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Anti-hyperglyceamic Effects of *Psidium guajava* LINN Crude Leaf Extracts and Fractions in Alloxan-induced Diabetic Mice.

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Abstract: In Nigeria, rural inhabitants often resort to herbal remedies and dietary control for the treatment and management of various forms of diabetes mellitus. This study was conducted to provide the rationale for the use of *Psidium guajava* leaves as a potent traditional anti-diabetic remedy. The crude leaf extracts of n-hexane, methanol, and ethyl acetate of *Psidium guajava* were separately prepared by cold maceration. Then, ethyl acetate crude extract of *Psidium guajava* leaves was fractionated by column chromatography to yield ethyl acetate bulked fractions: EF-I (1-75), EF-II (76-150), and EF-III (151-250). The n-hexane, ethyl acetate, and methanol crude leaf extracts and ethyl acetate bulked fractions (EF-I, EF-II and EF-III) were evaluated for anti-diabetic activity in alloxan-induced diabetic mice. The blood sugar levels of treated and untreated alloxan-induced diabetic mice were assayed as indices of anti-diabetic effect. The phytochemical constituents of both crude extracts and ethyl acetate fractions of *Psidium guajava* leaves and the mean lethal dose (LD_{50}) of ethyl acetate crude leaf extract of Psidium guajava were determined. The mean lethal dose (LD_{50}) of ethyl acetate crude leaf extract was calculated to be 1500mg/kg b.w. The results indicated that oral administration of ethyl acetate, n-hexane, methanol crude extracts, and ethyl acetate bulked fractions of *Psidium guajava* leaves at a dose of 100mg/kg b.w on treated groups exhibited much significant[$p<0.001$, $p<0.01$and $p<0.05$]anti-hyperglyceamic effect by ameliorating high blood sugar levels of alloxan-induced diabetic treated mice, while EF-II and EF-III showed non-significant[$p>0.05$] anti-hyperglyceamic activity for the reduction in blood sugar levels compared with the negative and positive control groups. The anti-diabetic potency of the crude leaf extracts and ethyl acetate fractions were in the order; EC>HC>MC>EF-I>EF-II>EF-III. The results of phytochemical screening of the crude extracts and ethyl acetate bulked fractions showed the presence of tannins, flavonoids, saponins, alkaloids, terpenoids, glycosides, and steroids while reducing sugar was absent. The results from this study gives credence to the use of *Psidium guajava* as an anti-diabetic agent in the management of diabetes mellitus

Keywords: *Type-2 diabetes mellitus, Psidium guajava, Anti-hyperglyceamic activity, Phytochemicals.*

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1. Introduction

Diabetes mellitus elucidates a metabolic disease characterized by abnormal hyperglycemia which alters the metabolism of lipids, carbohydrates, proteins arising from insulin-deficiency or insensitivity of target cell to insulin secreted in the body [37]. The prevalence of diabetes mellitus (DM) cases has been on the increase worldwide in recent years. The World Health Organization report released in 2000 estimated that over 171 million (2.8%) people are living with diabetes mellitus from the global population, and this figure has been projected to increase to 366 million (4.4%) by 2030[134][107]. Most especially, cases of type 2 diabetes mellitus (T2DM) have been increasing in contrast to cases of type 1 (T1DM), an autoimmune disease that often occurs due to the destruction of insulin-producing beta cells of the pancreas, and results to deficiency in insulin secretion[62][108]. On the other hand, T2DM has become a more serious problem in developing countries because of the trend of urbanization and consequent lifestyle changes, perhaps most importantly exemplified by a shift to the western-style diet, which is high in fat[85][110]. T2DM is mostly characterized by hyperglycaemia, insulin resistance (reduced insulin sensitivity), and obesity[[111][112]. Obesity is associated with not only T2DM but also hyperlipidemia and hypertension[118]120]. The coexistence of these diseases is well known as metabolic syndrome, a high-risk factor for cardiovascular disease[104][114]. It is known that obesity results from disequilibrium between energy intake and expenditure[117], and obesity is known to be a strong risk factor for Type-2 diabetes associated with insulin resistance[14][113].

1.1. Origin, distribution, and morphology of Guava (*Psidium guajava* Linnaeus)

Psidium guajava Linnaeus, widely known as Guava, is a member of the Myrtaceae family and a semi-deciduous tree native to tropical and subtropical countries with about 133 genera more than 3,800 species[48]. It is native and widely distributed in South and Central America. The Guava plant is now cultivated and naturalized throughout the tropics and subtropics in Africa, South Asia, Southeast Asia, the Caribbean, subtropical regions of North America, Hawaii, New Zealand, Australia, and Spain. It is well known in the islands for its edible fruit. However, the plant is cultivated today from the west coast of Africa to the Pacific region, including India and China, with varieties originally introduced over the past 300 years from the United States[61]. The two most common varieties of guava fruits are the red (variety: *Porifera*) and the white (variety: *Porifera*)[48]. The Guava plant is a low evergreen tree that grows up to 6 to 25 feet high, with wide-spreading branches and square downy twigs; it is widely grown for its fruit in the tropics. Generally, the guava plant has spread widely throughout the tropics because it thrives in a variety of soils, propagates easily, and bears fruit quickly. The guava berry is an important tropical fruit that is mostly consumed fresh. The fruit consists of a fleshy pericarp and seed cavity with fleshy pulp and numerous small seeds of varying hardness depending on the cultivar[60][70][79]. The flowers are white, incurved petals, 2 or 3 in the leaf axils; they are fragrant, with four to six petals and yellow anthers. The fruit is small, 3 to 6 cm long, pear-shaped, reddish-yellow, or creamy-white when ripe[48]. It is commonly used as food and processed as juice and jam. The guava fruits are either eaten fresh or made into drinks, ice cream, and preserves. Guava fruit is still enjoyed as a sweet treat by indigenous peoples throughout the rainforest, and the leaves and bark of the guava tree have a long history of medicinal uses that are still employed today[91][116]. The tree is easily identified by its distinctive thin, smooth, copper-colored bark that flakes off, showing a greenish layer beneath. It is the hardest among tropical fruit trees and excels most of the other fruit crops in productivity and adaptability. Moreover, guava scores over other fruits in ascorbic acid, pectin, and other mineral contents. Guava cultivars, however,

display a great diversity in tree size, bearing habit, and yield, as well as in fruit size, shape, quality, and ripening season[61].

Figure 1. Structure of guava leaves

(Source: United States Department of Agriculture, Natural Resources Conservation Service, 2013. Taxonomic Classification of *Psidium guajava* Linnaeus)[130].

Kingdom: Plantae

Sub kingdom: Viridaephytae (green plants)

Infra kingdom: Streptophytae (land plants)

Division: Tracheophyta (vascular plants)

Subdivision: Spermatophyta (seed plants)

Infra division: Angiospermae (flowering plants)

Class: Magnoliopsida (dicotyledons)

Superorder: Rosidae

Order: Myrtales

Family: Myrtaceae

Genius: Psidium Linnaeus

Species: guajava Linnaeus

Variety: *Psidium guajava* pyrifera (white guava)

1.2. Pharmacological Properties of *Psidium guajava* Linn.

Different parts of the guava tree have been shown to possess various pharmacological activities due to their use in folk medicine to treat different ailments[22][25]. The potential pharmacologic activities of the extract from the fruit, leaf, bark, or roots include antioxidant, hepatoprotective, anti-allergy, anti-microbial, anti-genotoxic, anti-plasmodial, cytotoxic, antispasmodic, cardioactive, anti-cough, anti-diabetic, anti-inflammatory, and anti-conceptive activities in vitro and animal models**[47][23].** The extract of the whole plant of Psidium Guajava, excluding roots, was reported to possess anti-bacterial, anti-fungal, anti-viral, hypoglycaemic, and diuretic activities**[128].** In other studies, the anti-diarrhoeal**[80],** anti-pyretic**[95][116],** anti-hypertensive**[8],** and bio-antimutagenic properties of guava leaf extract have been demonstrated**[84]**. Interestingly, guava leaves have also attracted attention as a folk remedy for diabetes in Japan and East Asia**[9][89]** and in Africa**[94].** Based on the need to maintain and promote good health, and prevent lifestyle-associated disease, the Japanese Ministry of Health, Labor and Welfare published "Foods for Specified Health Uses" (FOSHU), which are foods whose

claims of physiological effects on the human body have been officially approved and such foods were legally permitted to be used as dietary products for health preservation[6][105][119]. Guava leaf tea, Bansoureicha that contains aqueous guava leaf extract, was approved as FOSHU and recommended for individuals with pre-diabetes[26,26]. The product has become widely accepted and commercially available in Japan**[58][97]**

Antioxidant properties of Guava.

Antioxidants are substances that can prevent or reduce oxidative damage of biomolecules (lipids, proteins, and nucleic acids) by reactive oxygen free radicals such as superoxide, hydroxyl, peroxyl, alkoxyl, and non-radicals such as hydrogen peroxide, hypochlorous[123,124]. It is known that Guava, compared with other fruits and vegetables, is also rich in antioxidants that help to reduce the incidence of degenerative diseases such as arthritis, arteriosclerosis, cancer, brain dysfunction, heart disease, and inflammation**[42][120][121].** Besides preventing or delaying oxidative damage of these essential biomolecules like lipids, proteins, and nucleic acids caused by reactive oxygen species, antioxidants were reported to retard aging**[42][46][49].** They scavenge radicals by inhibiting initiation and breaking of chain reaction, suppressing the formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen**[115][28].** Studies have shown that the most abundant antioxidants present in fruits are polyphenols and ascorbic acid. The polyphenols contain a significant amount of flavonoids, which are mainly present in ester and glycoside forms[43][123]. In the case of guava, free ellagic acid and glycosides of myricetin and apigenin are found to be present[66][126]. Studies have shown that Guava fruit and leaf possess antioxidant and free radical scavenging capacity[18][27].

Anti-hyperlipidemic activity of Guava.

It has been reported that streptozotocin-induced diabetic rats treated with raw peel extract of *P. guajava* fruit for twenty-one days showed a significant decrease in serum triglycerides, total cholesterol, very-low-density lipoprotein (VLDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and an increase in serum high-density lipoprotein (HDL) cholesterol**[100][28].** A long-term clinical trial that investigated the effects of the consecutive intake of guava for eight weeks on the parameters of hyperlipidemia, diabetes, and safety in twenty-three subjects with borderline or mild hyperlipidemia with or without type 2 diabetes mellitus showed much significant reduction in total serum cholesterol level, serum triglycerides level in subjects with hyper-triglyceamia and that of phospholipid in subjects with hyper-phospholipidemia[127,131,132]. In addition, the extract significantly reduced blood glycosylated heamoglobin(HbA1c%)[30][31]. Also, it raised serum adiponectin level in each subject with adiponectinemia and hyperglyceamic conditions while levels of high-density lipoprotein cholesterol, non-esterified fatty acids (NEFAs), and lipid peroxide were not significantly reduced**[6][31].** In addition, research studies have shown that aqueous extract of *Psidium guajava* contains components with LDL-cholesterol that exhibit anti-glycation activity, suggesting its contribution to preventing neurodegenerative and cardiovascular diseases**[18,54,139].** Studies with humans have found that the consumption of Guava for 12 weeks reduced blood pressure by 8%, total cholesterol levels by 9%, triacylglycerides by almost 8%, and induced an 8% increase in the levels of HDL-cholesterol[33,34]. The authors attributed these effects to the fruit's high potassium and soluble fiber contents**[119][120].** Daily oral administration of red (pink) guava puree supplements showed a significant effect by reducing total cholesterol, low-density lipoprotein-cholesterol and triglycerides levels[139], and resulted to increase in high-density lipoprotein-cholesterol level in high fat diet-

induced obese rats[90][35][133]. Red (pink) guava is reported to have high crude fiber and mineral content, especially potassium, sodium, magnesium, phosphorus, zinc, and iron[8][40][140].

Hepatoprotective and Cardioprotective effects of Guava.

It has been reported that aqueous extract of lyophilized guava fruit peel exhibited a heptaprotective effect in a diabetic test subjects by maintaining a significant reduction in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and creatinine levels in streptozotocin-induced diabetic rats[100][42]. In another study, it was noted that the oral administration of guava pulp and rat food supplemented with guava seeds significantly diminished the levels of AST and ALT enzymes in normal Wistar rats[38][45]. A study of aqueous extract of *P. guajava* showed hepatoprotective activity in acute experimental liver injury induced by a combined mixture of carbon tetrachloride, paracetamol, and thioacetamide[108,46]. The effects observed were compared with a known hepatoprotective agent, silymarin, and histological examination of the liver tissues that supported hepatoprotective activity[108][56][136]. Other similar studies have also shown a cardioprotective effect of aqueous extract of *P. guajava* in myocardial ischemia-reperfusion injury in isolated rat hearts, which was primarily linked to its radical-scavenging action[137][53]

Anti-obese property of Guava.

Obesity is the most common nutritional disorder affecting people in the developed world[14][58]. A study has established that obesity results from an imbalance between energy intake and expenditure, and linked obesity, as a predisposing risk factor to the development of Type-2 diabetes mellitus, which is often associated with insulin resistance[14][57]. Studies have shown an increasing prevalence of obesity among persons, which poses a global health challenge and is linked to some diseases. It is considered a risk factor associated with the development of major human diseases, including cardiovascular disease, diabetes, and cancer[60][65]. Significantly, daily consumption of fat-enriched diets often tends to promote obesity[66][69]. In addition, increased intake of high caloric (energy and fat) food promotes body fat storage, greater body weight, and adiposity in humans and animals[14][35]. Over-the-counter remedies like nutritional supplements are extremely popular, especially for weight reduction, obesity, and healthy vitality[64][73]. Inhibition of digestion and absorption of dietary fats have been used as targets in obesity treatment[133]. Red guava purée intake has the beneficial effect of lowering body weight and suppressing obesity in diet-induced obese rats[92][90]. It is reported that moderate feeding of pink guava purée caused changes in dietary fatty acids and carbohydrates[102][70].

Anti-hypertensive activity of Guava.

A study on patients with essential hypertension showed the guava extracts administered to subjects before meals in a randomized and single-blind fashion for twelve weeks led to a significant net decrease in blood pressure values and a significant increase in high-density lipoprotein-cholesterol (HDL-C) after twelve weeks of Guava fruit substitution in hypertensive test subjects[102][74,75]. A similar study has also demonstrated that red guava exhibit an anti-hypertensive property capable of reducing blood pressure[8][78]. In addition, it was reported that polyphenols prevented cardiac hypertrophy and the production of reactive oxygen species and improved vascular function in an antihypertensive experimental rats[3][88][98]. Red guava extract showed a significant lowering effect on systolic blood pressure of high-fat diet-induced obese rats[90].

Anti-diarrheal properties of guava.

It is evident that Guava leaf extracts and fruit juice exhibited a significant recovery rates among test subjects infected with infantile diarrhea in a clinical control experimental study[132][115]. The study further reported that 62 infants treated with a separate oral administration of both leaf extracts and fruit juice had a significant recovery rate of 87.1% within 3 days, and the study concluded that guava extracts and fruit juice have an excellent anti-diarrheal effect against infantile rotaviral enteritis[132][100].

Anti-mutagenic and anti-cancer properties of guava.

Few research studies have established anti-mutagenic and anticancer activities of *P. guajava* leaves. Notably, it has been reported that *Psidium guajava* pulp, peel, and seeds extracts exert anticancer effects on both hematological and solid neoplasms, which were implicated as a result of antioxidant properties exhibited by the plant due to the presence of antioxidant compounds[12][107].

1.3. Aim of Study

This study investigated the anti-hyperglyceamic activities of n-hexane, methanol, ethyl acetate crude leaf extracts and ethyl acetate bulked fractions of *Psidium guajava* in alloxan-induced diabetic mice.

Objectives of the Study.

To evaluate phytochemical constituents of Psidium guajava LINN *n-hexane, methanol, ethyl acetate crude leaf extracts and ethyl acetate bulked fractions .*

To determine the mean lethal (acute) dose (LD50) of Psidium guajava LINN *ethyl acetate crude leaf extract.*

To assess anti-diabetic activities of P. guajava LINN ethyl acetate bulk fractions (EF-I, EF-II, and EF-III), n-hexane, ethyl acetate, and methanol crude leaf extracts to an approved reference anti-diabetic drug and negative control.

2. Materials and Methods

2.1. Materials

The test materials used in this research study include wistar albino mice (animal), Guava plant material, chemical reagents, and laboratory equipment.

2.2. Animal

Thirty-six (36) Wistar albino mice of body weight between 50-65g used for the study were purchased from the Animal House of the Department of Zoology, University of Nigeria, Nsukka Campus. The mice were fed with poultry starter feeds, drinking water *ad libitum*, and acclimatized for seven (7) days. The mice were further divided into nine (9) groups consisting of four (4) mice per group.

2.3. Drug/Chemical Reagents

All chemicals and anti-diabetic drug (Glucophage) used for this study were based on the recommended standard and analytical grade.

2.4. Instruments/Equipment

The equipment used for this study was domiciled at the Chemistry Department, Federal University of Agriculture, Markurdi and the Medical Biochemistry Department, University of Nigeria, Enugu State, Nigeria.

2.5. Plant Material

The plant leaves of *Psidium guajava* (Guava) used for this research study were obtained from the Botanical garden of the University of Nigeria, Nsukka Campus, Enugu State, Nigeria. The whole plant was identified by Dr. N. O Nweze, a renowned taxonomist in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka Campus, Enugu State, Nigeria.

2.6. Experimental design

The thirty-six (36) Wistar albino mice (50-65g) body weight were randomly divided into nine groups comprising four (4) mice per group (n=4/group). Group 1 was not induced with diabetes but received diluted dimethyl sulphoxide (5ml/kg). Alloxan monohydrate (Merck, Germany) was dissolved in cold normal saline and administered intraperitoneally at a dose of 120mg/kg per body weight to groups 2, 3, 4, 5, 6, 7, 8, and 9.

Table 2.6. Administration of Crude Extracts and Ethyl acetate bulked fractions (EF-I, EF-II, and EF-III) of Psidium Guajava leaves on alloxan-induced diabetic mice.

Group (4 mice per group)	**Sample (Dosage)**
Group 1 (Normal control)	*Dimethyl sulphoxide(DMSO) + distilled water (5ml/kg b.w)*
Group 2(negative control)	*(DMSO) + distilled water (5ml/kg b.w)*
Group 3(diabetic treated/ positive control)	*Glucophage (5mg/kg b.w)*
Group 4(diabetic treated mice)	*Ethyl Acetate Fraction I(EF-I)(100mg/kg b.w)*
Group 5(diabetic treated mice)	*Ethyl Acetate Fraction II(EF-II) (100mg/kg b.w)*
Group 6(diabetic treated mice)	*Ethyl Acetate Fraction III(EF-III) (100mg/kg b.w)*
Group 7(diabetic treated mice)	*Ethyl Acetate Crude Extract (100mg/kg b.w)*
Group 8(diabetic treated mice)	*Methanol Crude Extract (100mg/kg b.w)*
Group 9(diabetic treated mice)	*N-hexane Crude Extract (100mg/kg b.w)*

The alloxanized groups were hyperglycemic when blood glucose levels rose to ≥ 120mg/dL. Group 3 received a standard anti-diabetic drug (Glucophage, 5mg/kg per body weight) while group 2 received dimethyl sulphoxide (5ml/kg per body weight). The Group 4 mice received ethyl acetate bulked fraction I, EF-I (1-75) at a dose of 100mg/kg per body weight. The same oral treatment was given to groups 5, 6, 7, 8, and 9 with ethyl acetate fractions; EF-II, EF-III, ethyl acetate crude extract, methanol extract, and hexane extract respectively at the same dose of 100mg/kg as showed on table 2.6. The oral administration of Glucophage, dimethyl sulphoxide, fractions, and crude extracts of Psidium guajava leaves lasted for 14 days during which the blood glucose concentrations of the mice were measured from the 1st to 14th day. The Psidium guajava leaf extracts were dissolved in fresh dimethyl

sulphoxide and administered orally. The hypoglycemic effect of the crude extracts and ethyl acetate fractions were investigated on alloxan-induced diabetic mice.

2.7. Ethical Clearance

The ethical clearance and permission to undertake this research were granted by the Research Ethics Committee, College of Medicine, University of Nigeria, Enugu State, Nigeria. The experiment was conducted in accordance with the regulations of the Institutional Animal Research Ethics Committee, University of Nigeria, Enugu State, Nigeria.

2.8. Preparation of Plant Material (*P. guajava)*

The guava leaves were chopped and blended with a manual grinder into a fine powdery weighing three (3kg) kilograms.

2.9. Extraction of Plant Material ((*P. guajava)*

The leaf extraction was carried out by cold maceration[1][2]**.** Each one kilogram (1kg) fine powder of dried *Psidium guajava* leaves was soaked in a separately labeled glass flask (500ml) containing 400ml of analytically graded n-hexane, ethyl acetate, and methanol reagent, respectively. Each preparation was filtered through a grade 1 Whatman filter paper. Each filtrate was separately collected with a separate crucible before drying by evaporation under a steady air current for about 24 hours until a soft mass (extract) was obtained. Each crude extract obtained was weighed and recorded. The extracts were carefully air-dried to remove all traces of solvents under a thermal water bath. The percentage (%) yields of n-hexane, ethyl acetate, and methanol crude extracts were calculated, respectively.

2.10 Column Chromatographic Fractionation of Ethyl Acetate Crude Leaf Extract (*Psidium guajava)*

Column glassware made up of a simple straight glass tube 60cm long with a diameter size of 3.5 cm was used for column fractionation of ethyl acetate crude extract of *Psidium guajava* leaves. Firstly, the column glassware was rinsed with distilled water and later air-dried. A dried cotton wool plug was inserted into the bottom of the glass column just below the tap. The column chromatography was performed on silica gel (Machery Nagel, Germany) with a mesh pore size, 70-230nm. The wet loading method, the silica gel was packed into the glass column before fractionating ethyl acetate crude extract of *Psidium guajava* leaves to obtain fractionated bioactive constituents. The research procedure for the gravity elution column chromatography was carried out accordingly[51]. These include;

Column Development.

The dry silica gel powder (Machery Nagel, Germany) with a mesh pore size of 70-230nm weighing 150 g was carefully packed into the glass column by the slurry packing(wet loading) method. The slurry silica gel was prepared by mixing dry silica gel powder and a solvent volume of 200ml n-hexane (JHD®) in a glass beaker. The mixture was properly stirred for 10 minutes until silica gel was properly pre-absorbed. A ball of cotton wool was plugged just above the tap, and 1.5mm thick acid-treated sand (BDH®) was added and allowed to settle onto the plugged cotton wool. The glass

column was equilibrated with few drops of n-hexane to achieve a marked level. The slurry was carefully poured into the column glassware and gently allowed to settle in the glass column. A vibrator was used to ensure that the column glass set-up was properly packed. The n-hexane solvent level was above the silica gel-packed column.

Preparation of Plant Material for Column Fractionation.

The ethyl acetate crude leaf extract of *Psidium guajava* was placed into a clean beaker, and properly stirred followed by the addition of a solvent mixture of dimethyl ether and ethyl acetate (2:1) to pre-absorb the mixture, and air-dried. A fine powder of ethyl acetate crude extract of *P. guajava* leaves was obtained after air drying. The fine powder of ethyl acetate crude extract of *Psidium guajava* leaves weighing 1.5g was added onto a 1.5mm thick acid-treated sand layer (BDH, England) just above the wet silica gel in the column.

Solvent System.

The column fractionation was carried out at different solvent systems (mixtures) using pure analytical graded reagents in a sequence and polarity strength. The volume ratios of n-hexane: ethyl acetate used in the fractionation process with varying percentage volume ratios (100%:0% – 50%:50%). At the beginning of column fractionation, a solvent volume ratio of 500ml volume of n-hexane (100%): ethyl acetate (0%) was used to elute non-polar compounds. The sequence and polarity of volume ratios of the solvents were considered to avoid column cracking and also achieve efficient separation. At the end stage of the fractionation, the column was thoroughly washed with 500ml of 99.9% methanol to obtain methanol fraction separately. The solvent system (mixture) was continuously added and a gradual flow rate of 10 drops per minute was obtained.

Fraction Collection.

The serial labeled glass vials (5ml) were used for collecting solvent fractions (eluents). The collection of fractions was achieved after a successive elution to obtain a total number of two hundred and fifty (250) vial fractions. The solvent vial fractions were kept uncovered to air-dry for 24 hours after collection. The solute deposited in each vial was re-dissolved with a 5ml ethyl acetate solution (JHD®, China). The vial fractions were grouped into three main bulk fractions; EF-I, EF-II, and EF-III according to the retention factor (R_f) values of the thin layer chromatography fingerprints (TLC) plates (Chromatogram).

2.11 Thin Layer Chromatography (TLC)

Thin-layer chromatography (TLC) is one of the laboratory techniques applied in organic research laboratories[32]. It is used to achieve a quick separation of organic compounds and ascertain the purity level of a given mixture. TLC also helps to detect and analyze organic one or more compounds by comparing with known samples, in terms of its purity with identified samples, and monitoring the progress of separation during extraction or purification process[32]. The TLC comprise of a small glass or plastic plate coated with a thin layer of a dry solid silica gel or alumina gel which serves as the most common stationary phases. Mobile phases could be an organic solvent or solvent mixture. The sample mixture is spotted near the bottom of the plate as a small, and then placed in a developing chamber containing a little volume of solvent (mobile phase). The solvent travels up the plate and carries the sample mixture along with it through capillary action. As a result of the solubility of various components of the mixture in the mobile phase, the strength of their adsorption to the

stationary phase and each component in the mixture moves at a different rate. As the solvent moves near the top of the plate (solvent front). It is allowed to evaporate, leaving behind the components of the mixture at various distances from the point of origin. The ratio of the distance traveled by a compound to the distance traveled by the solvent is the R_f value (retention factor). This value is characteristic of the compound, the solvent, and the stationary phase[32]. The retention factor, R_f = distance traveled by sample/ distance traveled by solvent.

***Procedure**:*

Thin-layer chromatography (TLC) was performed on a silica gel pre-coated plate (GF254, Merck, UK). A solvent volume ratio of n-hexane in ethyl acetate (5ml:5ml) was used as the mobile phase for each fraction; EF-I, EF-II, and EF-III. The plates were sprayed with a mixture of 20% sulphuric acid dissolved in methanol and then heated to about 120°C for 3 minutes to visualize spots. This is suitable for the detection of most polar compounds such as carbohydrates etc. A total of two hundred and fifty (250) eluted fractions were further bulked into three main groups of ethyl acetate fractions based on the separation pattern of constituent compounds (R_f values) and labeled EF-I (1-75),EF-II(76-150)andEF-III(151-250)accordingly.

3 Results

3.10 Actual and Percentage Yield of Plant Extracts and Column Fractions

The plant extraction yielded 1.5g (21% w/w) of n-hexane crude leaf extract, 3.0g (42% w/w) ethyl acetate crude leaf extract and 2.51g (35% w/w) methanol crude leaf extract of *Psidium guajava.*

Table 1.1. Yield of *Psidium guajava* Crude Leaf Extracts

S/No	Type of Extract	Amount of Extract (gm)	% Yield
1.	N-hexane	1.5	21
2.	Ethyl acetate	3.0	42
3.	Methanol	2.51	35

Table 3.2. Bulking of Column Fractions of *P. guajava* Ethyl Acetate Crude Leaf Extracts

S/No	Ethyl acetate Bulked Fractions	Range of Combined Fractions
1.	Bulk Fraction I (EF-I)	1-75
2.	Bulk Fraction II (EF-II)	76-150
3.	Bulk Fraction III (EF-III)	151-250

The column fractionation yielded 250 column fractions which were bulked together into three main bulked fractions (EF-I, EF-II, and EF-III) respectively.

Table 3.3. Retention Factor Values (R_f) of Ethyl Acetate Fractions of *Psidium guajava* Leaves

S/N	Bulk Fractions	Retention Factor(R_f) Values
1	Bulk Fraction I(1-75)	0.33
2	Bulk Fraction II(76-150)	0.19
3	Bulk Fraction III(151-250)	0.23

Table 3.4. Qualitative Phytochemical Screening of Ethyl Acetate Fractions of *Psidium guajava* Leaves.

PYTOCHEMICAL CONSISTUENTS	ABUNDANCE		
	ETHYL ACETATE BULK FRACTION (EF-I)	ETHYL ACETATE BULK FRACTION (EF-II)	ETHYL ACETATE BULK FRACTION (EF-III)
Reducing Sugar	-	-	-
Tannins	+	+	+
Steroids	++	+	+
Flavonoids	++	+	+
Saponins	++	+	-
Alkaloids	++	+	+
Terpenoids	++	+	+
Glycosides	-	-	-

**Interpretation: -Absence, + Trace, ++Moderate, +++ Abundant*

Table 3.5. Qualitative Phytochemical Screening of Ethyl Acetate, Methanol and n-Hexane Crude Extracts of *Psidium guajava* Leaves.

PYTOCHEMICAL CONSISTUENTS	ABUNDANCE		
	N-HEXANE CRUDE EXTRACT (HC)	ETHYLACETATE CRUDE EXTRACT (EC)	METHANOL CRUDE EXTRACT (MC)
Reducing Sugar	-	-	-
Tannins	++	++	+++
Steroids	++	+++	++
Flavonoids	+	+++	++
Saponins	+	+	+
Alkaloids	+	++	+
Terpenoids	+	+	++
Glycosides	+	-	-

**Interpretation: -Absence, + Trace, ++Moderate, +++ Abundant*

3.6 Determination of Mean Acute (Lethal) Toxic Dose (LD_{50})

The mean acute toxic dose was determined in 2two phases I and II at different dosages of ethyl acetate crude leaf extract of Psidium guajava. The acute toxicity study was determined in mice by Lorke's research protocol[79]. The tests involved two phases. The first phase was used to determine the toxic range. The three groups 1, 2 and 3 comprising of three (3) mice was separately administered

10mg/kg b.w, 100mg/kg b.w, and 1000 mg/kg b.w doses of ethyl acetate crude leaf extract solubilized in 5% (v/v) dimethyl sulphoxide. The treated mice were observed after 24hrs for any lethal signs, abnormal behaviour and death. There was no lethal signs and death recorded. In the second phase of the toxicity study, each group was separately administered with different doses of 1500, 2000, and 5000 mg/kg per body weight of ethyl acetate crude leaf extract respectively. The treated mice were observed after 24hrs for lethality or signs of acute intoxication. The acute toxic dose (LD_{50}) was calculated. The result obtained is shown below:

Table 3.6. Outcome of Acute Toxicity Study

Phase	Groups of Mice (3 mice per group)	Dosage (mg/kg b.w.)	Mortality	Behavioural Changes
Phase I	*Group 1*	10	0/3	Nil
	Group 2	100	0/3	Nil
	Group 3	1000	0/3	Nil
Phase II	*Group 1*	1500	1/3	yes
	Group 2	2000	2/3	yes
	Group 3	5000	2/3	yes

3.7 Anti-hyperglyceamic Effect of Column Chromatographic Fractions of *Psidium guajava* Ethyl Acetate Crude Extract in Alloxan-induced Diabetic Mice

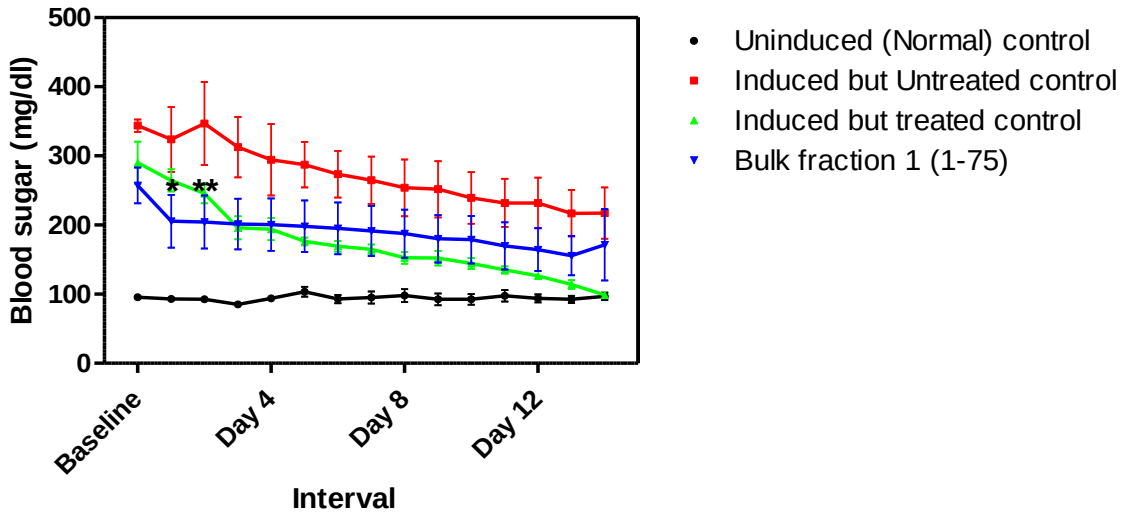

Figure 3.7.1. Blood sugar measurements of alloxan-induced diabetic mice treated with Bulked Fraction, **EF-I (1-75)** *(100mg/kg b.w)* of *Psidium guajava* Ethyl Acetate Crude Leaf Extract.

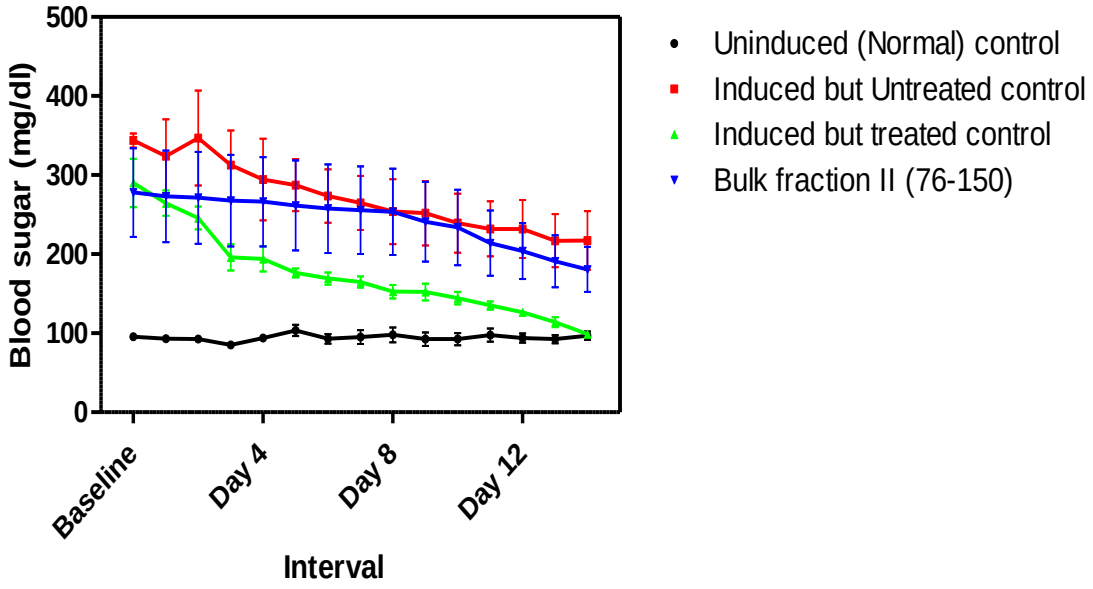

Figure 2.7.2. Blood sugar measurements of alloxan-induced diabetic mice treated with Bulked Fraction, **EF-II (76-150)** *(100mg/kg b.w)* of *Psidium guajava* Ethyl Acetate Crude Leaf Extract.

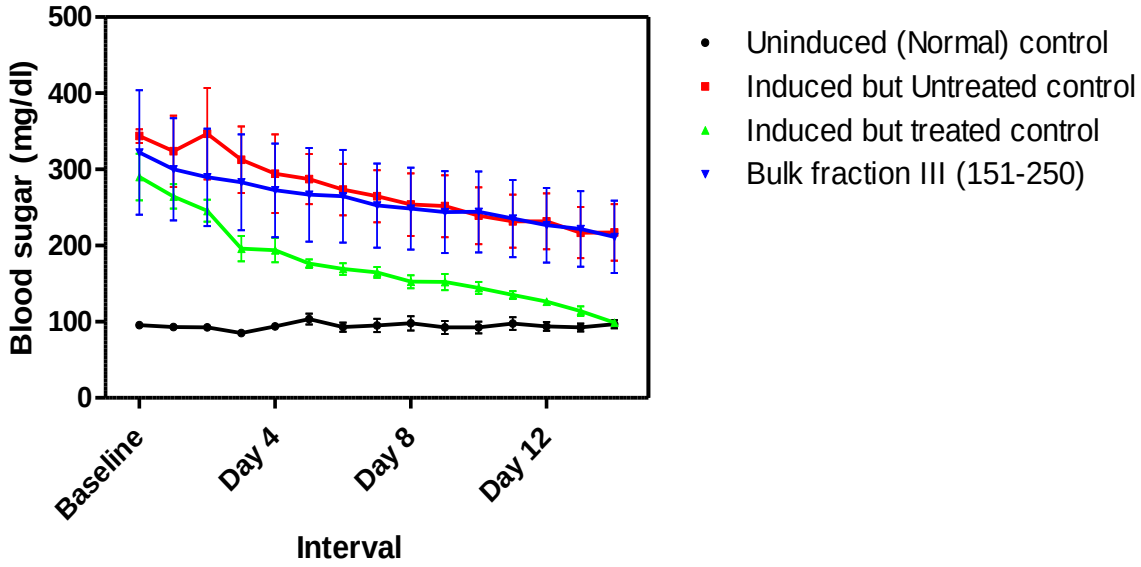

Figure 3.7.3. Blood sugar measurement of alloxan-induced diabetic mice treated with Bulked Fraction, **EF-III (151-250)** *(100mg/kg b.w)* of *Psidium guajava* Ethyl Acetate Crude Leaf Extract.

3.8 Anti-hyperglyceamic Effects of Ethyl Acetate, n-Hexane and Methanol Crude Leaf Extracts of *Psidium guajava* in Alloxan-induced diabetic mice.

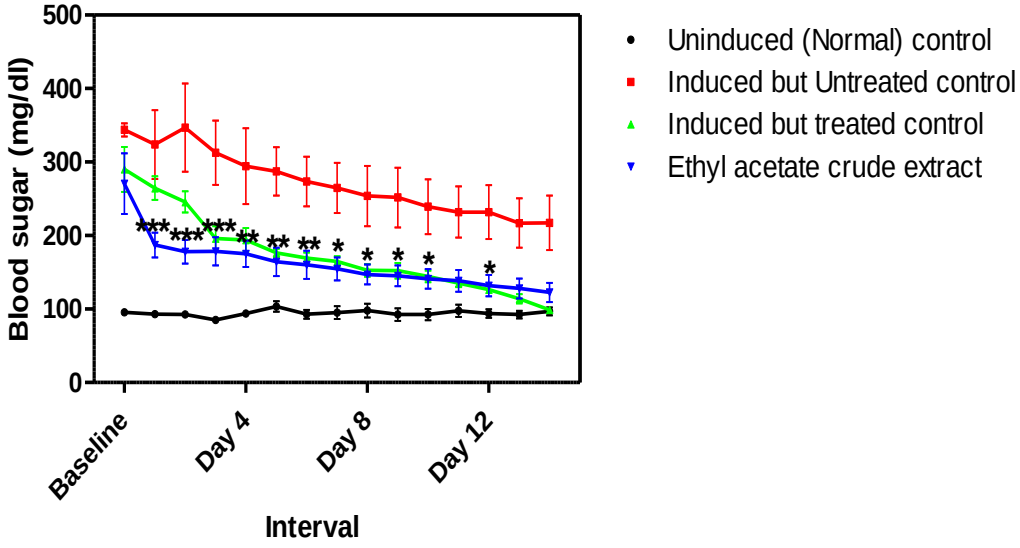

Figure 3.8.1. Blood sugar measurements of alloxan-induced diabetic mice treated with **Ethyl Acetate Crude Leaf Extract (**100*mg/kg b.w*) of *Psidium guajava*.

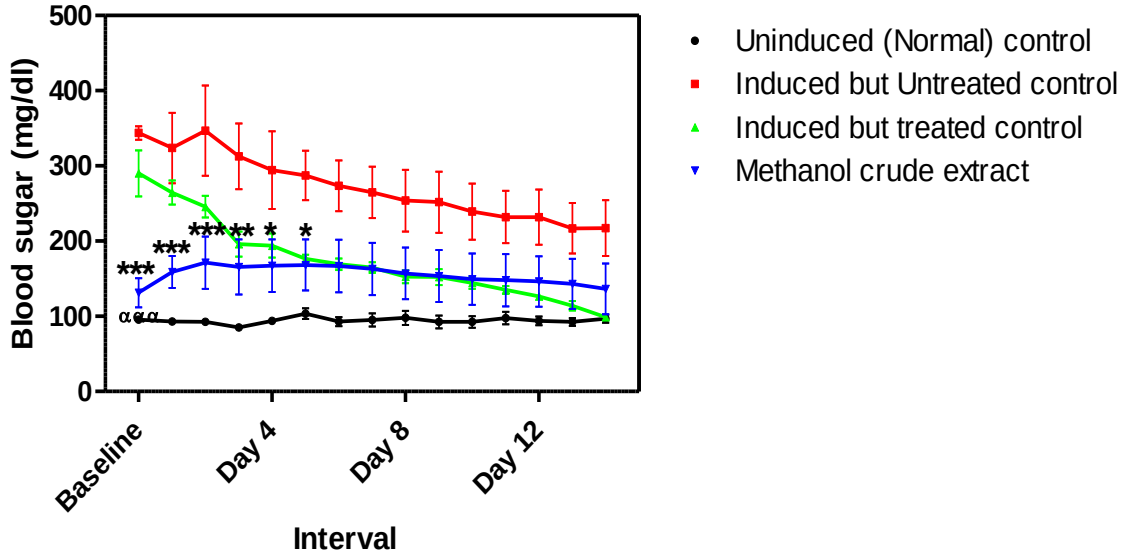

Figure 3.8.2. Blood sugar measurements of alloxan-induced diabetic mice treated with **Methanol Crude Leaf Extract** (100 *mg/kg b.w*) of *Psidium guajava*

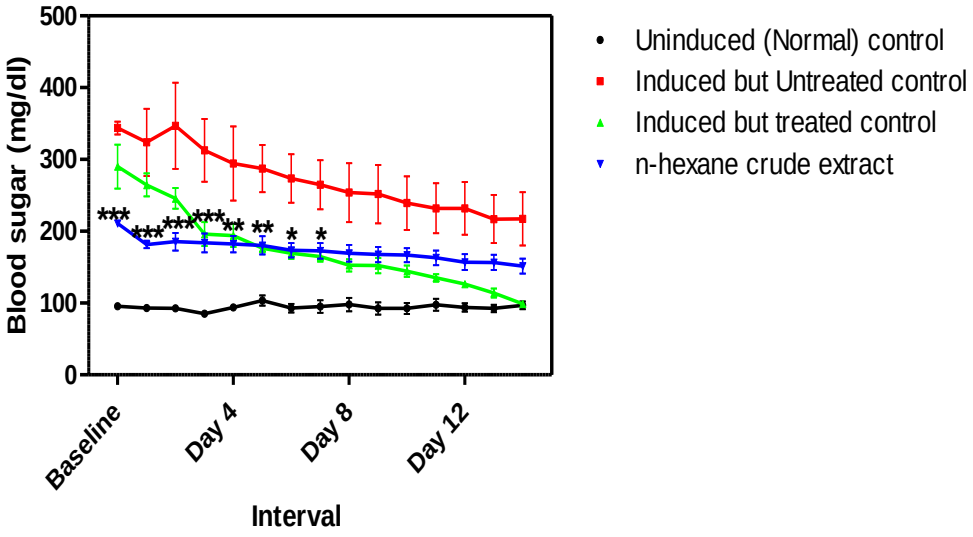

Figure 3.8.3 Blood sugar measurements of alloxan-induced diabetic mice treated with **n-Hexane Crude Leaf Extract** (100 *mg/kg b.w)* of *Psidium guajava.*

3.9 PERCENTAGE BLOOD GLUCOSE REDUCTION ON TREATMENT DAYS

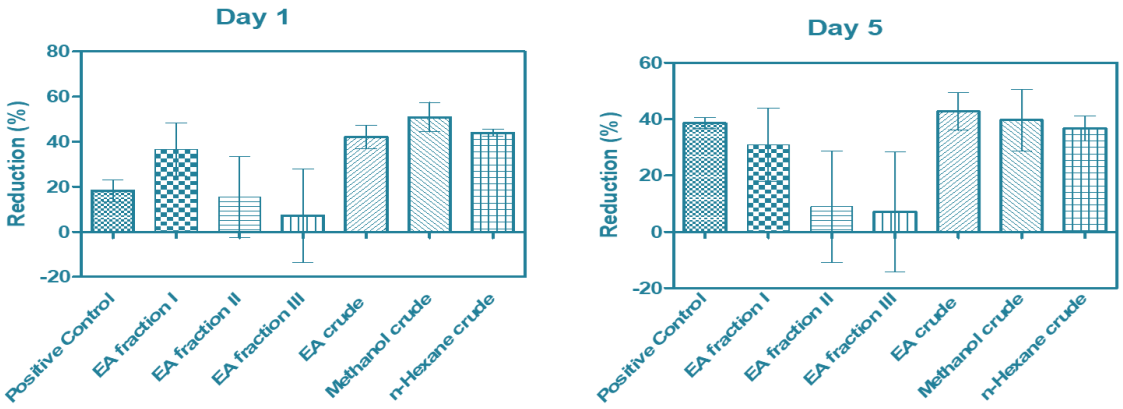

Figure 3.9.1 (A and B): Percentage reduction of Blood sugar on First (1st) and Fifth (5th) treatment days. Each bar represents the mean percentage reduction. (Error bars are the standard error of the mean (SEM). Each bar was not significantly different from the positive control. *(One-way ANOVA with Dunnett's multiple comparisons, using Graph pad Prism 5).*

The Figure 3.9.1 (A and B) showed the results of percentage mean blood glucose(Mean±SEM) reduction of treatment groups on the first(1st) and fifth(5th) day of oral treatment. The positive control(group 3) mice were treated with Glucophage at a dose of 5mg/kg b.w while group 4, 5, and 6 were treated with ethyl acetate fractions (EF I), (EF II) and (EF III) at a dose of 100mg/kg b.w and group 7, 8 and 9 treated with ethyl acetate, methanol and n-hexane crude extracts of *P. guajava* leaves at a dose of 100mg/kg/b.w respectively. The oral treatment of alloxan-induced diabetic mice with the crude and partially purified chromatographic fractions on Day 1, group 8 mice treated with

methanol crude(MC, 100mg/kg b.w) leaf extract of *P. guajava* showed the highest percentage blood glucose reduction with a mean (Mean±SEM) (45%), followed by n-hexane, HC(42%) and ethyl acetate crude extract (40%), EF I (39%) while EF II(18%) and EF III(10%) had least percentage blood glucose reduction. A statistical comparison of the anti-hyperglyceamic effect between all treatment groups and positive control using a One-way ANOVA (Dunnett's multiple) showed that each bar(Mean±SEM) was not significantly different from the positive control group(18% mean percentage blood glucose reduction). This implies that groups treated with EF I, EF II, EF III, ethyl acetate, methanol, and n-hexane crude extract of *P. guajava* leaves had a significant anti-hyperglyceamic effect with percentage reduction in high blood glucose level compared with the standard reference drug (Glucophage, 5mg/kg b.w) in the order of MC>HC>EC>EF-I>EF-II while the EF-III had a least anti-hyperglyceamic effect with percentage glucose reduction. On the second(2^{nd}) day of oral treatment of alloxan-induced diabetic mice with the crude and partially purified chromatographic column fractions, group 7 mice treated with ethyl acetate crude100mg/kg b.w) leaf extract of *P. guajava* showed the highest percentage blood glucose reduction with a mean (Mean ±SEM) (43%), followed by methanol, MC (40%) and n-hexane extracts(39%), EF I (31%) while EF II(15%) and EF III(13%) had least percentage blood glucose reduction. A statistical comparison between all treatment groups and positive control(group 3) using Dunnett's multiple One way ANOVA showed that each bar (Mean±SEM) was not significantly different from the positive control group(40% mean percentage blood glucose reduction). This implies that groups treated with EA fraction I, EA fraction II, EA fraction III, ethyl acetate, methanol, and n-hexane crude extract of P. guajava leaves had a significant anti-hyperglyceamic effect compared with the standard reference drug (Glucophage) administered at a dose of 5mg/kg b.w in the order of EC>MC>HC>EF-I >EF-II while the EF-III had least percentage blood glucose reduction.

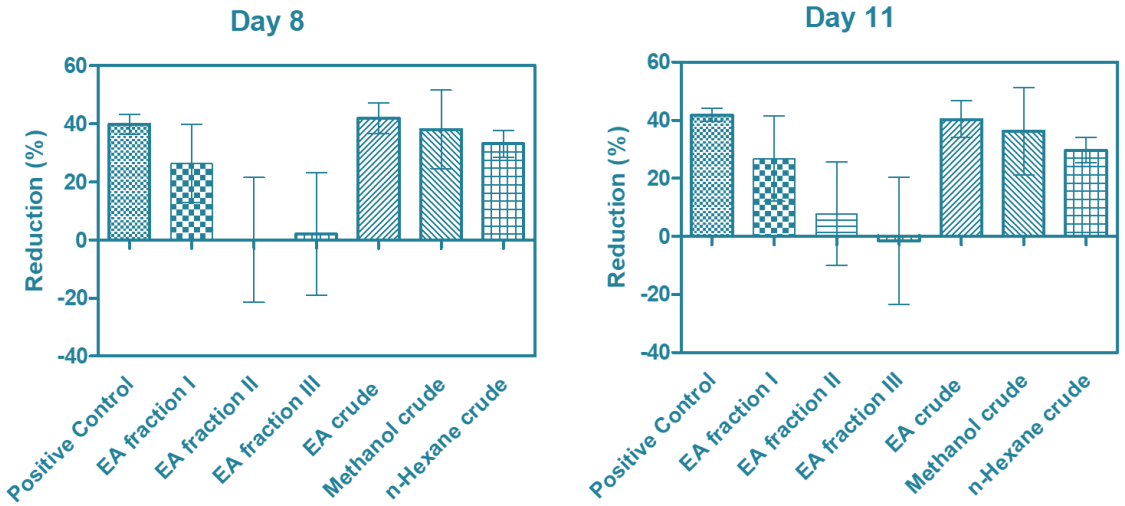

Figure 3.9.2 (**A and B):** Percentage reduction of Blood sugar on Eighth (8^{th}) and Eleventh (11^{th}) treatment days. Each bar represents the mean percentage reduction (Error bars are the standard error of the mean. Each bar was not significantly different from the positive control). *(One-way ANOVA with Dunnett's multiple comparisons, using Graph pad Prism 5).*

The **Figure 3.9.2** (A and B) showed the results of mean percentage blood glucose reduction (mean± SEM)for all treatment groups on the eighth (8^{th}) and eleventh (11^{th}) day of oral treatment with reference drug (Glucophage,5mg/kg b.w), crude extracts(n-hexane, ethyl acetate, methanol) and ethyl acetate fractions (EF-I, EF-II and EF-III) of P. guajava leaves respectively. On the eighth

(8th) day of oral treatment of alloxan-induced diabetic mice with the crude and partially purified chromatographic fractions. Group 7 mice treated with ethyl acetate(EC, 100mg/kg b.w) crude leaf extract of *P. guajava* showed the highest percentage mean blood glucose reduction with a mean(Mean±SEM) (43%), followed by methanol, MC(39%) and n-hexane extract, HC (30%), EF I (25%), EF III(5%) while EF II showed least mean percentage blood glucose reduction. A statistical comparison of the anti-hyperglyceamic effect of all treatment groups and positive control using One-way ANOVA (Dunnett's multiple) showed that each bar (Mean±SEM) was not significantly different from the positive control group(40%mean percentage blood glucose reduction). This implies that groups treated with EA fraction I, EA fraction II, ethyl acetate, methanol, and n-hexane crude leaf extract of *P.guajava* had a significant anti-hyperglyceamic effect compared with the standard reference drug(Glucophage administered at a dose of 5mg/kg b.w) in the order of EC>MC>HC>EF-I >EF III>EF-II, even though EA fraction II(EF-II) had least percentage blood glucose reduction. On the eleventh (11th) day of oral treatment of alloxan-induced diabetic mice with the crude and partially purified chromatographic fractions. The group 7 mice treated with ethyl acetate crude (100mg/kg b.w) leaf extract of P. guajava showed the highest percentage blood glucose reduction with a mean (Mean±SEM) (40%), followed by methanol(38%) and n-hexane extract(30%), EF I (25%) while EF II(18%) had least percentage blood glucose reduction and EF III had -5% with no significant mean blood glucose reduction. A statistical comparison of the anti-hyperglyceamic effect between all treatment groups and positive control using One-way ANOVA (Dunnett's multiple analysis) showed that each bar (Mean±SEM) was not significantly different from the positive control group(42% mean percentage blood glucose reduction). This implies that all treatment groups treated with EF I, EF II, EF III, ethyl acetate, methanol and n-hexane crude extract of *P.guajava* leaves at a dose of 100mg/kg b.w had a significant anti-hyperglyceamic effect compared with the standard reference drug (Glucophage) administered at a dose of 5mg/kg b.w in the order of EC>MC>HC>EF-I >EF-II >EF-III.

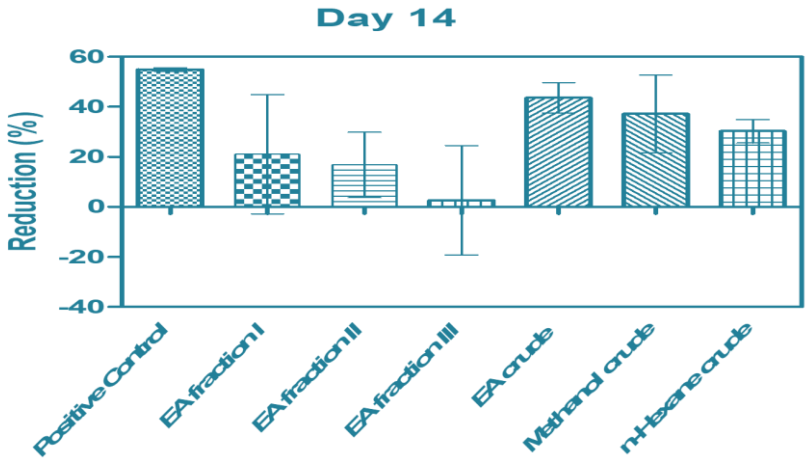

Figure 3.9.3. Percentage reduction of Blood sugar on Fourteenth (14th) treatment day. Each bar represents the mean percentage reduction; error bars are the standard error of the mean. Each bar was not significantly different from the positive control. *(One-way ANOVA with Dunnett's multiple comparisons, using Graph pad Prism 5).*

The **Figure 3.9.3.** showed the result of percentage blood glucose reduction (Mean±SEM) for the treatment groups on the fourteenth (14th) day of oral treatment of alloxan-induced diabetic mice(group 3) with reference drug(Glucophage) at a dose of 5mg/kg b.w. Group 7, 8, and 9 treated with ethyl acetate, methanol, and n-hexane at a particular dose of 100mg/kg b.w, and group 4, 5, and 6 were treated with ethyl acetate fractions(EF-I, EF-II & EF-III) at a 100mg/kg b.w of *P. guajava* leaves respectively.

On the Fourteenth (14^{th}) day of oral treatment of alloxan-induced diabetic mice with the crude extracts and partially purified ethyl acetate fractions. The group 7 mice treated with ethyl acetate crude(100mg/kg b.w) leaf extract of *P. guajava* had the highest percentage mean blood glucose with 43%(Mean±SEM), followed by methanol, MC(35%), and n-hexane extract, HC (30%), EF I (20%) while EF II(18%) while EF III(4%) had least percentage blood glucose reduction. A statistical comparison of the anti-hyperglyceamic effect of all treatment groups and positive control (group 3) using a One way ANOVA (Dunnett's multiple) showed that each bar (Mean±SEM) was not significantly different from the positive control group (59%) mean percentage blood glucose reduction). This implies that each group treated with EF I, EF II, EF III, ethyl acetate, methanol, and n-hexane crude extract of *P.guajava* leaves had much significant anti-hyperglyceamic effect compared with the standard reference drug (Glucophage) administered at a dose of 5mg/kg b.w in the order of EC>MC>HC>EF-I >EF-II while the EF-III had least percentage blood glucose reduction.

4 DISCUSSION

Diabetes mellitus is a metabolic condition categorized with several etiologies and chronic hyperglycemia caused by disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both[135][19]. Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin[77][16][17]. It has been considered an incurable metabolic disorder affecting about 2.8% of the global population[11]. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, the search for newer drugs continues because the existing synthetic drugs have several limitations[7][13].

Alloxan treatment is a method widely used to induce hyperglyceamic condition (diabetes mellitus) in test animals by the use of alloxan, a glucose analog which is selectively toxic to pancreatic beta cells due to its accumulation in the beta cells[15]. The mechanism of action of Alloxan is enhanced by the presence of cysteine amino acid residues which contains two –SH groups that forms a disulfide bond thereby inactivating enzyme. As a result of alloxan reduction, diuric acid is then re-oxidized back to alloxan, hence establishing a redox cycle for the generation of reactive oxygen species and superoxide radicals[135][20]. The cytotoxic action of alloxan is mediated mainly by these reactive oxygen species (ROS)[102]. Reactive oxygen species cause the fragmentation of the DNA of pancreatic islets, which takes place in the beta cells exposed to alloxan monohydrate[17][21].

The preliminary acute toxicity (LD_{50}) studies of ethyl acetate crude leaf extract of *Psidium guajava* was carried out in different phases to establish the lethal oral dosage of ethyl acetate crude leaf extract of *Psidium guajava*. The result showed that more than 500mg of ethyl acetate crude extract of the plant leaves was safe for consumption as a decoction.

This study further evaluated the anti-hyperglycemic effects of methanol, ethyl acetate, and n-hexane crude extracts and ethyl acetate bulked fractions of *Psidium guajava* Linn leaves in alloxan-induced diabetic mice and the results showed a significant anti-hyperglyceamic effect in reduction of high blood glucose levels in test groups treated with a dose of 100mg/kg of each crude leaf extract of P. guajava as compared with negative and positive controls.

However, the result of anti-hyperglycemic effect of ethyl acetate crude extract (EC) of *P. guajava* on alloxan-induced diabetic mice(group 7) exhibited much significant[$P<0.001$, $P<0.01$; $P<0.05$]blood glucose reduction throughout the oral treatment which lasted on the twelfth(12th) day of oral treatment, while alloxan-induced diabetic mice(group 9) treated n-hexane crude extract (HC,

100mg/kg b.w) of Psidium guajava leaves showed a significant[P<0.001, P<0.01; P<0.05]anti-hyperglyceamic control which lasted on 7th day of oral treatment compared with negative control(group 2). The alloxan-induced diabetic mice (group 8) treated with 100mg/kg of methanol extract of P. guajava leaves also showed significant [P<0.001,P<0.01; P<0.05] anti-hyperglyceamic control which lasted on the fifth(5th) day of oral administration compared with negative control(group 2).

The group 4 mice treated with ethyl acetate fraction I(EF-I) (100mg/kg b.w) showed a significant [P<0.05] anti-hyperglyceamic effect compared with the negative control(group 2) that lasted on the second (2^{nd}) day of oral treatment, while EF-II showed invariably a least significant glucose control on the test group as compared with control groups. The EF-III exhibited a non-significant [P>0.05] hypoglycaemic effect as compared with negative control (group 2), despite a gradual decline in high blood glucose level throughout the period of oral administration. However, the pattern of response or decrease in random blood glucose concentration of diabetic treated groups differed significantly over the period (days) of oral administration of each crude extract and ethyl acetate fractions respectively.

The high anti-hyperglycemic effect observed among different treatment groups with crude extracts and ethyl acetate bulked fraction I(EF-I) administered at a specific dose of 100mg/kg b.w can be attributed to the presence of rich anti-diabetic bioactive compounds present in the crude extracts and bulked ethyl acetate fraction I (EF I) of *Psidium guajava* leaves, since the qualitative phytochemical screening of crude leaf extracts and ethyl acetate bulked fraction I(EF I) showed a significant presence of alkaloids, flavonoids, steroids, and terpenoids which have been identified. In general, there is increasing biological knowledge on the specific modes of action of medicinal plants in the treatment of diabetes, but most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, and flavonoids that are frequently implicated as having an anti-diabetic effect[21][24]. However, the methanol crude extract showed a moderate significant decrease in high blood glucose levels in treated mice (group 8), while ethyl acetate fractions II and III showed a non-significant decrease in blood glucose level of alloxan-induced diabetic groups 5 and 6 respectively when compared with diabetic untreated control (group 2) due to the little or trace amount of these bioactive compounds. There are possible explanations for these findings, despite that there is no clear explanation of in *vitro* mechanism of action of *Psidium guajava* crude extracts and ethyl acetate bulked fraction as an anti-diabetic agent[82,83][82]. It may be hypothesized that major phytochemical constituent(s) of the crude extract and ethyl acetate bulked fractions may have delayed or reduced glucose absorption from the gastrointestinal tract into the circulatory system, through inhibition of carbohydrate digestion, or inhibition of Na+-glucose co-transporters and facilitated glucose transporters on the luminal (mucosal) side of the absorptive cells of intestinal epithelial cells, or inhibition of Na^+K^+ - ATPase on the serosa side of intestinal epithelial cells[94][104][87]. Moreover, the crude extracts of Psidium guajava leaves may have triggered the recovery of partially destroyed β-cells or possibly Psidium guajava leaf crude extracts of n-hexane, ethyl acetate, and methanol and ethyl acetate bulked fraction I, EF-I(1-75) may have initiated cell proliferation after induction of diabetes with alloxan monohydrate (120mg/kg i.p) on test mice. There was a non-significant reduction in blood glucose levels of alloxan-induced diabetic mice (groups 5 and 6) treated with ethyl acetate bulk fractions, EF-II and EF-III compared with negative and positive controls, despite presence of trace amounts of these phytochemical constituents. The non-significant anti-hyperglyceamic effects showed by ethyl acetate bulk fractions, EF-II and EF-III was due to loss of potent anti-bioactive compounds during the fractionation process and these bioactive compounds

have been implicated to elicit pharmacologic activity or synergistic that enhances anti-diabetic activity of fractions.

5 CONCLUSION

This study has showed that ethyl acetate crude leaf extract of *Psidium guajava* Linn exhibited a very high potent anti-diabetic effect followed by methanol, n-hexane crude leaf extracts and ethyl acetate bulk fraction I(EF-I) by lowering high blood glucose level in alloxan-induced diabetic treated mice compared with diabetic untreated(negative control) in the order of EC>MC>HC>EF-I>EF-II>EF>III. The result findings showed that the ethyl acetate bulked fractions, EF-II and EF-III exhibited a non-significant anti-hyperglyceamic effect compared with negative control mice (group 2), and despite the gradual decrease in high blood glucose concentration. In addition, a comparison of a percentage mean glucose reductions (Mean±SEM) of all diabetic treated groups with EF-I and all crude extracts administered at a dose of 100mg/kg b.w which showed a non-significant difference from the positive control mice(group 3) treated with glucophage (5mg/kg b.w), a standard anti-diabetic drug.

The phytochemical screening of methanol, ethyl acetate, n-hexane crude leaf extracts, and ethyl acetate bulk fractions of *Psidium guajava* showed a rich abundance of alkaloids, steroids, flavonoids, tannins, and saponins and absence of glycosides and reducing sugar were observed in EF-I, EF-II, EF-III, EC, HC, and MC. The LD_{50} of ethyl acetate crude leaf extract of *Psidium guajava* was determined within the range of 1200- 1500mg/kg / body weight, thus, suggesting a wide safety margin for use in animal model experiments. In conclusion, the result findings from this study given credence to the use of *Psidium guajava* leaves as a traditional remedy for the treatment and management of diabetes mellitus.

6 RECOMMENDATION

In future studies, it is recommended that emphasis should be made towards the use of advanced chromatographic techniques for both fractionation and purification of crude extracts of *Psidium guajava* leaves. The use of advanced chromatographic technique like High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS) and molecular spectroscopy should be used to further isolate, identify and characterize potent anti-diabetic drug compound(s) in the purified fraction that may be linked to the anti-diabetic property of *Psidium guajava*. It is also necessary to carry out *in-vivo* and *in-vitro* anti-diabetic studies of anti-hyperglyceamic activities of ethyl acetate fractions of *Psidium guajava* leaves with varying doses (200*mg/kg b.w*, 400*mg/kg b.w* and 600*mg/kg b.w*) on a specific cultured cell line and alloxan-induced diabetic mice over a prolonged duration, in order to assess the dose-dependent anti-hyperglycemic effect of each fraction. The medicinal value of *Psidium guajava* plant should be given more attention towards developing new anti-diabetic drug and other therapeutic drug researches.

AUTHORS' CONTRIBUTIONS.

This work was extensively conducted by Mr. Eze Uchenna Nwabunwanne under the supervision of Associate Prof. Anthonius A. Eze from the Department of Medical Biochemistry, Faculty of Basic Medical Sciences, University of Nigeria, Enugu, Nigeria. The Author conducted the literature search, experimental design and *in vivo* bioassay, statistical analyses, proof-reading and editing of the manuscript. All co-authors contributed to the success of this research by sharing knowledge, research experience and financial resources.

CONFLICTS OF INTEREST: We declare that there are neither conflicts of interests nor funding sponsor in executing this research paper.

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