analysis, using a HP 6890 Gas chromatographic apparatus fitted with pulse flame photometric detector (PFPD) using Nitrogen as carrier gas. Split ratio 20:1 with flow rate of 1.0ml/min, inlet temperature 250^{0}C, and

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column type HP-5 with 30m x .25mm x .25 µm column dimensions was used. Oven temperature: initial temperature @ 50^0C for 2 min, detector temperature maintained 320^0C; pressure 20psi and compressed air pressure 30psi.

Determination of Mineral Contents

Wet digestion of sample (5ml) using a mixture of concentrated HNO_3 and 60% (v/v) $HClO_4$ was carried out according to the method of A.O.A.C (2006) where the organic matter in the sample was digested and afterwards diluted to a final volume of 25 ml with deionized water. The levels of Na, Ca and P, in the sample were thus evaluated using an atomic absorption spectrophotometer (AAS) (Buck Scientific model 210 VGP) and flame photometer (Jenway model).

Qualitative Phytochemicals Analysis

Qualitative phytochemical screenings were carried out following standard protocols. A modified method of Ankita and Sapan, (2018) was used in these analyses. Essentially, phytochemical screenings were done to identify the presence of plants secondary metabolites. The saponin, alkaloides, tannin, flavonoids, glycoside, phenol and steroid were determined following these protocols

Analysis of Bioactive Compounds by Gas Chromatography Flame Ionization Detection (GC-FID) Technique

The analysis of bioactive compounds were performed using a gas chromatography flame ionization detector (GC-FID) system (Burk scientific M910) as described by Alcalde-eon *et al*. (2006). The GC-FID analysis was carried out on the *P. guajava* leaves extract to identify and quantify bioactive compounds present in the sample. A syringe was used to draw 0.1 ml of the fraction and injected into the gas chromatography (GC) machine equipped with FID. In principle, FID uses a flame to ionize organic compounds containing carbon. Following separation of the sample in the GC column, each analyte passes through a flame, fueled by nitrogen and zero air, which ionizes the carbon atoms.

Instrumentation

Analysis was performed on a GC-FID system (Buck scientific M910). The GC was equipped with a HP-5MS of 30 m length and 0.25 mm internal diameter capillary column (RESTEK 15 METER MIX-1), with 0.25 µM film thickness. The carrier gas was Nitrogen (at 5 pounds per square inch (P.S.I)) set to flow at 1.5 ml/min. The injector was operated in spitless mode at the 280 °C temperature. The chromatographic working conditions were optimized for complete separation of the target compounds. The oven was programmed from 50 °C (3.0 min.) to 310 °C at the rate of 5 °C/min. and maintained at this temperature for 5.0 min. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentrations of the different phytochemicals were expressed in microgram per gram (µg/g) and parts per million (ppm).

Collection of Organisms

Escherichia coli, *Shigella sp*, *Lactobacillus sp* and *Staphylococcus aureus* were the bacteria organisms used. These bacterial species were isolated from urine samples at the Microbiology Laboratory, Enugu State University of Science and Technology, Agbani.

Re-Isolation of Isolates.

After inoculating the isolates, a sterile wire loop was used to distribute them on nutrient agar slants and incubated at 37 °C for 24 h. After each usage, the colonies were stored on these nutrient agar slants at 4 °C.

Inoculum Preparation. A total of one loopful of each isolate in a 24 h incubation chamber at 37 °C using 5ml of sterile nutrient broth was cultured. Inoculum density or turbidity of the bacterial suspensions were adjusted to

that of 0.5 ml McFarland standard (CLSI, 2012; Kebede *et al*., 2021).

Reconstitution of the Extract.

In separate sterile test tubes, to 2 g of the extract, 5ml of Dimethyl Sulfoxide (DMSO) was added to one tube to get the concentration down to 200 mg/ml. A 2ml of the initial sample was divided among four test tubes containing two milliliters of dimethyl sulfoxide (DMSO), yielding a concentration of 200, 100, 50 and 25 mg/ml by weight; this was done to provide two-fold dilutions.

Antibacterial Assay of the Plant Extract.

Antibacterial assay on the isolates was determined by agar well diffusion method (Irobi *et al*., 2014). The four isolates were streaked on Muller Hinton agar plates with 0.1 ml each, after having been matched with 0.5 McFarland standard. Sterile cork borers, measuring 6mm in diameter each, were used to penetrate the plates. The pipetted sterile extract ranging in concentration from 200 to 25 mg/ml were introduced to the holes in increments of 0.1ml. The antimicrobial agents diffuse in the agar medium and inhibit the growth of the microbial strain tested, then, the clear zone or zone of inhibition were observed. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well (Kebede *et al*., 2021). The culture plates were incubated at 37 ^{o}C for one day after being pre-diffused on the work surface for 30 min. Thereafter, millimeter-scale readings of the inhibitory zones was done using ruler.

Determination of the Minimum Inhibition Concentration (M.I.C)

The agar well diffusion method was used to determine the minimum inhibitory concentration of the extract. Multiple concentrations were achieved by 2-fold dilution for the antimicrobial susceptibility test: 200, 100, 50 and 25 mg/ml. Before being put onto the Muller Hinton plates, 0.1ml of each isolate was matched to 0.5 McFarland's turbidity standard. Holes were then created using a 6mm cork borer. A total of 0.1ml of extract was added to each well. The plates of these cells were incubated for the formation of the zone of inhibition after 24 h of incubation at 28 °C. The concentrations at which the extract became inhibited is called the Minimum Inhibitory Concentration (MIC) (Obodo *et al*., 2025).

Statistical Analysis

Data collected were subjected to statistical analysis using IBM Statistical Package for Service Solution (SPSS) version 21.0. Descriptive statistics were used and the differences in mean were considered significant at $p < 0.05$. Experiments were performed in triplicate ($n = 3$) and results were expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Guava (Psidium guajava) leaves extract exhibit a rich phytochemical profile, as well as essential nutrients as shown by proximate and physicochemical compositions. The proximate parameter of the *Psidium guajava* leaves showed high percentage (%) of carbohydrate (66.01) and protein contents (18.73), moderate levels of moisture (8.24), ash (3.63) and crude fibre (2.30) with low levels of fat (1.11) (Table 1).

Table 1: The Proximate Parameters of the Guava (Psidium guajava) Leave Extract

Proximate Composition (%)	Results
Ash	3.63±0.01
Moisture	8.24±0.02
Fiber	2.30±0.10

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Protein	18.73±0.15
Fat	1.11±0.01
Carbohydrate	66.01±0.01

Key: % = percentage. Values are means of triplicate results ± SD.

Guava leaves are a rich source of various health-promoting micro - and macronutrients as well as bioactive compounds. According to Shabbir *et al*., 2020, the leaves contained 82.47% moisture, 3.64% ash, 0.62% fat, 18.53% protein, 12.74% carbohydrates, 103 mg ascorbic acid, and 1717 mg gallic acid equivalents (GAE)/g total phenolic compound. The moderate content of moisture recorded in this finding is closed to low moisture content of the leaves reported by Adefagha and Obah, (2011) and this would hinder the growth of micro-organisms, thereby increasing the extracts storage stability. The presence of protein, carbohydrate and fat indicate that the leaves extract of P. guajava L. may assist in growth, tissue repair and energy production in the body. The protein content of the *Psidium guajava* compared favorably with the work of Eze and Obinwa (2014) on the phytochemical and nutritional evaluation of *Psidium guajava*. The low amount of fat showed that it is not a good source of lipids. Accumulation of fats can cause arteriosclerosis and aging. These high protein and low-fat characteristic of *Psidium guajava* leaves reported in this study, have been previously reported by Adefagha and Obah (2011). 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 along with other beneficial nutrients and phytochemicals

Table 2: Physicochemical Composition of the Guava (Psidium guajava) Leaves Extract

Physicochemical Composition	Results
Temperature (^{0}C)	27.63±0.35
pH	5.97±0.04
Total solid (TS)	0.98±0.01
Solid-non-soluble fat (SNF)	0.37±0.02
Specific gravity	0.73±0.02
Titratable acidity (TTA)	0.32±0.15

Values are means of triplicate results ± SD.

The physicochemical compositions of guava leaves were analyzed and results revealed that total solid (TS) was 0.98, pH (5.97), temperature (27.63), titratable acidity (0.32), solid non-soluble fat (SNF) (0.37) and specific gravity of 0.73 (Table 2). These findings agreed with the reports of Rashmi and Shukla (2017). Similarly, Dani (2023) reported moisture of 89.28%, pH 3.35 and TSS (brix) of 11.56 on the physical and chemical characteristics of guava (Psidium guajava L.) Taiwan pink variety. The presence of these physicochemical properties indicate the high nutritional contents of guava leaves.

Table 3: Vitamin Composition of the Guava (Psidium guajava) Leaves Extract

Vitamin Composition (mg/100g)	Results
B	5.78±0.02

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B 1	4.77±0.01
B 6	6.63±0.02
C	6.37±0.55
Carotenoid	5.67±0.03

Key: mg/100g = milligram per 100 grams; ppm = parts per million; mg/kg = milligram per kilogram. Values are means of triplicate results ± SD.

Table 4: Mineral Composition of the Guava (Psidium guajava) Leaves Extract

Mineral Composition (ppm, mmg/kg)	Results
Sodium (Na) (ppm)	34.68±0.01
Calcium (Ca) (ppm)	6.06±0.01
Phosphorus (P) (mg/kg)	7.79±0.00

Key: ppm = parts per million; mg/kg = milligram per kilogram. Values are means of triplicate results ± SD.

Guava leaves are the rich source of minerals, such as calcium, potassium, sulfur, sodium, iron, boron, magnesium, manganese, and vitamins C and B. The mineral contents (Table 3) of the guava leaves (GLs) studied had shown high level of Sodium (34.68 ppm) and moderate concentrations of Calcium (6.06 ppm) and Phosphorus (7.79 mg/kg), while the vitamins contents (mg/100g) (Table 4) were; B (5.78±0.02), B 1 (4.77), B 6 (6.63), C (6.37) and carotenoid (5.67).

Adrian *et al*. (2015) had reported higher concentrations of macro- and micronutrients such as Mg, Na, S, Mn, and B in guava leaves. These high mineral contents of guava leaves make them highly suitable choice for human nutrition and also as animal feed to prevent micronutrient deficiency. 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, while the concentrations of vitamins C and B were 103.0 and 14.80 mg per 100g DW, respectively. The concentration of minerals and vitamins in this study were not in agreement with results obtained by other researchers which could be due to differences in the extraction method used. Consumption of Ca- and P-rich guava leaves (GLs) reduces the risk of deficiency-related diseases like hypocalcemia, hypophosphatemia, and osteoporosis. It had been reported that 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 (Kumar *et al*., 2012).

The rate of extraction of phytochemicals from medicinal plants depends on the solvent dielectric constant. According to Nawaz *et al*. (2020), extraction and purification are generally affected by such factors including; time, temperature, solvent concentration and solvent polarity. The phytochemical compositions of the *P.guajava* leaves studied had shown the distributions of bioactive metabolites. The qualitative phytochemical screenings revealed the presence of alkaloids, saponins, flavonoids, glycoside, tannin, phenol and steroid which are known to exhibit medical and physiological activities. All the bioactive compounds detected showed moderate concentration of the metabolites except glycoside which was high (Table 5). Available reports on indigenous medicinal plants including; neem (*Azadirachta indica*) (Amadi *et al*., 2017), lemon grass (*Cymbopogon citatus*) (Wifek *et al*., 2016) etc had equally detected a number of bioactive substances reputed to hold protective and therapeutic properties owing to the presence of these pharmacologically active components such as alkaloids, flavonoids, phenols, tannin, saponin, and glycoside. Yusuf *et al*. (2020) reported the presence of terpenoids,

reducing sugars, and phenols in substantial quantities, thus giving credence on the anti-oxidants, antimicrobial, anti-inflammatory, analgesic, anti-arthritic and wound healing effect of methanol and ethyl-acetate extracts of *Azanza garckeana.* Our findings agreed with the earlier studies, according to Bamishaiye et al. (2011), not all the phytochemicals are present in all plant parts in large amount and those present differ according to the type of the extracting methods used, the plant species, and the geographical location. The presence of these bioactive compounds recorded in this investigation, point to the plant's medicinal value. It's anti-oxidant, anti-inflammatory, anti-diabetic, anticancer, and anti-malaria properties of this plant had been linked to the presence phytonutrients and bioactive compounds

Table 5: Qualitative Phytochemicals of Guava (Psidium guajava) Leaves.

Phytochemicals	Qualitative
Alkaloides	+ +
Saponins	+ +
Flavonoids	+ +
Glycosides	+ + +
Tannins	+ +
Phenols	++
Steroids	+ +

Key: + + = moderate, + + + = high,

The presence of saponin in our finding has supported the use of the plant in managing inflammation. The inhibitory effect of saponin on inflamed cells and its ability of precipitating and coagulating red blood cells have been reported (Waziri and Saleh, 2015). Saponins which are glycosides have been found to have inhibitory effects on Gram-positive organism, *S. aureus*. Saponin was also detected in *Moringa oleifera* leaves and have been shown to possess some beneficial (cholesterol lowering) properties (Bamishaiye et al., 2011). The presence of steroids in *Psidium guajava* leaves was of great importance as they are of interest in pharmacy due to their relationship with such compounds as sex hormones. Steroids increase protein synthesis, promoting growth of muscles and bones (Waziri and Saleh, 2015).

The *Psidium guajava* leaves also contained alkaloides in moderate level which are nitrogen-containing naturally occurring compounds, commonly found to have antimicrobial properties due to their ability to intercalate with DNA; they are very low in concentration when compared with those of *Moringa oleifera* (Bamishaiye, *et al*., 2011). Tannins are polyphenolic compounds that bind to proline rich *protein* that interferes with protein synthesis (Sanches *et al*., 2005) and has shown to have antibacterial activity (Min *et al*., 2008). The flavonoids are hydroxylated polyphenolic compounds known to be produced by plants in response to microbial infections. This response mechanism has been extensively studied and found to have antimicrobial activity against an array of microorganism's *in vitro* studies (Cowan, 1999). The capability of flavonoid has been attributed to their ability to form complexes with extracellular and soluble proteins and bacterial cell walls (Trease and Evans, 1989). On the other hands, alkaloids play some important metabolic role in living organisms as anticancer, antimalarial, analgesic, antispasmodic and bactericidal, antioxidant and stimulating functions (Achikanu *et al*., 2022). Therefore, the phytochemical analysis revealed that the aqueous (water) extracts have chemical compounds that have been found to possess antibacterial activities, which supported the findings obtained from antibacterial analysis.

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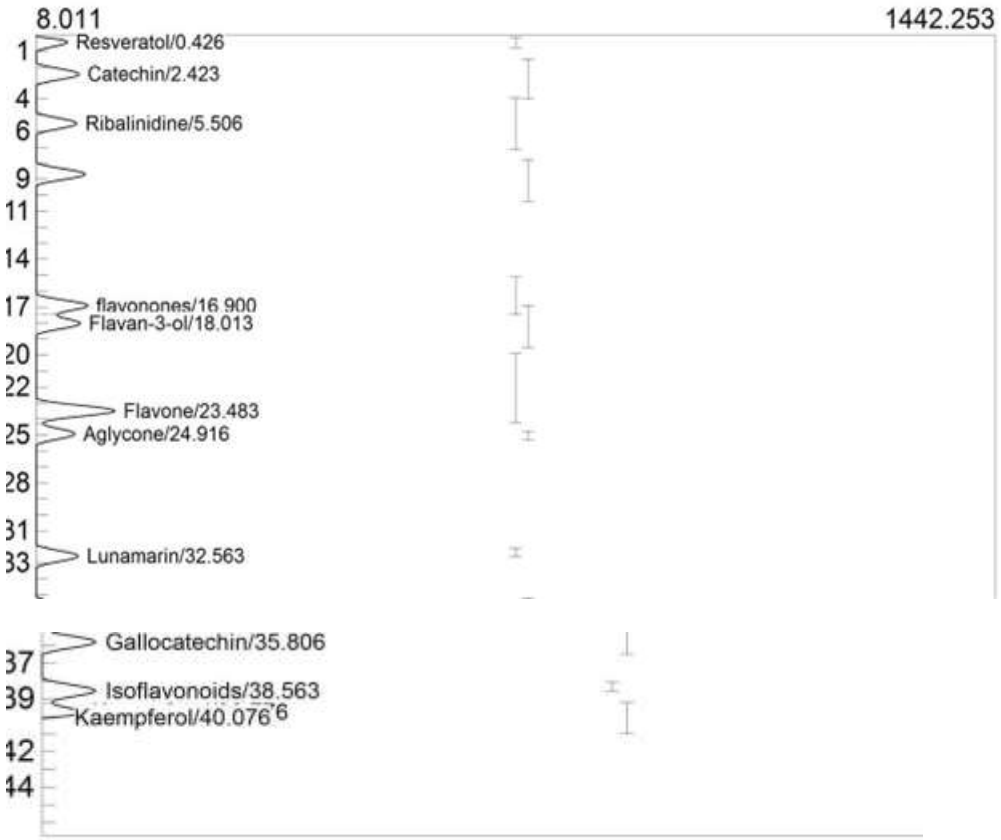
Temperature program:

Init temp	Hold	Ramp	Final temp
50.00	5.000	10.000	180.00
180.00	2.000	5.000	220.00
220.00	0.000	5.000	310.00

Events:

Time Event



Component	Retention	Area	Height	External	Units
Resveratol	0.426	6108.0233	55.456	6.7431	ug/ml
Catechin	2.423	3584.7027	72.032	10.1194	ug/ml
Ribalinidine	5.506	3438.8984	68.471	12.3962	ug/ml
flavonones	16.900	4273.9892	85.054	9.1471	ug/ml
Flavan-3-ol	18.013	3668.8441	73.766	10.3883	ug/ml
Flavone	23.483	6312.0334	125.373	17.2746	ug/ml
Aglycone	24.916	3275.3754	65.634	7.7131	ppm
Lunamarin	32.563	3545.0768	70.367	33.6262	ppm
Gallocatechin	35.806	3739.7866	74.523	13.4230	ppm
Isoflavonoids	38.563	3726.4203	74.465	5.0253	ppm
Kaempferol	39.776	2050.3854	57.237	10.9861	ug/ml
Kaempferol	40.076	66.5142	35.450	0.3564	ug/ml
		43790.0498		137.1988	

Figure1: GC-FID Graph (Chromatogram) Showing Different Constituents of Bioactive Compounds Identified in Ethanolic Fraction of Guava Leaves.

Key: Retention = Retention Time (min.); Area = Peak Area (cm^2); Height = Peak height of each compound; External = Concentration measured in their various units.

The GC-FID Screening

The chromatogram of phytochemical analysis of ethanolic extract of guava leaves using GC-FID techniques is as shown in Figure 1. A total of 12 peaks were detected and 12 bioactive compounds were identified and quantified using their peaks and retention times. These results are comparable with that of Duru (2020), who reported similar finding in the concentration of phytochemical of ethanolic extract of *Z. mays* husk using GC-FID. Ethanol has proved its effectiveness in the extraction of the various compounds of flavonoid including; resveratrol, flavonones, flavan-3-ol, and flavone; the quinoline alkaloids such as lunamarine, and ribalinidine. Other compounds detected are aglycone, gallocatechin, isoflavonoid and kaempferol, a compound of flavonol exhibiting different concentrations. The forkloric claims of this plant's usage in medicine for the stimulation of the cardiac and uterine muscles in childbirth might be related to these alkaloids' activities.

The compound lunamarin was found to be the most abundant with a concentration of 33.6262 ppm and a retention time (RT) of 32.562 min, followed by flavone with a concentration of 17.2746 µg/ml and retention time of 23.483 min. However, the compound kaempferol was the least with a concentration of 0.3564 µg/ml and a retention time of 40.076 min. Kaempferol is a natural flavonol, a class of flavonoid known to increase intracellular ATP content under hypoxic conditions. It scavenges different types of radicals, inhibits reactive oxygen species (ROS) – generating enzymes and increases the expression of antioxidant enzymes (Achikanu *et al*., 2022). Resveratrol is also a flavonol that has been reported to have shown anticancer activity as well as in preventing heart damage after a cardiac arrest (Gal *et al*., 2021). It also assists in the reduction of oxidative damage of the liver during ethanol intoxication. Ribalinidine, a quinoline alkaloide, is known to have radical scavenging function, and pharmacological activities (Duru, 2020). Flavan-3-ol oligomers and monomers have been reported to be potent antioxidant compounds. Flavanone is a flavonoid that has been linked to cardiovascular disease and cancer prevention. Intake of catechin – rich foods for instance, have been associated with the prevention and treatment of chronic diseases in humans, such as inflammatory bowel disease (IBO) (Achikanu *et al*., 2022). Spartein, lunamarine and ribalinidine are quinoline alkaloids known to be pharmacologically active compounds with biological activities such as antimalarial, anti-inflammatory, antimicrobial, anti-protozoal, antioxidant as well as metal chelating activities. In this report, the identification of lunamarin and ribalinidine have proved the extract's biological activities potential. Lunamarine and ribalinidine have equally been reported to have radical scavenging function, while Lunamarin possess anti-amoebic activity (Duru, 2020; Achikanu *et al*., 2022). In essence, the presence of these various bioactive compounds in *Psidium guajava* leaves may be responsible for their numerous physiological potentials and biological activities.

Table 6: Antibacterial Activity of Ethanolic Extract of P. guajava Leaves Against Clinical Bacteria Isolates

Bacterial Isolates	Diameter zone of inhibition (mm)			
	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml
E. coli	17	14	12	7
Shigella sp	8	4	2	1
Lactobacillus sp	20	17	14	6
Staphylococcus aureus	9	6	3	1

Key: mm = millimeter, mg/ml = milligrams per milliliter

The result of the antimicrobial properties of the extract on clinical bacterial isolates using alcoholic solvent (ethanol) is shown in Table 6. The antibacterial activities of phytoextract tested against four (4) organisms: Gram negative (G-ve) organisms (*E. coli*, *Shigella sp)* and Gram positive (G-ve) organisms (*Lactobacillus sp* and *Staphylococcus aureus*) revealed that the extract inhibited *E. coli* at 200, 100, 50 and 25 mg/ml concentrations

with clear zones of 17, 14, 12 and 7 mm, respectively, *Shigella species* at 200, 100, 50 and 25 mg/ml concentration gave clear zones of 8, 4, 2 and 1 mm, respectively. Similarly, *Lactobacillus species* at 200, 100, 50 and 25 mg concentration exhibited 20, 17, 14 and 6 mm zones of clearing, whereas, *Staphylococcus aureus* exhibited zones of clearing of 9, 6, 3 and 1 at concentrations (mg/ml) of 200, 100, 50 and 25. The extract was more potent against two of the organisms: *E. coli* and *Lactobacillus,* whereas, *Staphylococcus aureus and Shigella* organisms showed reduced potency as shown by their zones of clearing or inhibitions by the extract.

The reports on the antibacterial activities of leaves of *Psidium guajava* are available in literature (Fugaban, 2016; Pandey *et al*., 2017; Sandeep *et al*., 2023). This research result would contribute in a way as humans continue to source for total cure for infectious diseases especially with the growing trends of antimicrobial resistivity. The investigation had shown that depending on the species of bacteria, Gram-ve bacteria has higher sensitivity than Gram+ve bacteria against most studied plant extracts regardless of the type of solvent. As detailed in Table 6, the diameters of inhibition zones in crude extract for *E. coli* ranged from 7-17 mm, *shigella sp* (1-8 mm), while the diameters of inhibition zones for *Lactobacillus sp* ranged from 6-20 mm and *S. aureus* (1-9 mm).

This variation mighty be due to the difference in the cell wall and cell membrane compositions between Gram+ve and Gram-ve bacteria. These bioactive 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). Hence, in this study, the high sensitivity of Gram-ve organisms to GL extracts against Gram+ve ones could be due to the fact that development of the cells of Gram-ve bacteria were more inhibited, leading to disruption and lysis of cells, cumulating in the clearing mechanisms of these organisms. This finding was in agreement with Ceyhan-Güvensen and Keskin (2016) and Mickmaray (2019), while results differed from the findings of Yildirim *et al* (2013) who found that the Gram+ve bacteria commonly seem to be more susceptible to the inhibitory effects of the plant extracts than the Gram-ve bacteria. It was reported earlier that Gram-ve bacteria are usually more resistant to the plant-origin antimicrobials and even show no effect, compared to Gram+ve bacteria (Stefanello *et al*., 2008; Tajkarimi *et al*. 2010). The resistance of the Gram-ve bacteria could be attributed to its cell wall structure. Gram-ve bacteria have an effective permeability barrier, comprised of a thin lipopolysaccharide exterior membrane, which could restrict the penetration of the extruding plant extract. In contrast, Gram+ve bacteria have a mesh-like peptidoglycan layer which was more accessible to permeation by the extracts (Qa'dan *et al*., 2005; Stefanello *et al*., 2008). Elisha *et al*. (2017) reported that the plant extracts which have high antibacterial activity against Gram-ve bacteria do not necessarily have high activity against other Gram+ve bacteria.

Table 7: Minimum Inhibitory Concentration (MIC) of P. guajava Leaves Extract.

	Concentration (mg/ml)				MIC
Isolates	200	100	50	25	
E. coli	-	-	-	+	50
Shigella sp	+	+	+	+	200
Lactobacillus sp	-	-	-	+	50
Staphylococcus aureus	+	+	+	+	200

Key: (-) = No bacterial growth; (+) = Bacterial growth

Minimum Inhibitory Concentration (MIC) of P. guajava Leaves Extract

The least concentration that inhibited the growth of *E. coli* and Lactobacillus sp was at 50mg/ml, whereas at the highest dose of 200mg/ml, Shigella sp and Staphylococcus aureus isolates were least inhibited. This suggests

that the extract was more effective at inhibiting of E. coli and Lactobacillus sp with low MIC values than Shigella sp and Staphylococcus aureus because a smaller amount is needed to inhibit their growth. These organisms are considered normal flora and may not be of pathogenic strains. It had been reported that lower MIC value signifies greater antibacterial potency (smaller amount of extract is needed to inhibit growth) (Buah et al., 2023). These findings support the potential of P.guajava as an antibacterial agent, particularly against E. coli and Lactobacillus sp and could be relevant in exploring natural alternatives to synthetic drugs which are expensive, not easily accessible with potential side effects.

CONCLUSION

The present work demonstrates the quantification of bioactive compounds using GC-FID technique and antimicrobial potential of Psidium guajava leaves extract using ethanol as solvents. The plethora of these phytonutrients are responsible for the pharmacological activities employed in our traditional health-care management. The results indicated that the extract was more potent against two of the organisms: E. coli (Gram-ve) and Lactobacillus sp. (Gram+ve), whereas, Staphylococcus aureus (Gram+ve) and Shigella (Gram-ve) organisms showed reduced potency as shown by their zones of clearing or inhibitions by the extract. The minimum inhibitory concentration (MIC) results suggest that the extract was more effective at inhibiting of E. coli and Lactobacillus sp with low MIC values than Shigella sp and Staphylococcus aureus. Comparisons with related data from the literature indicate that according to the different methodologies of studies on antibacterial activity, the most diverse outcomes can be obtained. This study provides scientific insight to further determine the antimicrobial principles and investigate other pharmacological properties of guava. On the basis of the present findings, P. guajava leaves possess the capabilities of being a good candidate in the search for a natural antibacterial agents against infections and/or diseases caused by E. coli, Shigella sp, Lactobacillus sp and S. aureus.

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