

Original Research Article

Systemic Evaluation of Anti-diabetics, Anti-Inflammatory and Secondary Metabolite Potentials of *Aframomum melegueta* [Roscoe] K.

Abstract

This research work helps to investigate systemic evaluation of anti-diabetics, anti-inflammatory and Secondary metabolite potential of stem, leaf-sheath, Fruit-pulp and seed Extracts of *Aframomum melegueta*. Diabetes mellitus is characterized as metabolic disorders which increases glucose levels (hyperglycemia) of blood, due to failings in insulin secretion and related resistance to its actions. Fresh plant samples of *Aframomum melegueta* were harvested from the reserved forest of Akungba Akoko Ondo State (Nigeria) on February 4th, 2020 early in the morning. Dried and pulverized samples were extracted by cold ethanol maceration method for 48 hours. The medicinal potentials were determined using Kim's and Demoraes, Leelaprakash's and Perry's, Priya's and Babu's method of analysis. The leaf-sheath has the highest inhibition percentage for α -glucosidase (79.58%) in this study while the stem has the lowest inhibition (25.60%) percentages. In all the parameter investigated, the seed extract has the highest inhibition percentage (46.21%, 33.25%, 45.11% and 39.84%) while Stem has the lowest percentage (8.23, 21.01, 22.18, and 20.52%). It is definite that the seed has the largest percentage concentration of Anti-Inflammatory potentials in *Aframomum melegueta* (Heat induced haemolysis, Antiproteinase, Hypotonicity-induced haemolysis, and Inhibition of albumin denaturation). The most prominent components observed in *Aframomum melegueta* were Flavonoids, Terpenoids and Cardiac glycosides while fewer components present were Anthocyanin and Beta cyanin. This research work supports herbal usage of *Aframomum melegueta* to reduce the menace of diabetics, inflammatory activity of malignant cells caused by diabetes mellitus in human system and in formulation of synthetic drug the plant supplements for therapeutic purposes.

Keywords: Systemic Evaluation, Anti-diabetics, Anti-Inflammatory, Secondary metabolite
Aframomum melegueta

Introduction

Diabetes mellitus is characterized as metabolic disorders by increased glucose levels (hyperglycemia) of blood because of failings in insulin secretion, insulin action or both. Insulin is produced by beta cells of the pancreas which use glucose from medicinal plants or digested food as an energy source. The pancreas either cannot make insulin or the insulin made by it is not enough and cannot function properly. Glucose builds up in the blood at a high level in this regard and are frequently secreted through urine which is classical symptom of diabetes mellitus (1).

Different clinical form of diabetes has been recognized. Type 1 diabetes (Insulin-dependent diabetes mellitus) (IDDM) or juvenile-onset diabetes, Type 2 diabetes (non-insulin dependent diabetes mellitus (NIDDM) or adult-onset diabetes. Majority of diagnosed cases of diabetes is associated with several risk factors such as obesity, impaired glucose tolerance, family history of diabetes, prior history of gestational diabetes, physical inactivity, cardiovascular diseases, hypertension and race/ethnicity (3). Other particular types of diabetes result from specific hereditary disorders, drugs, malnutrition, surgery, infections, and different ailments are also present, though in extremely uncommon cases. Diabetes mellitus if untreated for long time causes retinopathy, neuropathy, nephropathy, heart attack, stroke and peripheral vascular disease (3).

Constant hyperglycemia—a major characteristic of diabetes mellitus (DM)—has been linked to overproduction of reactive oxygen species (ROS). Excessive generation of ROS activates cytokines reduction, apoptotic proteins, and transcription factors, resulting in chronic inflammation and increased apoptosis, an underlying factor in the development of diabetic complications (4). Evidence suggests that oxidative damage, pro-inflammatory responses, and apoptosis are key players in pathological conditions relating to diabetic cardiomyopathy(2,3).

Diabetic cardiomyopathy (DCM) is a pathological condition of the heart observed in subjects with diabetes, and it is independent of other cardiovascular pathologies such as hypertension, ischemic heart injury, coronary artery disease, and congenital heart diseases. DCM is a major cause of heart failure(5). The progression of diabetes mellitus to diabetic cardiomyopathy involves the interplay of several mechanisms: oxidative damage, inflammation, apoptosis, mitochondrial dysfunction, and over activation of Raas (Renin-angiotensin aldosterone system). Many diabetic cases may be complicated with some inflammatory

conditions that can necessitate the combined treatment of inflammation with it to enhance good well-being.

Inflammation is a normal biological process in response to tissue injury, microbial pathogen infection, and chemical irritation. Inflammation is initiated by migration of immune cells from blood vessels and release of mediators at the site of damage. This process is followed by recruitment of inflammatory cells, release of ROS, RNS, and proinflammatory cytokines to eliminate foreign pathogens, and repairing injured tissues. In general, normal inflammation is rapid and self-limiting, but aberrant resolution and prolonged inflammation cause various chronic disorders (6). Inflammation is a form of defense and protection from infections and tissue damage;

The uncontrolled regulation of immune responses can lead to excessive tissue damage (7,8). Pro-inflammatory cytokines with other indices of inflammation have been shown to be elevated in the hearts of type II diabetes (T2D) subjects (9,10). Diabetes triggers lower levels of systemic inflammation in the cardiomyocytes as an early response to myocardial injury due to the overproduction of mitochondrial ROS. This systemic inflammation triggers the recruitment of leukocytes and causes the secretion of pro-inflammatory cytokines and chemokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor alpha (TNF-) (11).

Further exposure to high concentrations of glucose results in increased production of advanced glycation end products (AGEs). AGEs are regulators of endothelial cell permeability and migration of monocytes and ultimately activate nuclear factor kappa-light-chain enhancers of activated B cells (NFkB)(12).NFkB primarily stimulates the expression of more pro-inflammatory cytokines (TNF-,IL-6, IL-18) in the heart, which are associated with hypertrophy, fibrosis, and left ventricular dysfunction. This cascade of reactions is repeated severally, leading to a sustained immune response and thereby causing further injury and cardiomyopathy(13).

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue (14). Cells and

tissues degeneration, signified by inability of wounds to heal fast, or not healed at all, in diabetic patients is much pronounced.

In the other word, this natural effects of Diabetics and inflammation may be corrected naturally with natural phyto-medicinal plant such as *Aframomum melegueta*. *Aframomum melegueta* Schum (Zingiberaceae) also known as Guinea pepper, grains of paradise, or alligator pepper (indigenous names include Atare in Yoruba, Ose-oji in Igbo, and Citta in Hausa) is a perennial herb widely cultivated for its valuable seeds in the tropical region of Africa(14). It grows up to 1.5m in height, with purple flower that develop into long pod containing small, reddish brown aromatic and pungent seed. The seeds extract are used as a spicy and have a wide range of folkloric uses in traditional medicine. They are used as a remedy for treating stomach ache, diarrhoea, and snakebite(14). The seeds and leaf are very rich in the nonvolatile pungent compounds gingerol, shogaols, paradol, and related compounds. The study of Mohammeda *et al.*, (15) shows the bioassay-guided isolation of possible bioactive compounds responsible for the antidiabetic action of *A. melegueta* fruit which is complementary to the efficacy of this plant source.

The basis of this research is to search for more effective, safer, readily available, less expensive and less toxic Antidiabetic, Anti-Inflammatory agents and rich secondary metabolite. There are numerous natural plant products from *Aframomum melegueta* medicinal plants which are not only good and beneficial for human health but also crucial for the existence of our well-being. Attentions has been shifted to *Aframomum melegueta* as new discovery in the treatment and management of anti-inflammatory and anti-diabetic agents from medicinal plants

Material and Methods

2.1 Collection and Identification of *Aframomum melegueta* sample

freshly plant samples of *Aframomum melegueta* (Stem, leaf sheath, Fruit pulp (mesocarp) and seed) were harvested from the reserved forest of Akungba-Akoko Ondo State (Nigeria) in February 4th,2020 around 6.24am in the morning The plant specimen were identified and authenticated at Department of Plant Science and Biotechnology, Adekunle Ajasin University Akungba-Akoko, Nigeria where the voucher specimens were kept on record. Voucher number AAU-2489 was recorded for the plant extract for future reference (14).

2.2, Preparation of *Aframomum melegueta* sample.

Dried and pulverized stem, leaf sheath, Fruit pulp (mesocarp) and seed sample of *Aframomum melegueta* were extracted by cold ethanolic maceration method for 48 hours at room temperature in a Winchester bottle. The *Aframomum melegueta* sample was filtered with Whatman No. 1 filter paper. The filtrate was concentrated *in-vacuo* using vacuum rotary evaporator at 40°C and later concentrated to dryness in a hot-air oven at 40°C. The extract was stored in a refrigerator at 4°C (14).

2.3. Determination of Anti-diabetic potential of *Aframomum melegueta* [Roscoe] K. Schum, (Stem, leaf sheath, fruit pulp (mesocarp) and seed extracts)

1. Alpha-Glucosidase Inhibitory Assay

The effect of the plant extracts on α -glucosidase activity was determined according to the method described by Kim *et al.*, (2005) using α -glucosidase from *Saccharomyces cerevisiae*. The substrate solution p-nitrophenylglucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, and pH 6.9. 100 μ L of α -glucosidase (0.3 U/mL) was pre-incubated with 50 μ L of the sample for 10 min. Then 50 μ L of 3.0 mM (pNPG) as a substrate dissolved in 20 mM phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37°C for 20 min and stopped by adding 2 mL of 0.1 M Na₂CO₃. The Alpha -glucosidase activity was determined by measuring the yellow-colored paranitrophenol released from pNPG at 405 nm (16).

2. α -Amylase Inhibitory assay

The method used involved estimating the amount of reducing sugar produced by the activity of each enzyme on buffered starch. α -amylase was assayed as reported by Demoraes *et al.* (1999). The substrate for assay was 0.5 ml of 0.5% soluble starch, buffered with 0.2 ml of 0.1 M sodium acetate (pH 5.6). Crude enzyme extract (0.3ml) was added to the mixture, mixed and incubated at 40°C for 30min in a water-bath. DNSA (colorimetric) method as used by Miller (1959) was thereafter employed for estimation of reducing sugars produced. One ml of DNSA solution was added to the mixture and boiled for 5 min. Four ml of distilled water was introduced after cooling before absorbance is read at 540 nm in spectrophotometer. Blank that consisted of 0.3 ml

distilled water, 0.5 ml of 0.5% soluble starch, 0.2 ml of buffer was subjected to similar treatments (17).

2.4. Determination of Anti-Inflammatory potential of *Aframomum melegueta*[Roscoe] K.

Schum, (Stem, leaf sheath, fruit pulp (mesocarp) and seed extracts)

1. Inhibition of albumin denaturation

The anti-inflammatory activity of the sample was studied by using inhibition of albumin denaturation technique (15). The reaction mixture consists of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The sample extracts were incubated at 37 °C for 20 min and then heated to 51 °C for 20 min, after cooling the samples the turbidity was measured at 660nm. UV Visible Spectrophotometer (15).

2. Determination of Antiproteinase

The test was performed according to the modified method of Leelaprakash *et.al.*(2010). The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1ml test sample of different concentrations (100 - 500µg/ml). The mixture was incubated at 37°C for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. (15,17),

3. Determination of Heat induced haemolysis

The reaction mixture (2ml) consisted of 1 ml test sample of different concentrations (100 - 500 µg/ml) and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 °C for 30min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples (15,17).

3. Determination of Hypotonicity-induced haemolysis

Different concentration of extract (100-500µg/ml), reference sample, and control were separately mixed with 1ml of phosphate buffer, 2ml of hyposaline and 0.5ml of HRBC suspension. Diclofenac sodium (100µg/ml) was used as a standard drug. All the assay mixtures were incubated at 37⁰C for 30mins and centrifuged at 3000rpm. The supernatant liquid was decanted and the haemoglobin content was estimated by a spectrophotometer at 560nm (18).

2.5 Qualitative analysis of Secondary metabolite of *Aframomum melegueta* (Phytochemicals) screening

Test for flavonoids

About 0.5 mL of aqueous Stem, leaf sheath, Fruit pulp (mesocarp) and seed extracts of *Aframomum melegueta* [Roscoe] K. Schum, was shaken with pet ether to remove the fatty materials. The defatted residue was dissolved in 20 mL of 80% ethanol and filtered. Then 3 mL of the filtrate was mixed with 4 mL of 1% KOH. A dark yellow colour was observed, which indicates the presence of flavonoids(19).

Test for saponins

About 0.5 mL of Stem, leaf sheath, Fruit pulp (mesocarp) and seed extracts of *Aframomum melegueta* [Roscoe] K. Schum was dissolved in 2 mL of boiling water in a test tube, allowed to cool and shaken to mix thoroughly. Foam appeared indicating the presence of saponins (19).

Test for alkaloids

About 0.5 mL of Stem, leaf sheath, Fruit pulp (mesocarp) and seed extracts of *Aframomum melegueta*[Roscoe] K. Schum was mixed with about 8 mL of 1% HCl, warmed and filtered. Then 2 mL of filtrate was treated separately with Mayer's reagent. Turbidity was observed to indicate the presence of alkaloids (20).

Test for tannins

About 0.5 mL of Stem, leaf sheath, Fruit pulp (mesocarp) and seed extracts of *Aframomum melegueta* [Roscoe] K. Schum was boiled with 20 mL of distilled water in a test tube and then filtered. 0.1% FeCl₃ was added to the filtrate. Appearance of brownish green coloration showed the presence of tannins (20).

Test for coumarins

About 0.5 mL of aqueous Stem, leaf sheath, Fruit pulp (mesocarp) and seed extracts of *Aframomum melegueta* [Roscoe] K. Schum was taken in a small test tube and covered with filter paper moistened with 1 N NaOH. The test tube was placed for few minutes in boiling water. Then the filter paper was removed and examined in UV light for yellow fluorescence to indicate the presence of coumarins (20).

Test for anthocyanin and betacyanin

To 2 mL of the Stem, leaf sheath, Fruit pulp (mesocarp) and seed extracts of *Aframomum melegueta* [Roscoe] K. Schum, 1 mL of 2N sodium hydroxide was added and heated for 5 min at 100 °C. Formation of yellow colour indicates the presence of betacyanin (21).

Test for glycosides

About 2 mL of Stem, leaf sheath, Fruit pulp (mesocarp) and seed extracts of *Aframomum melegueta* [Roscoe] K. Schum was mixed with 3 mL of chloroform and 10% ammonium solution was added. Formation of pink colour was not identified, which indicates the absence of glycosides (21).

Test for cardiac glycosides

To 0.5 mL of Stem, leaf sheath, Fruit pulp (mesocarp) and seed extracts of *Aframomum melegueta* [Roscoe] K. Schum 2 mL of glacial acetic acid and few drops of 5% ferric chloride were added. This under layered with 1 mL of concentrated sulphuric acid. Formation of brown ring at interface indicates the presence of cardiac glycosides (22).

Test for terpenoids

To 0.5 mL of Stem, leaf sheath, fruit pulp (mesocarp) and seed extracts of *Aframomum melegueta* [Roscoe] K. Schum, 2 mL of chloroform was added and concentrated Sulphuric acid was added carefully. Formation of red brown colour at the interface indicates the presence of terpenoids (23).

Test for phenols

To 1 mL of the Stem, leaf sheath, fruit pulp (mesocarp) and seed extracts of *Aframomum melegueta* [Roscoe] K. Schum, 2 mL of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue colour indicates the presence of phenols (23).

Test for quinones

To 1 mL of Stem, leaf sheath, fruit pulp (mesocarp) and seed extracts of *Aframomum melegueta* [Roscoe] K. Schum, 1 mL of concentrated sulphuric acid was added. Formation of red colour indicates the presence of quinines (23).

Test for steriods

To 0.5 mL of the Stem, leaf sheath, Fruit pulp (mesocarp) and seed extracts of *Aframomum melegueta* [Roscoe] K. Schum, 2 mL of chloroform and 1 mL of sulphuric acid were added. Formation of reddish brown ring at interface indicates the presence of steroids (23).

2.6 Quantitative analysis of Secondary metabolite of *Aframomum melegueta* (Phytochemicals) screening

(i) Saponins

About 20grams each of dried plant samples were ground and, put into a conical flask after which 100 ml of 20 % aqueous ethanol were added. The mixtures were heated using a hot water bath. At about 55^oC, for 4 hours with continuous stirring, after which the mixture were filtered and the residue re-extracted with a further 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90^oC. The concentrate was transferred into a 250 ml separatory funnel and 20 rnl of diethyl ether were added and then shaken vigorously. The aqueous layer were recovered while the ether layer was discarded. The purification process was repeated three times. 60 mL of n-butanol were added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution were heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage of the starting material (24,25).

(ii) Flavonoids

About 10 g of the plant sample were extracted repeatedly with 100 ml of 80% aqueous methanol, at room temperature. The whole solution was filtered through Whatman filter paper No 42. The filtrates were later transferred into a crucible and evaporated into dryness over a water bath; the dry content were weighed to a constant weigh. (24,25).

(iii) Tannins

About 500 mg of the plant sample were weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the marked level. Then, 5 ml of the filtrate was transferred into a test tube and mixed with 2 ml of 0.1 M FeCl₃ in 0.1 M HCl and 0.008 M potassium ferrocyanide. The absorbance were measured at 120 nm within 10 minutes. The tannins content was calculated using a standard curve of extract (24, 25),

(iv) Alkaloids

Five grams of the plant sample were weighed into a 250 ml beaker and 200ml of 10% acetic acid in ethanol was then be added, the reaction mixture were covered and allowed to stand for 4 hours. This were filtered and the extract will be concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation is complete. The whole solution were allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue being the alkaloid, which was dried and weighed to a constant mass (24,25).

(v) Total phenol (Spectrophotometric methods)

2 g each of the samples were defatted with 1mL of diethyl ether using a soxhlet apparatus for 2 hours. The fat free samples were boiled with 50 mL of ether for the extraction of the phenolic components for 15 minutes. 5 mL of the extracts were pipetted into 5 mL flask and then 10 mL distilled water was added. 2 mL of ammonium hydroxide solution and 5 mL of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 minutes. For color development. This was measured at 505 nm (Sofowora, 1982). Standard alternative procedures and reagents were used for the study.

3.0 Results

The result of this research work is as shown in Tables and figures presented respectively demonstrating the medicinal potentials of *Aframomum melegueta*. Tables 1 and 2 shows the systemic evaluations of anti-diabetics and anti-Inflammatory potential of *Aframomum melegueta* [Roscoe] K. Schum stem, leaf sheath, fruit-pulp(mesocarp) and seed extracts.. Different parameters like α -Amylase Inhibition, α -Glucosidase Inhibitory potentials.

Table 1 shows the anti-diabetics potential of *Aframomum melegueta* [Roscoe] K. Schum stem, leaf sheath, fruit-pulp(mesocarp) and seed extracts. .it was observed that two parameter were investigated, the parameter are α -Amylase Inhibition and α -Glucosidase and their inhibition percentages.

In α -Amylase Inhibition, It was observed that the leaf sheath has the highest inhibition percentage (48.31%) while the stem has the lowest percentage (21.52%) respectively. Others are Seed (35.72%) and Fruit-pulp (mesocarp) (35.72%).

In α -Glucosidase inhibition, it was observed that the leaf has the highest inhibition percentage (79.58%) while the stem has the lowest inhibition (25.60%) percentages. Other are the Seed (63.32%) and Fruit-pulp (mesocarp) (59.16%). It is crystal clear that the leaf-sheath has the largest percentage concentration of anti-diabetics parameter in *Aframomum melegueta*(α - Amylase Inhibition and α -Glucosidase) followed by the stem in decreasing order of percentage (Leaf sheath<Stem< Seed<Fruit pulp (mesocarp)).

Table 1; Systemic Evaluation of Antidiabetic potentials of *Aframomum melegueta*[Roscoe] K. Schum Stem, leaf-sheath, fruit-pulp (Mesocarp) and seed extracts

| Plants part Used | α -Amylase Inhibition % | α-Glucosidase Inhibition % |
|-------------------------|--|---|
| Seed | 37.48 | 63.32 |
| Fruit pulp (mesocarp) | 35.72 | 59.16 |
| leaf sheath | 48.31 | 79.58 |
| Stem | 21.52 | 25.60 |

(Leaf sheath<Stem< Seed<Fruit pulp (mesocarp)).

Table 2 shows Systemic Evaluation of Anti-Inflammatory potential of *Aframomum melegueta* [Roscoe] K. Schum Stem, leaf-sheath, Fruit-pulp (mesocarp) and seed Extracts. It was observed that four parameter were investigated, the parameter are Heat induced haemolysis, Antiproteinase, Hypotonicity-induced haemolysis, and Inhibition of albumin denaturation their inhibition percentages. In all the parameter investigated, the seed extract has the highest

inhibition percentage (46.21%, 33.25%,45.11% and 39.84%) while Stem has the lowest percentage (8.23, 21.01, 22.18, 20.52%).It is definite that the seed has the largest percentage concentration of Anti-Inflammatory potentials in *Aframomum melegueta* (Heat induced haemolysis, Antiproteinase, Hypotonicity-induced haemolysis, and Inhibition of albumin denaturation) followed by the stem in decreasing order of percentage. (Seed<Stem< Leaf sheath<Fruit pulp (mesocarp)).

Table 3 shows the Qualitative Secondary metabolite potentials of *Aframomum melegueta* [Roscoe] K. Schum, Stem, leaf-sheath, Fruit-pulp(mesocarp) and seed extracts. It was observed that all Secondary metabolite were present qualitatively in *Aframomum melegueta* extracts, the secondary metabolite tested are Tannins, Saponins, Quinones, Terpenoids, Steroids, Flavonoids, Phenol, Alkaloids, Glycosides, Cardiac glycosides, Coumarins, Anthocyanin and Beta cyanin. The most prominent features observed in *Aframomum melegueta* were Flavonoids, Terpenoids and Cardiac glycosides while less features were present in Anthocyanin and Beta-cyanin. The leaf sheath and seed extracts has the most observable secondary metabolites. Less metabolites were present in stem and Fruit pulp (mesocarp).

Table 2. ;Anti-Inflammatory potentials of *Aframomum melegueta*[Roscoe] K. Schum Stem, leaf sheath, fruit pulp (mesocarp) and seed extracts

| Plant part | Heat induced haemolysis % | Antiproteinase inhibition % | Hypotonicity-induced haemolysis % | Inhibition of albumin denaturation % |
|-----------------------|---------------------------|-----------------------------|-----------------------------------|--------------------------------------|
| Seed | 46.21 | 33.25 | 45.11 | 39.84 |
| Fruit pulp (mesocarp) | 35.05 | 21.72 | 35.88 | 37.29 |
| leaf sheath | 21.11 | 28.62 | 32.48 | 31.21 |
| Stem | 8.23 | 21.01 | 22.18 | 20.52 |

(Seed<Stem < Leaf sheath<Fruit pulp (mesocarp)).

Table 3: Qualitative Secondary metabolite potentials of *Aframomum melegueta*[Roscoe] K. Schum. Stem, leaf sheath, fruit pulp (mesocarp) and seed extracts,

| Secondary metabolite | AqsLE | ELsE | AcLsE | AcLsE | AcLsE | EFPE | AcFPE | DeFPE | AqSE | ASE | AcSE | DeESE | AqSE | ESE | AcSE | DeESE |
|----------------------|-------|------|-------|-------|-------|------|-------|-------|------|------|------|-------|-------|------|------|-------|
| Tannins | +ve | + ve | - ve | - ve | -ve | + | - ve | +ve | ++ve | + | - ve | ++ ve | +ve | + | - ve | - ve |
| | | | | | | ve | | | | ve | | | | ve | | |
| Saponins | + ve | ++ | + ve | + ve | + ve | ++ | - ve | + ve | + ve | ++ | + | + ve | + ve | ++ | + ve | + ve |
| | | ve | | | | ve | | | | ve | ve | | | ve | | |
| Quinones | ++ ve | + ve | - ve | - ve | + ve | ++ | - ve | - ve | +++ | - ve | - ve | - ve | ++ ve | + | - ve | - ve |
| | | | | | | ve | | | ve | | | | | ve | | |
| Terpenoids | ++ ve | + ve | - ve | - ve | ++ ve | ++ | - ve | +ve | ++ | ++ | - ve | - ve | ++ ve | + | - ve | - ve |
| | | | | | | ve | | | ve | ve | | | | ve | | |
| Steroids | + ve | + ve | + ve | - ve | + ve | - ve | + ve | - ve | + ve | - ve | + | - ve | + ve | + | + ve | - ve |
| | | | | | | | | | | | ve | | | ve | | |
| Flavonoids | ++ve | ++ | ++ | - ve | ++ve | + | + ve | - ve | +++ | + | ++ | - ve | ++ve | ++ | ++ | - ve |
| | | ve | ve | | | ve | | | ve | ve | ve | | | ve | ve | |
| Phenol | ++ ve | - ve | + ve | + ve | ++ ve | - ve | + ve | + ve | ++ | +ve | + | + ve | ++ ve | - ve | + ve | + ve |
| | | | | | | | | | ve | | ve | | | | | |

| | | | | | | | | | | | | | | | | |
|---------------------------|-------|------|------|------|-------|------|-------|------|------|------|------|-------|-------|------|------|------|
| Alkaloids | + ve | + ve | - ve | - ve | ++ ve | + | - ve | - ve | + ve | - ve | - ve | - ve | + ve | + | - ve | - ve |
| | | | | | | ve | | | | | | | | ve | | |
| Glycosides | + ve | - ve | - ve | - ve | + ve | - | - ve | - ve | + ve | - ve | - ve | - ve | + ve | - ve | - ve | - ve |
| | | | | | | ve | | | | | | | | | | |
| Cardiac glycosides | ++ ve | ++ | ++ | - ve | + ve | +++ | ++ ve | - ve | +++ | ++ | ++ | - ve | ++ ve | ++ | ++ | - ve |
| | | ve | ve | | | ve | | | ve | ve | ve | | | ve | ve | |
| Coumarins | + ve | + ve | - ve | ND | + ve | + | - ve | -ve | + ve | + | - ve | -ve | + ve | + | - ve | +ve |
| | | | | | | ve | | | | ve | | | | ve | | |
| Antho cyanin | - ve | - ve | - ve | - ND | - ve | - ve | - ve | - ve | ++ve | + | - ve | ++ ve | - ve | - ve | - ve | - ve |
| | | | | | | | | | | ve | | | | | | |
| Beta cyanin | - ve | - ve | - ve | - ve | - ve | - ve | - ve | - ve | + ve | ++ | + | + ve | - ve | - ve | - ve | - ve |
| | | | | | | | | | | ve | ve | | | | | |

ey –

Aqueous LeafsheatExtract - AqLE

AqueousFruit pulp (mesocarp)Extract -AqFPE

Aqueous Seed Extract -AqSE

Ethanol Leafsheat Extract –ELSE

Ethanol Fruit pulp (mesocarp)Extract –EFPE

Ethanol Seed Extract- ASE

AcetoneLeafsheath Extract-AcLSE

Acetone Fruit pulp (mesocarp)Extract -AcFPE

Acetone Seed Extract -AcSE

Di ethyl ether Leafsheat Extract-DeELS

Di ethyl ether Fruit pulp (mesocarp)Extract –DeFPE

Di ethyl ether Seed Extract –DeESE

Aqueous Stem Extract –AqSE

Ethanol Stem Extract –ESE

Acetone Stem Extract -AcSE

Di ethyl ether Stem Extract-DeESE

Figure 1- Figure 9 shows different parameter investigated in systemic evaluation of Anti-diabetics and Anti-Inflammatory potential of *Aframomum melegueta*[Roscoe] K. Schum Stem, leaf sheath, Fruit pulp (mesocarp) and seed Extracts.

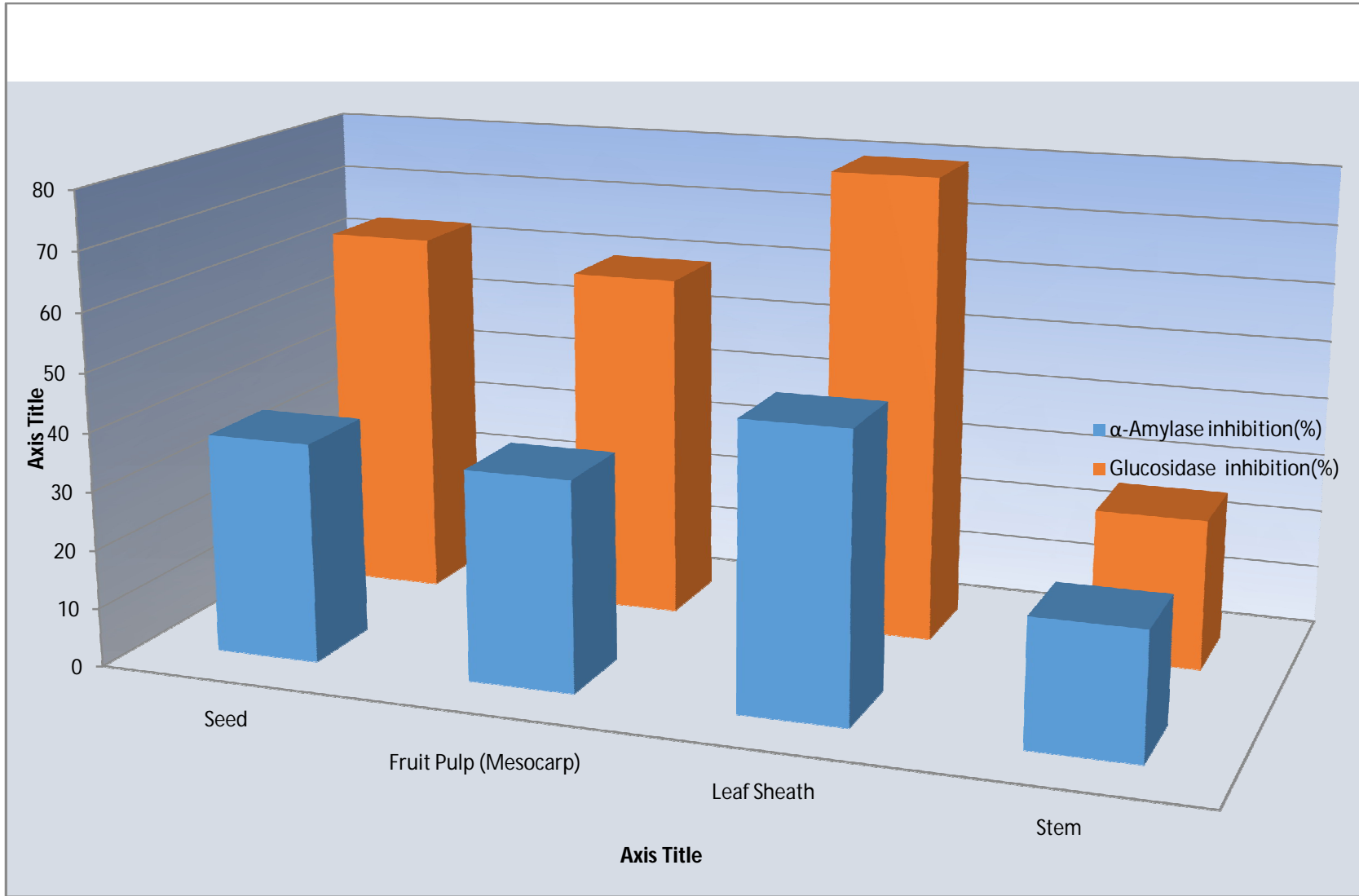


Figure 1: Over all parameter of Systemic Evaluation of Antidiabetic potentials of *Aframomum melegueta* (%) [Roscoe] K. Schum Stem, leaf sheath, fruit pulp (mesocarp) and seed extracts

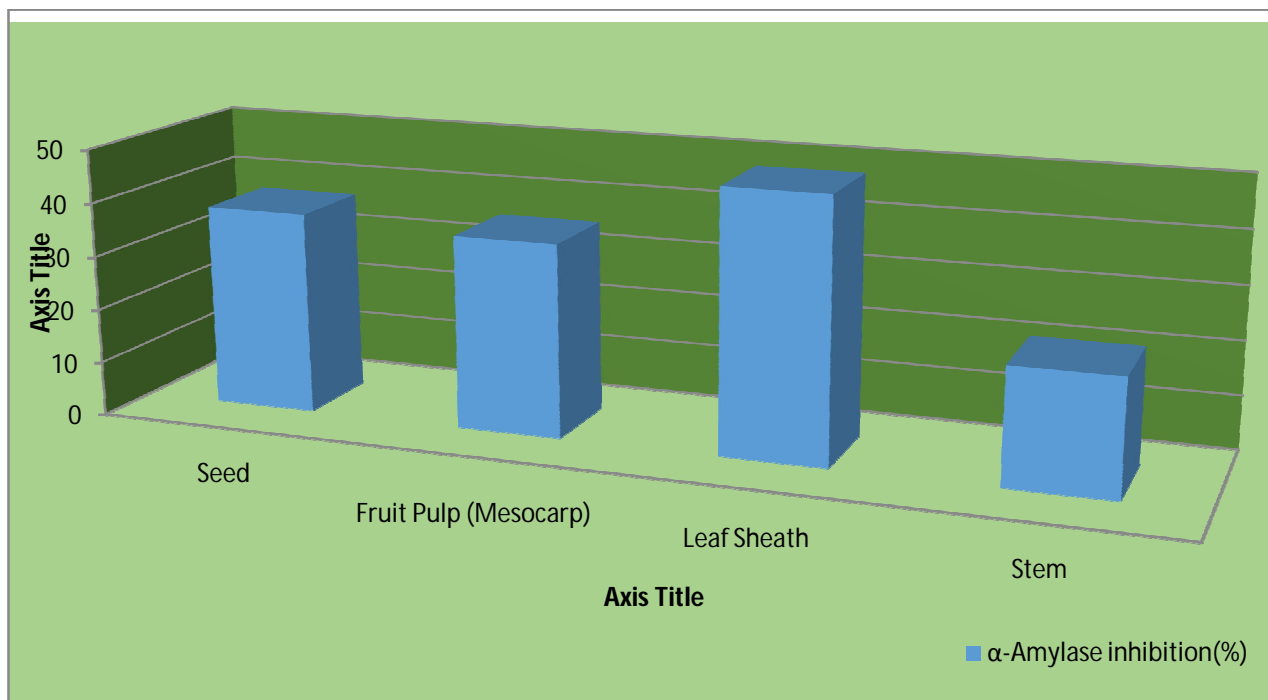


Figure 2: Systemic Evaluation of α -Amylase inhibition of *Aframomum melegueta* (%) [Roscoe] K. Schum Stem, leaf sheath, fruit pulp (mesocarp) and seed extracts

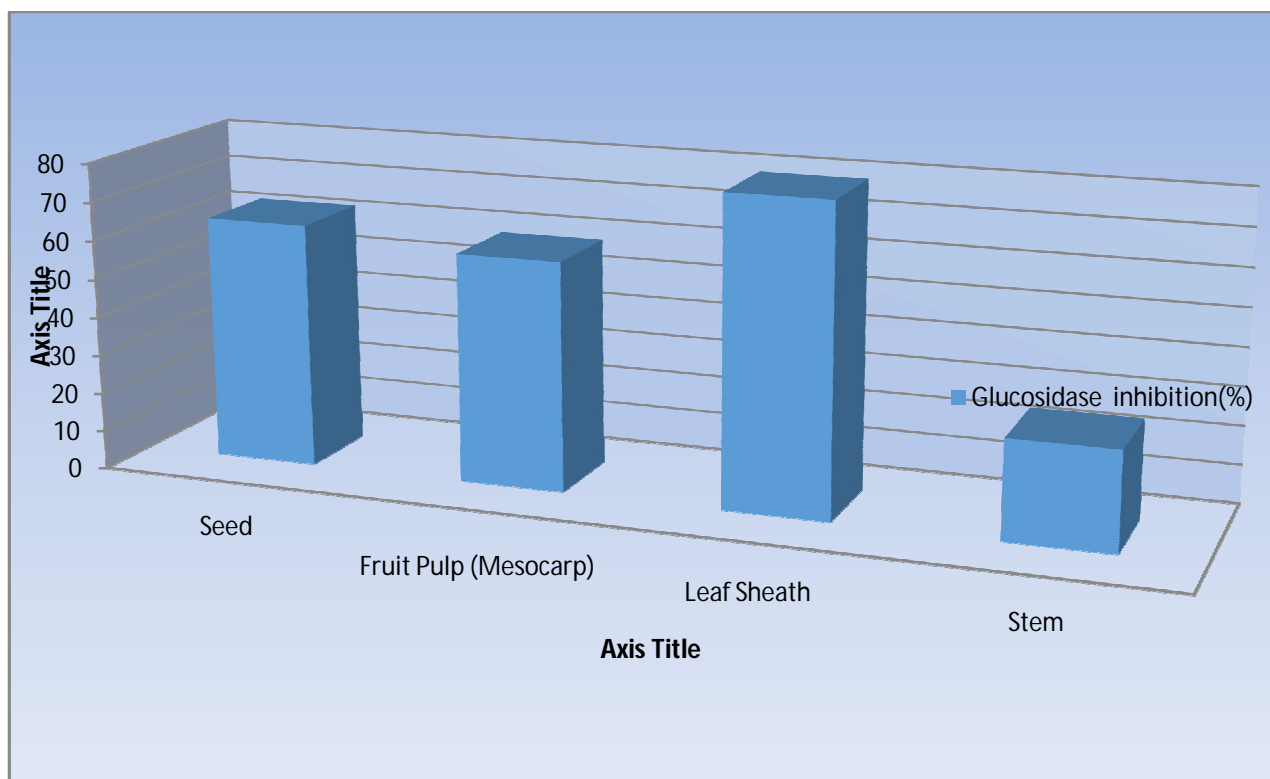


Figure 3: Systemic Evaluation of α -Glucosidase Inhibitory of *Aframomum melegueta* (%) [Roscoe] K. Schum Stem, leaf sheath, fruit pulp (mesocarp) and seed extracts

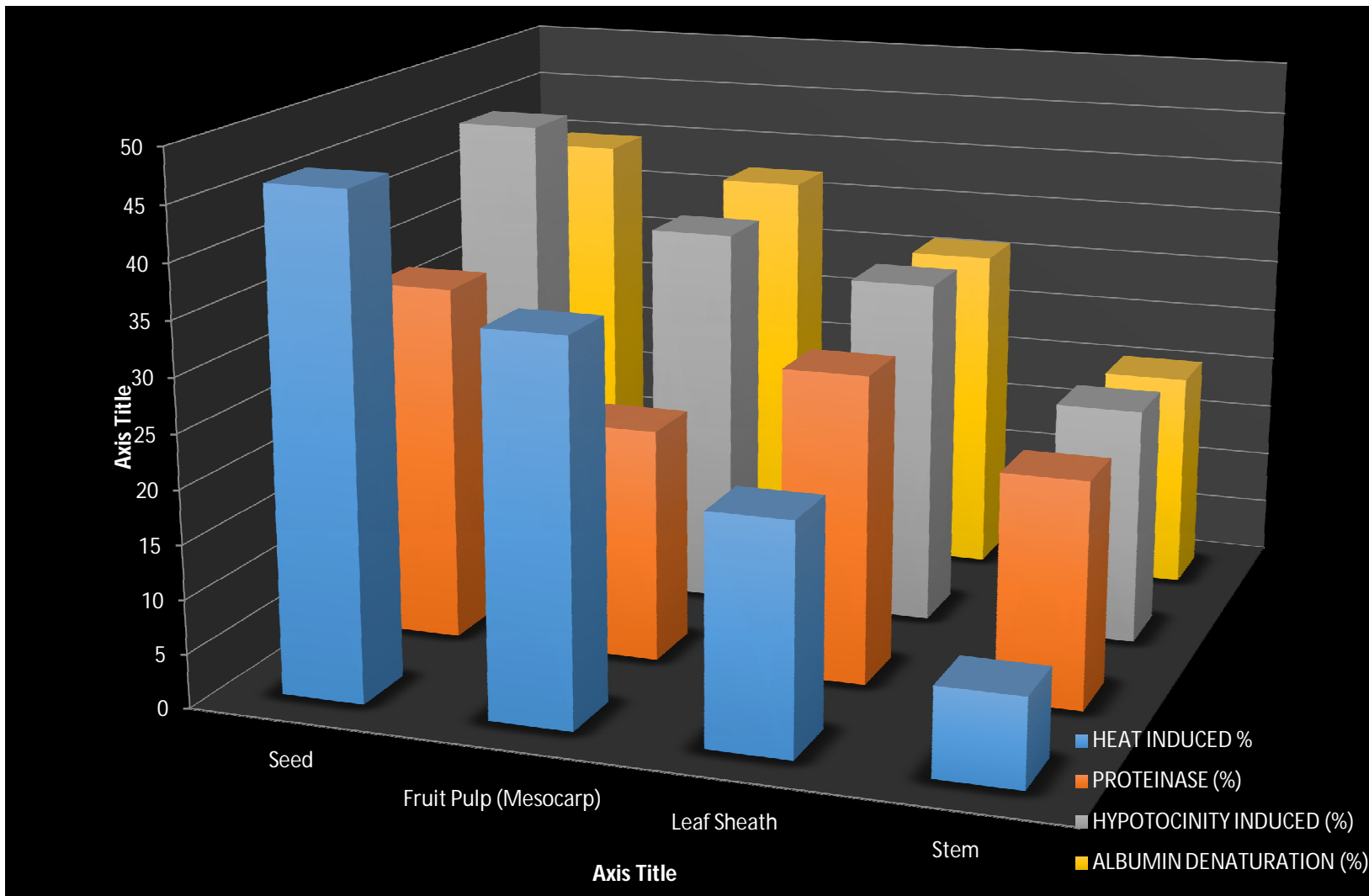


Figure 4: Over all parameter of Systemic Evaluation of Anti-inflammatory potentials of *Aframomum melegueta* (%) [Roscoe] K. Schum Stem, leaf sheath, fruit pulp (mesocarp) and seed extracts.

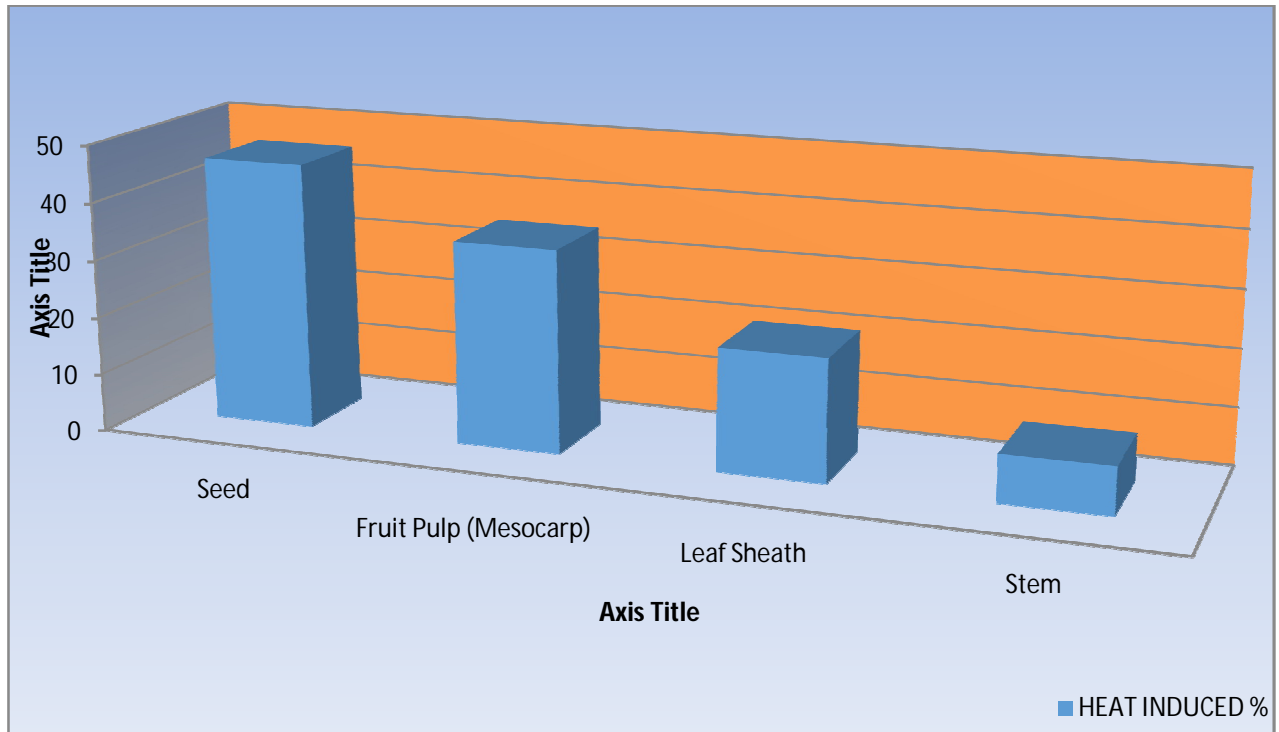


Figure 5: Systemic Evaluation of Heat induced haemolysis of *Aframomum melegueta* (Roscoe) K. Schum Stem, leaf sheath, fruit pulp (mesocarp) and seed extracts

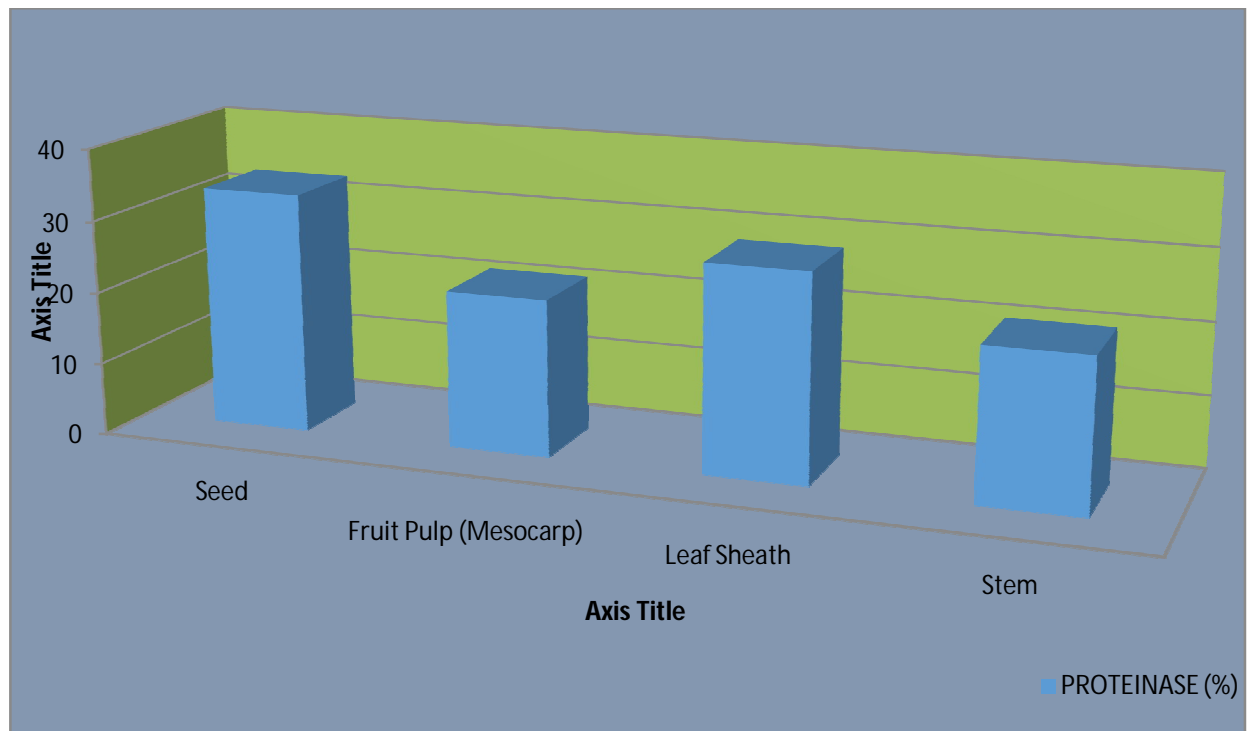


Figure 6: Systemic Evaluation of Antiproteinase inhibition of *Aframomum melegueta* (Roscoe) K. Schum Stem, leaf sheath, fruit pulp (mesocarp) and seed extracts

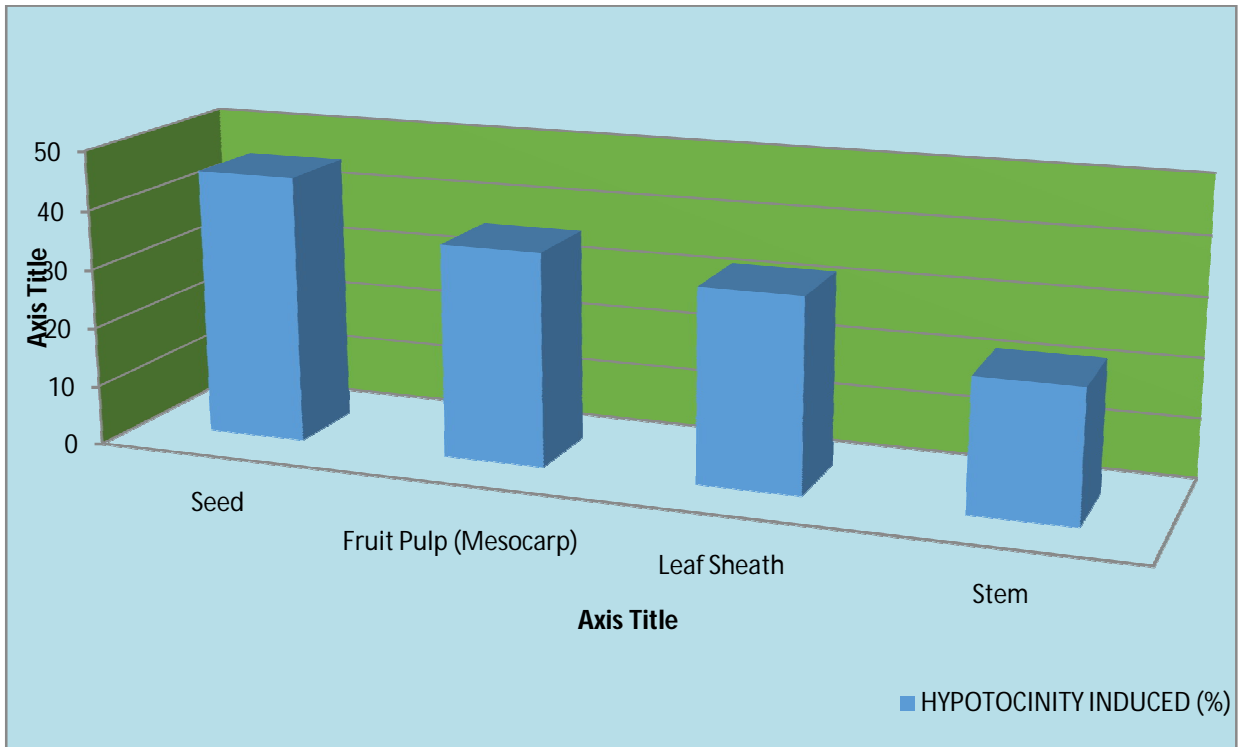


Figure 7: Systemic Evaluation of Hypotocinity-induced haemolysis of *Aframomum. melegueta* (%) Roscoe] K. Schum Stem, leaf sheath, fruit pulp (mesocarp) and seed extracts

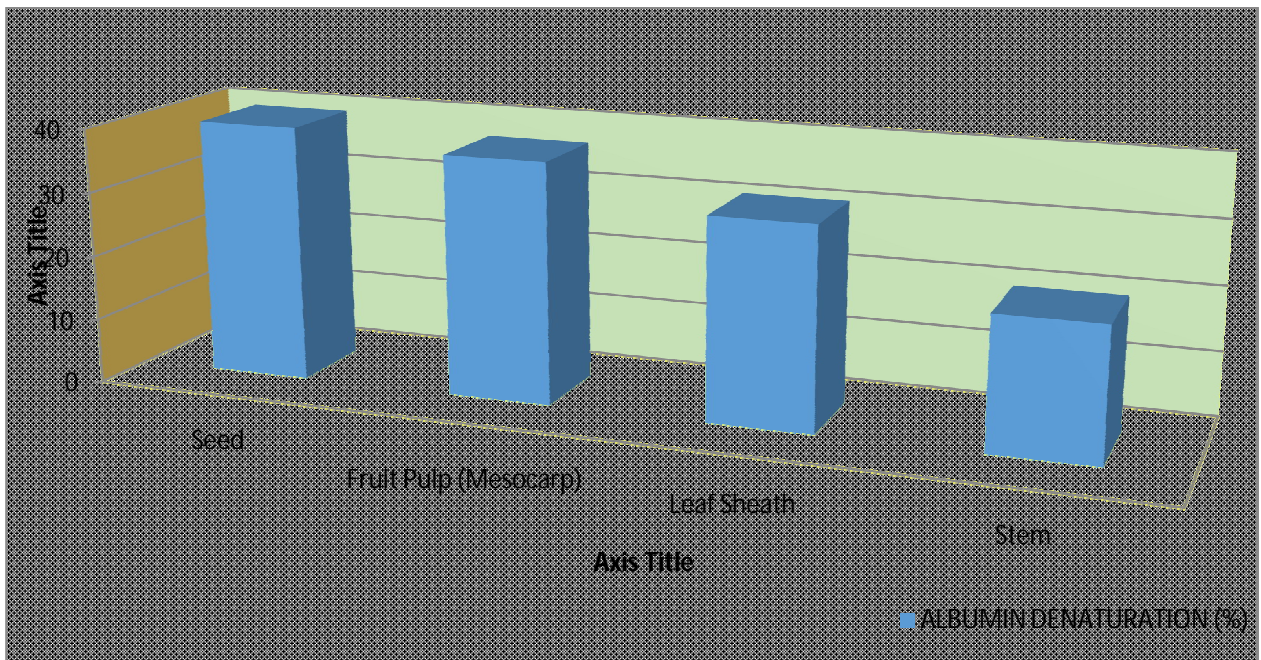


Figure 8: Systemic Evaluation of Inhibition of albumin denaturation of *Aframomum melegueta* (%) Roscoe] K. Schum (Stem, leaf sheath, Fruit pulp (mesocarp) and seed Extracts

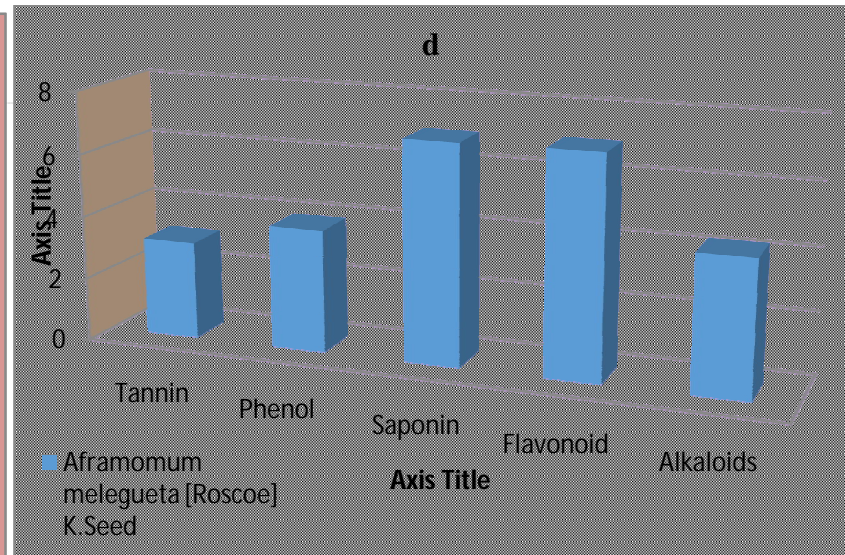
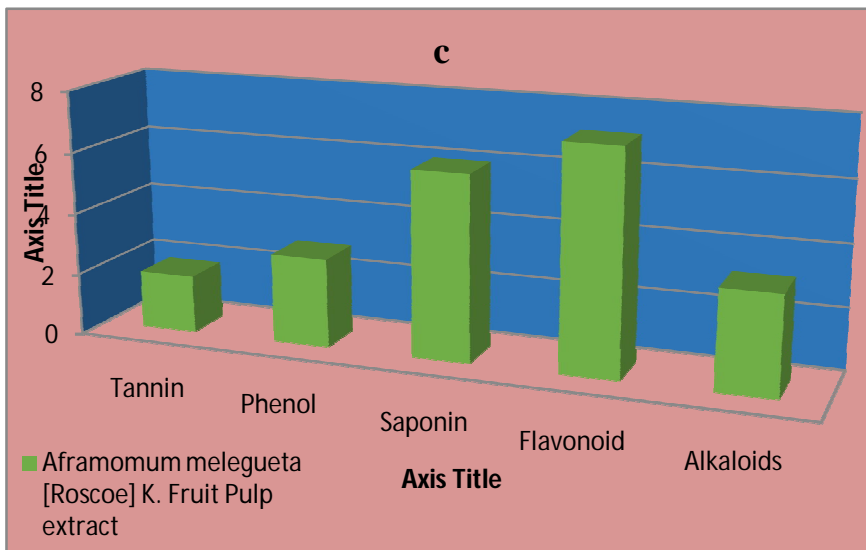
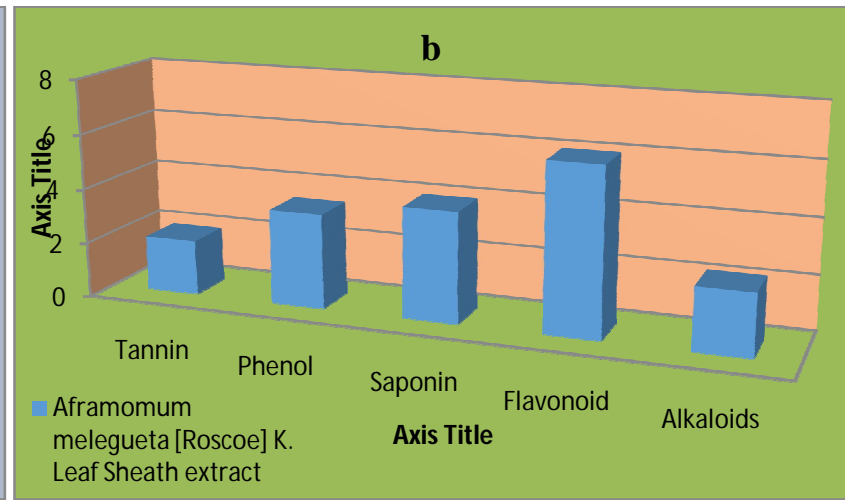
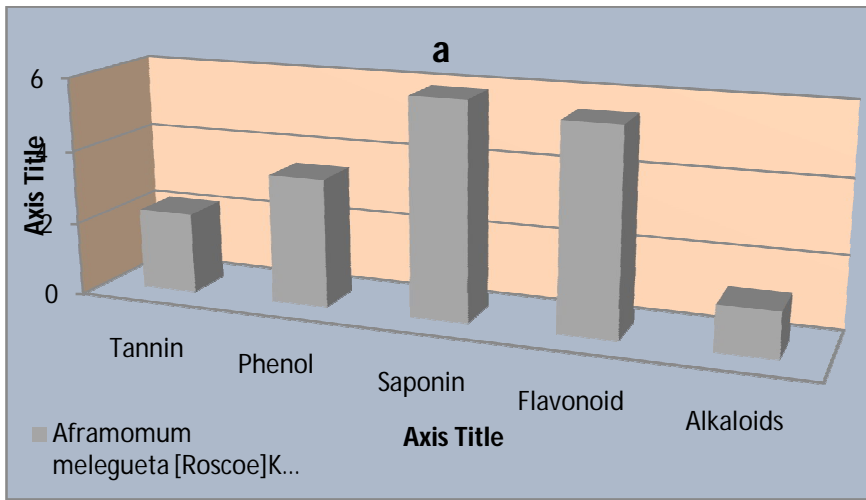


Figure 9(a-d) : Systemic Evaluation of Quantitative Secondary metabolite potentials of *Aframomum melegueta* [Roscoe] K. Schum. (a)Stem, (b)leaf sheath, (c)Fruit pulp (mesocarp) and (d)seed Extracts

4.0 Discussion

The purpose of this research work is to determine the anti-diabetics, anti-Inflammatory and qualitative secondary metabolite potential of *Aframomum melegueta* Stem, leaf-sheath, fruit-pulp (mesocarp) and seed extracts. Diabetes mellitus is a metabolic disorder, a life threatening disease which is increasing day by day. Insulin is key player to regulate carbohydrate, fat and protein metabolism. Diabetic mellitus is a menace in the third world country and affect several people in Africa, because most of our food is based on starch and carbohydrate, and less protein and mineral oil (23).

Aframomum melegueta is one of the medicinal plants used in Africa and Nigeria in particular for different purposes. Its uses are limitless but due to the advent of synthetic drug, its uses are more or less going into extinction. This research work evaluates its antidiabetics and anti-Inflammatory potentials of *Aframomum melegueta*. Qualitative secondary metabolite assay were determined, to ascertain the present of natural occurring chemical that can help develop herbal product, which will be cheap, readily available, less toxic and metabolized to human system. Some parameters like α -Amylase Inhibition and α -Glucosidase be determined during the course of this research work. Others are Heat induced haemolysis, Antiproteinase, Hypotonicity-induced haemolysis, and Inhibition of albumin denaturation their inhibition percentages which denote the inflammatory potentials of *Aframomum melegueta* (24).

α -Glucosidase inhibitors slow down the process of digestion and absorption of carbohydrates by competitively blocking the activity of glucosidase. Consequently, the peak concentration of postprandial blood glucose is reduced and the blood sugar level comes under control and α -glucosidase inhibitors fall under the third category of oral hypoglycemia agent and α -glucosidase inhibitors (24) in other word *Aframomum melegueta* is a natural oral hypoglycemia agent, if *A. melegueta* slows down carbohydrate digestion, absorption and maintain the blood sugar level, Diabetic mellitus may be the infection of the past. Very few α -glucosidase inhibitors are commercially available but its abundance in medicinal plants like *Aframomum melegueta* is

limitless (25) reported that glucosidase inhibitors, such as acarbiose and voglibose obtained from natural sources like *Aframomum melegueta*, can effectively control blood glucose levels after food intake and have been used clinically in the treatment of diabetes mellitus (26). Therefore, it is imperative, to search for alternatives like *Aframomum melegueta* that can display α -glucosidase inhibitory activity but without side reactions.

Insulin is produced by beta cells of the pancreas which use glucose from medicinal plants or digested food as an energy source. α -Glucosidase inhibitors slow down the process of digestion and absorption of carbohydrates by competitively blocking the activity of glucosidase, glucosidase inhibitors, such as acarbiose and voglibose obtained from natural sources like *Aframomum melegueta* can effectively control blood glucose levels after food intake and have been used clinically in the treatment of diabetes mellitus. secondary metabolites like flavonoids, saponini, cardiac glucoside, tannins and terpenoids were effectively inhibit alpha amylase and alpha glucosidase (25,26).

The enzyme alpha amylase and alpha glucosidase may be responsible for the breakdown of carbohydrates into glucose. Alpha amylase is responsible for hydrolysing the starch, which breaks down into glucose before absorption (26). Inhibition of alpha amylase can lead reduction in post prandial hyperglycemia and Alpha glucosidase is an enzyme present in the small intestine, used for the cleavage of disaccharides in to glucose (27). The ethanolic and aqueous extract of *Aframomum melegueta* extracts showed potential percentage of alpha amylase and alpha glucosidase inhibition (28).

It should be profound that Alpha amylase inhibitory effects of tannins were due to its ability to bind carbohydrates and proteins. The above studies proved that, the secondary metabolites like flavonoids, saponini, cardiac glucoside, tannins and terpenoids were effectively inhibit alpha amylase and alpha glucosidase(29). There are many naturally occurring constituents available in medicinal plants with alpha glucosidase inhibition activity. *Aframomum melegueta* is a typical example having alpha glucosidase inhibitory action. It leads to minimum absorption of mono saccharides. Thus inhibition of alpha glucosidase may be the challenging one to control diabetes (30).

Inflammation is one of the body's nonspecific internal systems of defense, the response of a tissue to an accidental cut is similar to the response that results from other types of tissue damage, caused by burns due to heat, radiation, bacterial or viral invasion. When tissue cells become injured they release kinins, prostroglandins and histamine. an increased vasodilation (widening of blood capillaries) and permeability of the capillaries. This leads to increased blood flow to the injured site. These substances also act as chemical messengers that attract some of the body's natural defense cells a mechanism known as chemotaxis (31).

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured (31). Denaturation of proteins is a well documented anti-inflammatory potential of *Aframomum melegueta* which is one of the cardinal and focal point of discussion in this research work. *Aframomum melegueta* was effective in inhibiting heat induced albumin denaturation. It was effective in inhibiting heat induced albumin denaturation (32). Seed extract has the highest percentage of antiproteinase inhibition. This probably denote if *Aframomum melegueta* are consumed orally, it can drastically reduce the inflammatory effect caused by diabetes mellitus in human system (33).

Neutrophils are known to be a rich source of serine proteinase and are localized at lysosomes. It was reported by (34) that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors. *A. melegueta* extract is rich proteinase inhibitors, one of the basic reason the plant may serve as a good proteinase inhibitor in diabetic mellitus patient. *Aframomum melegueta* extract was effective in inhibiting the heat induced haemolysis at different percentages However, *Aframomum melegueta* plants and its parts have the tendency to reduce the blood glucose level. This is due to the availability of tannins, terpenoids and flavonoids (35). Tannins and flavonoids have potential inhibitory effects on alpha amylase and alpha glucosidase (36). The alpha amylase inhibitory action of *Aframomum melegueta* extract was due to the availability of terpenoids (37,38). Alpha amylase inhibitory effects of tannins were due to its ability to bind carbohydrates and proteins. The above studies proved that, the secondary metabolites like flavonoids, tannins and terpenoids were effectively inhibit alpha amylase and alpha glucosidase (40,41).

5.0 Conclusion

From these findings it was concluded that the presence of secondary metabolite constituents in the ethanolic extract of *Aframomum melegueta* may be responsible for the effective inhibitory action of alpha amylase and alpha glucosidase enzymes. The inhibitory effect is more pronounced towards alpha glucosidase than alpha amylase. This research work supports the herbal usage and encourage the formulation of synthetic drug with herbal supplements in the treatment of diabetic mellitus. The seed extract shown potentially active anti-diabetic property. The seed may be used for the pharmaceutical purpose, this results also indicate that the ethanol extracts of *Aframomum melegueta* possess antidiabetics and anti-inflammatory properties. These activities may be due to the strong occurrence of secondary metabolites like polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols. Thus present findings confirmed the alpha-amylase and alpha-glucosidase inhibitory, Anti-Inflammatory and qualitative secondary metabolite potential of *Aframomum melegueta* [Roscoe] K. Schum, Leaf, Stem Bark, Seed Bark and Seed extracts.

FUNDING

This work was supported by TETFUND Institution Based Research program, Nigeria.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to TETFUND Institution Based Research program, Nigeria for sponsoring this research work and all the technical staff of the laboratory unit of the Department of Microbiology, Faculty of Science, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria, Obafemi Awolowo University, Ile Ife, Osun State, Nigeria, for their technical support

References

- 1) American Diabetes Association(ADA) (2010). Diagnosis and classification of diabetes mellitus. *Diabetes Care* **33** (Suppl1), S62.

- 2) Atkinson, M.A., Eisenbarth, G.S. and Michels, A.W. (2014).Type 1 diabetes. *Lancet*. **383**, 69-82.
- 3) Vijan, S. (2010). Type 2 diabetes. *Ann. Intern. Med.* **152**, ITC3-1.
- 4) Miranda-Díaz, A.G.; Pazarín-Villaseñor, L.; Yanowsky-Escatell, F.G.and Andrade-Sierra, J. (2016). Oxidative Stress in Diabetic Nephropathy with Early Chronic Kidney Disease. *J. Diabetes Res.*
- 5) Althunibat, O.Y.; al Hroob, A.M.; Abukhalil, M.H.; Germoush, M.O.; Bin-Jumah, M.and Mahmoud, A.M. (2019) Fisetin ameliorates oxidative stress, inflammation and apoptosis in diabetic car diomyopathy. *Life Sci.* 221,83–92.
- 6) Osuntokun O.T.,OluduroA.O., Idowu T.O. and Omotuyi A.O. (2017). Assessment of Nephrotoxicity, Anti-inflammatory and Antioxidant properties of Epigallocatechin, Epicatechin and Stigmasterol phytosterol (synergy) Derived from ethyl acetate stem bark extract of *Spondias mombin* on Wister Rats Using Molecular method of analysis, *Journal of Molecular Microbiology*, Vol.1 (1):103, Pp 1-11
- 7) Zhang, H.; Sun, S.-C. and NF- κ B (2015), in inflammation and renal diseases. *Cell Biosci.* 5, 63.
- 8) Liu, T.; Zhang, L.; Joo, D.; Sun, S.-C. and NF- κ B (2017), Signaling in inflammation, Signal Transduct. Target. Ther, 2,17023.
- 9) Mano, Y.; Anzai, T.; Kaneko, H.; Nagatomo, Y.; Nagai, T.; Anzai, A.; Maekawa, Y.; Takahashi, T.; Meguro, T. and Yoshikawa, T. (2011). Overexpression of Human C-Reactive Protein Exacerbates Left Ventricular Remodeling in Diabetic Cardiomyopathy. *Circ. J.* 75, 1717–1727.
- 10) Yu, X.-Y.; Chen, H.-M.; Liang, J.-L.; Lin, Q.-X.; Tan, H.-H.; Fu, Y.-H.; Liu, X.-Y.; Shan, Z.-X.; Li, X.-H. and Yang, H.-Z. (2011) Hyperglycemic Myocardial Damage Is Mediated by Proinflammatory Cytokine: Macrophage Migration Inhibitory Factor. *PLoS ONE*, 6, e16239.
- 11) Fuentes-Antrás, J.; Ioan, A.M.; Tuñón, J. and Egido, J. (2014), Lorenzo, Ó. Activation of Toll-Like Receptors and Inflamma some Complexes in the Diabetic Cardiomyopathy-Associated Inflammation. *Int. J. Endocrinol.* 1–10.
- 12) Goldin, A.; Beckman, J.A.; Schmidt, A.M. and Creager, M.A (2006). Advanced Glycation End Products. *Circulation*, 114, 597–605

- 13) Nishida, K. and Otsu, K. (2017). Inflammation and metabolic cardiomyopathy. *Cardiovasc. Res.*, 113, 389–398.
- 14) Osuntokun O.T., Olumekun V. O., Ajayi A.O., Omotuyi I.O. and Olonisakin A. (2020). Assessment of in-vitro Antioxidant/Enzymes Inhibitory Potentials of *Aframomum melegueta* [Roscoe] K. Schum (Grains of Paradise) Leaf, Stem Bark, Seed Bark and Seed Extracts, *Archives of Current Research International* 20(2): 40-57, 2020 Article no.ACRI.55957 ISSN: 2454-7077, Pp40-57
- 15) Mohammeda A., Gbonjubola V.A., Koorbanallyc N.A. and Islama M/S. (2017). Inhibition of key enzymes linked to type 2 diabetes by compounds isolated from *Aframomum melegueta* fruit. *Pharmaceutical Biology*, Vol. 55(1), 1010–1016. <http://dx.doi.org/10.1080/13880209.2017.1286358>
- 16) Leelaprakash G. and Mohan Dass S. (2011). *International Journal of Drug Development & Research* | Vol. 3 | Issue 3 | ISSN 0975-9344
- 17) De Moraes L.M.P., Filho S.A. and Ulhoa C.J. (1999). Purification and some properties of an α -amylase glucoamylase fusion protein from *Saccharomyces cerevisiae*. *World J. Microbiol. Biotech* Vol. 15: 561-564.
- 18) Perry N.S., Houghton P.J., Theobald A., Jenner P. and Perry E.K. (2000). In-vitro inhibition of human erythrocyte acetylcholinesterase by *Salvia lavandulaefolia* essential oil and constituent terpenes. *J. Pharm. Pharmacol.* 52(7):895–902.
- 19) Kim Y.-M., Jeong Y.-K., Wang M.-H., Lee W.-Y., and Rhee H.-I. (2005), “Inhibitory effect of pine extract on α -glucosidase activity and postprandial hyperglycemia,” *Nutrition*. Vol. 21(6): 756–761, 2005.
- 20) Boulekbache-Makhlouf L, Slimani S. and Madani K. (2012), Antioxidant effects and phytochemical analysis of crude and chromatographic fractions obtained from *Eucalyptus globulus* bark. *African J Biotech*;11(42):10048-55.
- 21) Priya G, and Chellaram C. (2014), Evaluation of antibacterial activity and phytochemical analysis of medicinal plant *Solanum trilobatum*. *Int J Pharm Bio Sci.*;5(3):354-9.
- 22) Babu K., Samundeeswari A. and Chitti Babu C.V. (2015). Studies on phytochemical screening, estimation of tannin and antioxidant activity of *Delonix elata* Lind. *Int J Curr Sci.*;15S:E37-42.

- 23) Sahaya S.S., Janakiraman N. and Johnson M. (2017), Phytochemical analysis of *Vitex altissima* L. using UV-VIS, FTIR and GC-MS. *Int J Pharm Sci Drug Res.*;4(1):56-62
- 24) Osuntokun O.T., Ajayi A., Adeoye M.I. and Odufunwa A.E. (2017).Assessment of Anti microbial and Phytochemical Properties of Crude Leaf and Bark Extracts of *Ceiba Pentandra* on Selected Clinical Isolates Found in Nigerian Teaching Hospital, *Journal of Bacteriology & Mycology*, Vol. 4(1), 00079. DOI:10.15406/jbmoa.20 17.04.00079.
- 25) DuW.Q. , ShiX.F. , QiuM.Y. (2005). Progress in treatment of diabetes drugs Chin. Hosp. *Pharm. J.*, 25, pp. 67-69
- 26) PlayfordR.J. , PitherC., Gao,R. (2013). Use of the α -glucosidase inhibitor acarbose in patients with 'Middleton syndrome': normal gastric anatomy but with accelerated gastric emptying causing postprandial reactive hypoglycemia and diarrhea *Can.J. Gastroenterol.*, 27, pp. 403-404
- 27) Abhijit S., Rashmi S., Srivastava, Nikita S., Yashwant M., Bhagyashri C. (2014),Anti-diabetic activity of *Tridax procumbens*. *J Sci Innovative Res.*;3(2):221-6.
- 28) Raman B.V., Naga Vamsi Krishna A., Narasimha Rao B., Pardha Saradhi M., Basaveswara Rao M.V. (2012), Plants with antidiabetic activities and their medicinal values. *Int Res J Pharm.* ;3(3):11-5.
- 29) Amin M.M., Sawhney S.S., Jassal M.M.S. (2015), In-vitro antidiabetic studies of various extracts of *Taraxacum officinale*. *Pharm Innov J.*;4(1):61-6.
- 30) Anuradha Devi V., Mallikarjuna K. (2016), *In-vitro* antimicrobial and antidiabetic activity of leaf extracts of *Schrebera swietenoides* and *Homalium zeylanicum*. *Int J Life Sci Pharma Res.*;6(3):1-7.
- 31) Mbaze L.M., Poumale H.M.P., Wansi,J.D. (2007), α -Glucosidase inhibitory pentacyclic triterpenes from the stem bark of *Fagara tessmannii* (Rutaceae)*Phyto chemistry*, 68, pp. 591-595
- 32) Gerard J Tortora, Sandra Reynolds, eds. Principles of Anatomy and Physiology. Harper Collins College Publishers, 1993, 7th edition: pp 695.
- 33) Shenoy S., Shwetha K., Prabhu K., Maradi R., Bairy K.L., Shanbhag T. (2010),Evaluation of anti-inflammatory activity of *Tephrosia purpurea* in rats. *Asian Pac J rop Med*; 3(3): 193-5

- 34) Mizushima Y., and Kobayashi M. (1968), Interaction of antiinflammatory drugs with serum preteins, specially with some biologically active proteins. *J of Pharma Pharmacol*; 20:169, 173.
- 35) Osadebe P.O., Omeje E.O., Uzor P.F., David E.K., Obiorah D.C. (2010), Seasonal variation for the antidiabetic activity of *Loranthus micranthus* methanol extract. *Asian Pac J Trop Med.* ;3(3):196-9.
- 36) Poongunra J., Pere H.K.I., Fernando W.I.T., Jayasinghe L., Sivakanesan R. (2015), α -Glucosidase and α -amylase inhibitory activities of nine Sri Lankan anti diabetic plants. *British J Pharm Res.*;7(5):365-74.
- 37) Vinodhini V., Himaja M., Sai S., Poppy D. (2015), *In-vitro* antidiabetic activity of *Tragia involucrata* Linn. Leaf extracts. *Int J Res Ayurveda Pharm.*;6(1):1-3.
- 38) Khan M.F., Rawat A.K., Pawar B., Gautam S., Srivastava A.K., Negi D.S. (2014), Bioactivity guided chemical analysis of *Melia azedarach* L. (Maliaceae), displaying antidiabetic activity. *Fitoterapia*;98:98-103.
- 39) Guevarra B.Q. (2005). A guidebook to plant screening phytochemical and biological. UST Publishing House, Philippines. Hawkins E. B., Ehrich S. D. and Goto Kola Editions. *University of Maryland Medical Center, Baltimore, USA*
- 40) Sofowora A. (2006). *Medicinal plants and traditional medicine in Africa*. 5th Edition. *Spectrum book limited Ibadan, Nigeria*. Page 289.
- 41) Osuntokun O.T., Yusuf-Babatunde M.A., Oladele, O.F. (2020) Components and Bioactivity of *Ipomoea batatas* (L.) (Sweet Potato) Ethanolic Leaf Extract, *Asian Journal of Advanced Research and Reports* 10(1): Article no. AJARR.56421 ISSN :2582-3248, <https://doi.org/10.9734/ajarr/2020/v10i130232>, Page: 10-26