# TOTAL FLAVONOID, TOTAL PHENOLIC AND ANT-DIABETIC ACTIVITY OF *zingiber officinale* POWDER SOLD IN LOCAL DESPENSARY

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## DECLARATION

This research study is my original work and has not been presented to any other Institution. No part of this research should be reproduced without the authors' consent or that of Mount Kenya University.

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Declaration by the supervisor(s)

This research has been submitted with my approval as The Mount Kenya University Supervisor(s).

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## DEDICATION

I dedicate this work to my dear parents, Mr.  and Mrs. Wachira especially my mother and siblings my friends and the general society  for their , support and guidance throughout my studies and my supervisor professor Bindu Madhavi for her continued guidance and moral support in the completion of my research project.

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## ABSTRACT

Diabetes mellitus is a common metabolic disorder that has affected about 2.8 % of the population worldwide. This disease is characterized by hyperglycemia which is the condition of having high glucose levels. Many conventional drugs used to manage this condition are very expensive in some cases and not void of side effects. Finding an alternative antdiabetic is paramount in the efforts compact diabetes. Plants or herbal medicines have shown to be potent against many ailments diabetes included. However, very few studies have been studied or evaluated. The current study evaluated the concentrations of the phenolic and flavonoid and the antidiabetic activity of *Zingiber officinale.* The total flavonoid and total phenolic content were conducted following standard in vitro assays: aluminum nitrate calorimetric and Folin–Ciocalteu method respectively. The antidiabetic activity was conducted by in vitro assay of alpha amylase inhibition. Catechin, gallic acid and metformin were used as standards for flavonoid, phenolic and amylase inhibition assays respectively. The results showed that 150.257±1.786 mg GAE/f dw and 121.396± 1.179 mg CE/g dw phenolics and flvanoids were recorded respectively. The *Zingiber officinale* significantly inhibited the activity of the amylase enzyme recording inhibition percentages of 45.465±7.965 %, 23.850±5.560 % and 11.940±2.520 % for 200 ug/ml, 150 ug/ml and 50 ug/ml respectively. The standard andiabetic drug metformin recorded inhibition of 60.075±3.47 %. In conclusion, *Zingiber officinale* is a potential antioxidant and antidiabetic agent.

# CHAPTER ONE: INTRODUCTION

## 1.1 Background information

Diabetes mellitus is a metabolic disorder that is characterized by hyperglycemia and altered biomolecules (lipids, carbohydrates and proteins) metabolism. This disorder affects both the social, psychological and physical ill health of the patients (Dewanjee et al., 2009). Diabetes is grouped into two categories; type 1 and diabetes type 2 based on the pathogenesis criteria. However, both the two types of diabetes are characterized by hyperglycemia which is the major sign and symptom of diabetes. Hyperglycemia is the condition whereby the level of glucose is very high as a result of lower activity of insulin secreting hormone. In the diabetes type 2 hyperglycemia results due to failure of insulin secretion or impairment of insulin action (Lin & Sun, 2010). As per the reports of the world health organization (WHO) most of the population across the entire world is in the middle of the diabetes epidemic. For instances the larger population of people in the southern Asia and western pacific are under greater risk with the majority of the patients being diagnosed with diabetes type 2. The diabetes mellitus is projected to affect a larger population of people that amount to about 592 million by the year 2035. This has been triggered by many factors including aging, high population growth size, high prevalence of obesity, rise in living standards and the spread of calorie rich, fatty and fast food.

Diabetes mellitus complication development is largely mediated by oxidative stress condition. This condition occurs upon reduced potency of the antioxidant defense system resulting into increased concentration of the free radicals. This results into diabetes whose pathogenesis is mediated by the reactive oxygen species. The

interference with the antioxidant defense system in the body is accompanied by enhanced lipid peroxidation, alteration of the antioxidant enzymes and disabling of the glutathione metabolism.

Diabetes management involves use of many modern synthetic a drugs and agents. Such drugs include acarbose and miglitol that have mechanism of action hinted at that inhibiting the activity of both alpha amylase and alpha-glucosidase enzymes enzymes (American Diabetes Association, 2016). These drugs have only resulted into minimal prevention of the diabetes cases. The commercially available drugs are as well out of rich for many people since they are expensive. Additionally some have resulted into side effects hence are unsuitable for use. The side effects include abdominal distention, flatulence, vomiting and diarrhea.

Herbal management of diabetes has been practiced since the early days prior to invention of the modern drugs for diabetes. Many of the herbal products that include fruits and foods are prescribed for compacting the detrimental effects of diabetes and related complications. These products are normally most sought for due to their high therapeutic effects a characteristic they owe to the phytochemicals. Even though many of the used remedies from the natural sources are used only few have been screened foresee vital bioactive compounds. However, the unscreened plants, fruits and vegetables are still used since the herbal management is considered safer, effective and easily available. The search for the a new safer and potent ant diabetic agent from plant is not only for treating diabetes but for other complication related to oxidative stress such as c cardiovascular diseases and cancer. *Zingiber officinale* plant is commonly identified by its local name as ginger.

It’s used as spice in many countries where the rhizome is used when fresh or grounded in powder. The rhizome contains essential oils that when extracted are used for various pharmacological activities. The effectiveness of the *Zingiber officinale* as antioxidant, antimicrobial, anti-inflammatory and ant analgesic has been reported. However, the information about its ant-diabetic activity is still scant. Therefore the study evaluated the antioxidant and ant-diabetic activity of the *Zingiber officinale* powder.

### 1.2 Problem statement

The two type of diabetes are recognized; type two and type one (Abena Sekyere, 2017). The diabetes type two characterized by hyperglycemia and abnormality in carbohydrate metabolisms is reported to cause high rate of morbidity and mortality globally (A Peter, 2017). Many deaths amounting to about 38 million people were reported in the year 2013 and the deaths were expected to double by the year 2035. Similarly, the high cost of managing diabetes results into economic burden to the patient and close relatives and friends (Kumar, 2014). Conventional strategies of managing the type two diabetes involves use of synthetic such as acarbose and miglitol that inhibits the activity of both alpha amylase and alpha-glucosidae enzymes (American Diabetes Association, 2016). However, these two agents have been accompanied by side effects such as abdominal distention, flatulence, vomiting and diarrhea. Alternatives with lesser or no toxic side effects to the user are inevitable.

## 1.3 Justification

Plants have been of great help in managing many aliments including diabetes type two for many centuries (Saikat et al., 2010). These plants contain various therapeutic properties that confer certain pharmacological activities such as antioxidant and ant diabetic (Marrelli et al., 2019). The phenolic compounds that include flavonoids are important antioxidant compounds in the body (Keter & Mutiso, 2012). These compounds scavenge for the free radicals reducing their deleterious effects. Additionally any antioxidant compounds of plants have a great role in protecting the body against pathogenesis of diabetes type two. This has been seen through the ability of the antioxidants to protect β-cells from attack by the reactive oxygen species and this reduces the chances of developing diabetes induced by free reactive oxygen species (Adefegha & Oboh, 2016). Therefore it's very important to evaluate the many potential antioxidant and ant diabetic herbs and plants that are able to reduce the reactive oxygen species and as well lower blood glucose levels which greatly contribute to genesis of diabetes. Hence this study aimed at evaluating the antioxidant and ant diabetic potentiality of ginger powder.

## 1.4 Objectives

### 1.4.1 General objectives

To evaluate the antioxidant and ant diabetic activity of ginger powder

### 1.4.2 Specific objectives

I. To evaluate the ant diabetic activity of the *Zingiber officinale* powder
II. To quantify the total phenolic and flavonoid content of *Zingiber officinale* powder.

## 1.5 Research questions

I. Does the *Zingiber officinale* powder have anti-diabetic properties?

II. What is the concentration of total phenolic and flavonoid content in total phenolic and flavonoid content?

## 1.6 Significance of the study

This study intends to find the lasting solution of the prevention of the diabetes and its related complications. This will bring forward alternative to the commercially available drugs that have shown to be effective to only certain limit and presents side effects upon use. Also the alternative that is easily available to the larger population that are able to access some of the commercially available ant diabetes drugs due to poverty. This alternative that is both effective and safer is available in the medicinal plants.

# CHAPTER TWO; LITERATURE REVIEW

## 2.1 Diabetes mellitus

Diabetes mellitus (DM) is a category of metabolic disorder that is characterized by hyperglycemia condition. This hyperglycemia condition arises as a result of impairment in the hormone responsible for insulin secretion or inhibition of its action (Eddouks et al., 2012). The impairment of the action of insulin results into no regulation of the levels of glucose in the blood. Hyperglycemia many either be acute or chronic which is usually associated with long term damage, function impairment, and collapse of various organs including eyes, kidneys, nerves, heart and blood vessels.

The symptoms of hyperglycemia which is the characteristic hallmark of diabetes include increased urination at short intervals, increased thirst, and hunger. When diabetes goes untreated for a long period of time, it results into complications which include both acute and chronic. The acute complications include; diabetic ketoacidosis and non ketotic hyperosmolar coma. The chronic complications includes; heart diseases, stroke, kidney failure, foot ulcers and damage to the eyes (Gao et al., 2017).

## 2.2 History of diabetes

The history of diabetes dates back about 3000 years ago hence it's regarded as one of the oldest form of diseases known by man as per the reports in the Egyptians manuscript. In the year 1936 the differentiation between type 1 and 2 diabetes was made. In the year 1986, diabetes type 2 was described as a component of metabolic syndrome (Gao et al., 2017).

### 2.3 Pathogenesis of diabetes

Many processes results into development of diabetes. These processes ranges from the autoimmune destruction of the pancreatic beta cells. The destruction of these cells results into reduced level of insulin and abnormalities that impair the action of insulin. The deficient of insulin on target tissues, results into dysfunction in metabolism process of carbohydrates, lipids, fats and protein. The reduced action of insulin, occurs as a result of reduced insulin secretion resulting into lower levels of insulin than the required one or failure of the tissues to respond to one or more point of the complex pathways of hormone reaction or both (Olokoba et al., 2015).

### 2.4 Classification of diabetes mellitus

Diabetes mellitus is classified into three categories; type 1, 2 and gestational diabetes.

#### 2.4.1 Diabetes type 2

This is the most common type of diabetes that was formally known as non-insulin dependent diabetes mellitus. This type of diabetes is characterized by hyperglycemia, insulin resistance and relatively reduced insulin deficiency. The pathogenesis of diabetes type 2 is caused by interaction between various factors such genetic, environmental and behavioral risk factors. Similarly, excessive obesity and lack of enough exercise was regarded as the primary cause of this second type of diabetes (Olokoba et al., 2015).

#### 2.4.2 Type one diabetes

This is the type of diabetes that occurs as result of the body not being able to produce enough insulin. This as well recognized as the insulin dependent diabetes or juvenile diabetes in the old days. Its cause is not known but it has been linked to

partly being inherited where genes such as HLA genotypes that are known to influence the risk of diabetes (Dewanjee et al., 2009).

### 2.4.3 Gestational diabetes

This is the third class of diabetes that cuts across the two major types of diabetes (type 1 and 2). This through combination of both inadequate insulin and reduced responsive of the body to insulin action. It's more prevalence in pregnant mothers who had no history of diabetes.

## 2.5 Zingiberaceae family

Zingiberaceae commonly known as ginger family, is a family of flowering plants comprising more than 1300 species divided into about 52 genera of aromatic perennial herbs with creeping horizontal or tuberous rhizomes, distributed throughout tropical Africa, Asia, and the America. Numbers of plants of this family showed significant antimicrobial activities. Many of the family's species are important ornamental, spice, or medicinal plants. Ornamental genera include the shell gingers (Alpinia), Siam or summer tulip (Curcuma alismatifolia), Globba, ginger lily (Hedychium), Kaempferia, torch-ginger Etlingera elatior, Renealmia, and ginger (Zingiber). Spices include ginger (Zingiber), galangal or Thai ginger (Alpinia galanga and others) (Parham et al., 2020).

# CHAPTER THREE: MATERIAL AND METHODS

## 3.1 Study design

This study was a laboratory based study that was conducted in the biochemistry laboratory. All the procedures were for the invite investigation.

## 3.2 Source of the *Zingiber officinale* powder

The *Zingiber officinale* crude powder was obtained from the supermarket stores located in Thika town, Kiambu County. The powder was then brought into the pharmacognosy laboratory and stored in a dry environment.

## 3.3 Preparation of the sample

The powder was extracted with methanol and the resultant solution used as the study sample. About 0.5 grams of the powder was weighed and 10 ml of analytical methanol added. This was shaken and left for about one hour and filtered. The resultant solution of *Zingiber officinale* served as the stock solution.

## 3.4 Data collection

### 3.4.1 Antioxidant activity

#### 3.4.1.1 Total phenolic content

The Folin – Ciocalteu method was used to quantify the quantity of the phenols in the *Zingiber officinale* powder. The standard total phenolic content determination protocols were followed (Sousa et al.,2016). The reaction mixture was prepared by mixing exactly 300 ul of the sample/ standard at different concentrations, and 1500 ul of Folin-Ciocalteu reagent (1ml in 10 ml of didtilled water). The two solutions were mixed by shaking for about five minutes and 1500 ul of sodium carbonate (7.5 %) prepared in distilled water was added. The resultant reaction mixture was incubated in the dark for 90 minutes and the absorbance measured at 760 nm wavelength. Gallic acid was used as the reference phenol and its standard curve was drawn from which the concentration of the phenols in the sample were drawn.

The concentration of the phenols was calculated following the formula and presented as Gallic acid equivalent per gram of dry weight (GAE, mg/g dw).

$$concentration\ \left(\text{GAE}, \frac{\text{mg}}{\text{g}}\text{dw}\right) = \frac{c \times v}{m}$$

C= concentration of phenol from standard curve,

V= volume of the sample

M= mass of the extract weighed.

**3.4.1.2 Total flavonoid content**

Standard procedures that employ the aluminum chloride calorimetric method were used (Al-Rifai et al.,2017). *Zingiber officinale* powder sample/ standard at 125 ul was mixed with 100 ul of sodium nitrate mixed and incubated at room temperature conditions for six minutes. Exactly 75 ul of sodium hydroxide (4% in distilled water) was added and lastly 750 ul of aluminum chloride followed. The reaction mixture was topped up to 2500 ul with distilled water. Absorbance of the reaction mixture measured at 510 nm using spectrophotometer. Catechin used as standard flavonoid and its curve drawn. From the curve the concentration of flavonoid determined and calculated using formula

$$Concentration\ \left(\text{CE}, \frac{\text{mg}}{\text{g}}\text{dw}\right) = \frac{c \times v}{m}$$

C= concentration of phenol from standard curve,

V= volume of the sample

M= mass of the extract weighed.

Concentration presented as milligrams of catechin per gram of dry weight.

### 3.4.2 Ant diabetic activity

#### 3.4.2.1 Alpha amylase inhibition assay

The ability of the *Zingiber officinale* powder to inhibit the activity of the pancreatic alpha amylase enzyme was performed by method of with minor modifications. Briefly this involved adding 150 ul of the plant sample at three concentration levels (prepared in phosphate buffer pH 6.9) into clean test tubes. This was followed by 50 ul of the alpha amylase enzyme solution (1% in phosphate buffer). The reaction mixture were then incubated in a thermostat set at 37 $^{0}$ C and upon elapse of this incubation period the reaction was initiated by addition of 200 ul of the starch solution (1 % in distilled water) and incubated for 30 minutes. The reaction was then stopped by adding 100 ul of 1 M hydrochloric solution. Lastly into the entire mixture 750 ul of iodine reagent was added. The blank was prepared to only contain all the reagent except the extract which was replaced with phosphate buffer. Metamofine chloride was used as the standard drug at the concentration level of. The absorbance of the mixture was measured at 580 nm against phosphate buffer as the blank and the percentage inhibitory activity of alpha amylase calculated using the formula

$$\%\ inhibition = (1 - \frac{Abs.C(untreated)}{Abs.test}) \times 100$$

### 3.5 Data analysis

The total phenolic and total flavonoid data were tabulated in the excel spread sheet and the respective standard curves drawn. From the curves the various

concentrations of the phenolic and flavonoid content were calculated and tabulated in the graph pad prism software for analysis. The alpha-amylase activity data was calculated for its percentage inhibition and as well entered manually in graph pad prism software. Similarly the percentage radical scavenging values were entered in the graph pad prism software as well. All the data was subjected to descriptive statistics and presented in the form of Mean ± SEM. Total phenolic content and flavonoid content were compared by student t-test while the antioxidant and the percentage inhibition of amylase were compared by the one-way anova. The means which were statistically different were checked with tuskeys post hoc test and $p<0.05$ was taken as the value of significant difference.

### 3.6 Ethical consideration

Since the study was conducted using in vitro assays no ethical clearance was required.

## CHAPTER FIVE: RESULTS AND DISCUSSION

### 4.1 Total phenolic and total flavonoid content

The quantity of the total phenolic and flavonoid content were determined from the two respective standard curves straight line equations. The total phenolic was calculated from the equation ($y = 0.0106x - 0.1493$; $R^2 = 0.9879$; Fig 4.1) while total flavonoid was calculated from the equation ($y = 0.0106x - 0.1493$; $R^2 = 0.9879$; fig 4.2). The total phenolic content in *Zingiber officinale* powder was 150.257±1.786 mg GAE/f dw while the total flavonoid content was 121.396± 1.179 mg CE/g dw. On comparison of the two phytochemicals the quantity of total phenolic content was significantly higher than the concentration of total flavonoid ($p<0.05$; fig 4.3).

**Table 4. 1 total phenolic and total flavonoid content**

| Total phenolic content | Total flavonoid content |
|---|---|
| 150.257±1.786 | 121.396± 1.179 |

**Figure 4. 1 Catechin standard curve**

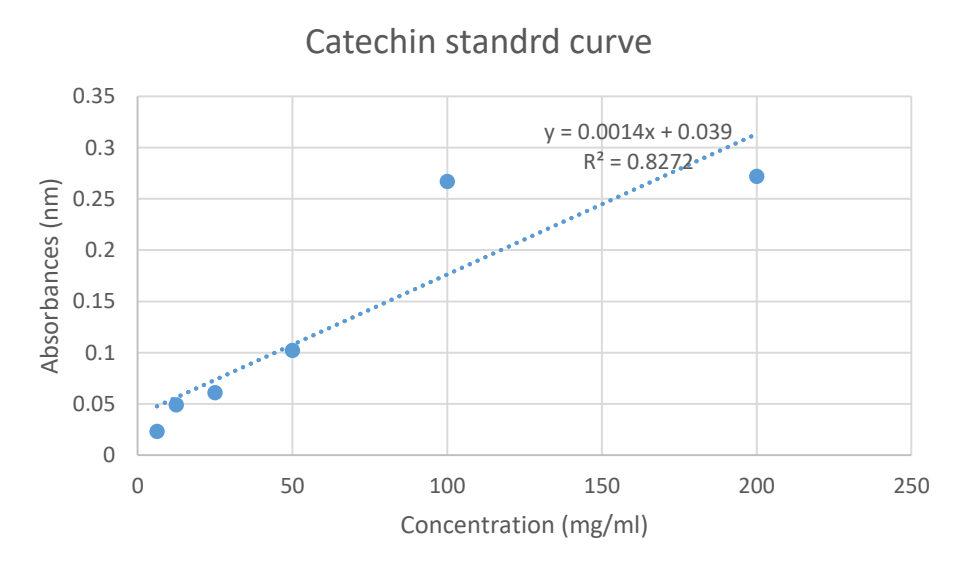


**Figure 4. 2 Gallic acid standard curve**

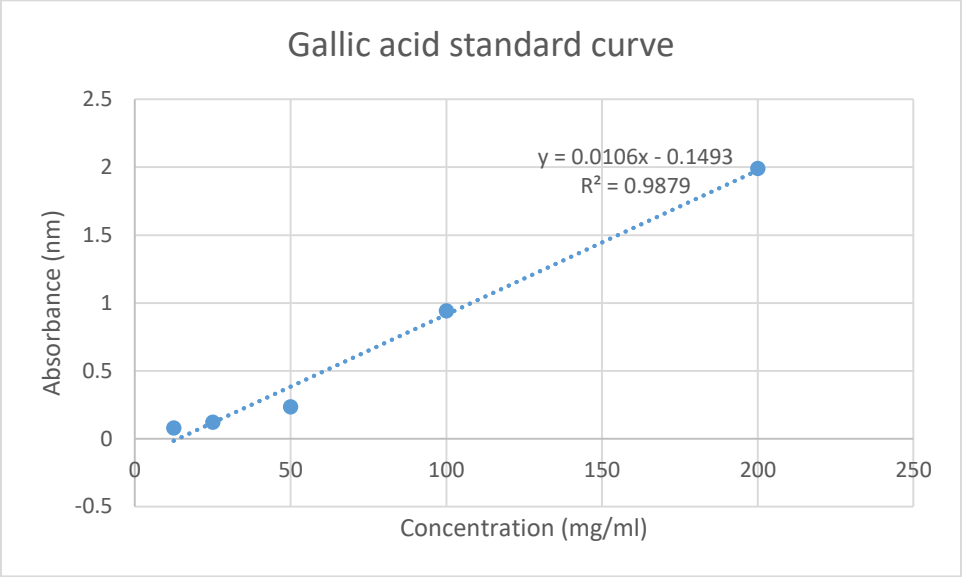
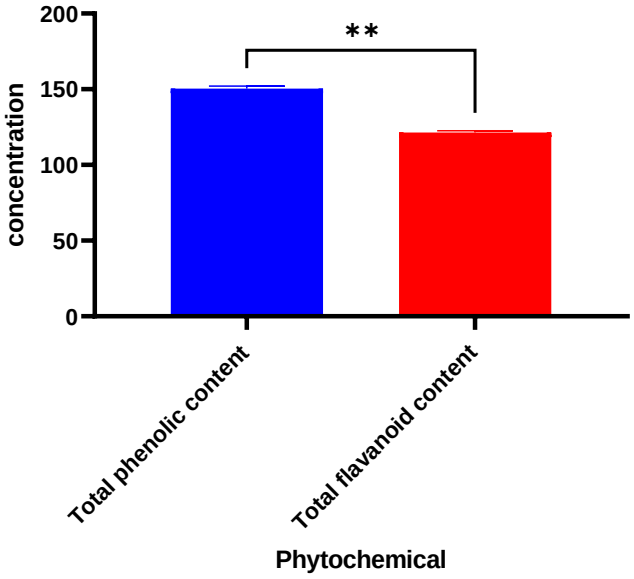



**Figure 4. 3 Total phenolic and total flavonoid content**




### 4.2 Inhibition of the alpha amylase enzyme activity

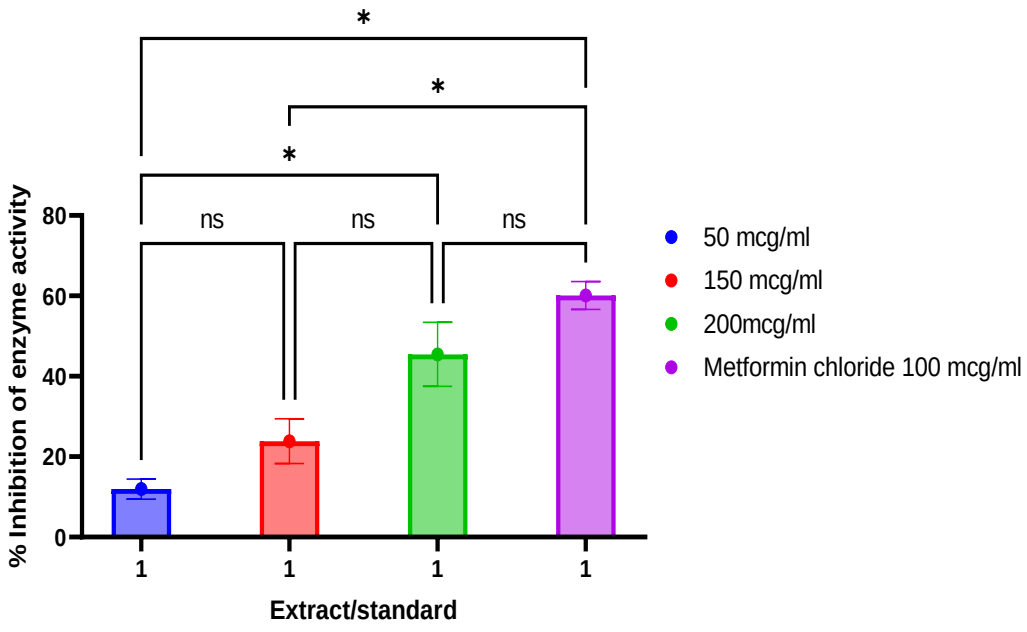
The results for the ant diabetic activity of *Zingiber officinale* powder are presented in table 4.2 and figure 4.4. The ability of the extract to inhibit the activity of the alpha amylase enzyme was evaluated at three concentration levels 50 mcg/ml, 150

mcg/ml and 200 mcg/ml and metformin at concentration level of 100 mcg/ml as the standard ant diabetic drug. The results showed that the standard drug recorded the highest percentage inhibition of 60.075±3.475 % followed study sample at 200 mcg/ml, 150 mcg/ml and 50 mcg/ml which recorded percentage inhibition of 45.465±7.965 %, 23.850±5.560 % and 11.940±2.520 % respectively (table 4.2).

**Table 4. 2 Percentage inhibition of alpha amylase enzyme**

| Extract | % INHIBITION |
|---|---|
| 50 mcg/ml | 11.940±2.520 |
| 150 mcg/ml | 23.850±5.560 |
| 200 mcg/ml | 45.465±7.965 |
| Standard (metformin 100 mg/ml) | 60.075±3.475 |

**Figure 4. 4 Percentage inhibition of alpha amylase enzyme**



### 4.3 Discussion

Free radicals are very beneficial under normal levels that do not surpass the levels of antioxidants. Their generation is mainly through the normal physiological processes such as metabolism in the body. During this processes they are produced as by products. However on the high levels of the free radicals they cause negative impact that include damage on the biomolecules include carbohydrates, proteins and nucleic acids. The high concentration of the free reactive oxygen species causes oxidative stress a main influencer in the pathogenesis of many chronic conditions such as diabetes. Finding the agents with ability to scavenge free radicals will be relieve for the diabetes patients as pathogenesis of diabetes is as well mediated by the reactive oxygen species.

Similarly, alpha amylase and alpha glucosidase are key enzymes in carbohydrate metabolism found in the small intestine. These enzymes converts polysaccharides to monosaccharaides that are present in the consumed carbohydrates. These monosaccharaides include to glucose which is elevated in the blood as a result of its absorption in the small intestine. The agents that are able to inhibit the activity of these agents are very beneficial in control of diabetes.

In the present study the antioxidant activity, total phenolic, total flavonoid and ant diabetic activity of *Zingiber officinale* powder was evaluated in this study. The results showed high total phenolic and flavonoid content in the studied samples. This phytochemicals are beneficial as antioxidants hence can be potent ant diabetic as well. The *Zingiber officinale* powder showed high showed moderate inhibition of the alpha amylase enzyme activity that was seen by the digestion rate of the starch. The ant diabetic activity was as well in adores dependent manner with the

higher dose recording the highest inhibition. However, the inhibition of the extract was lower than that of the standard commercially available ant diabetic drug.

# CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

## 5.1 Conclusion

Diabetes is causing much discomfort among many people including both family and friends. The available drugs are as much more expensive hence not readily available to the majority of the people. The side effects as well as the reduced potency has nailed the last stop on their prioritization by many people. The alternatives from plants are slowly gaining popularity among people. Their characteristics that include easy availability, less costly, safe and high efficacy has contributed to their use. The current study, evaluated the antioxidant and ant diabetic activity of *Zingiber officinale* powder. The extract was able to inhibit the activity of alpha amylase enzyme. The high activity can be attributed to the high concentration of total phenolic and flavonoid content. These phytochemicals are best known for their different pharmacological including antioxidant and ant diabetic activity.

## 5.2 Recommendation

From the study, the following recommendation can be made;

I. In vivo ant diabetic activity of *Zingiber officinale* powder be evaluated

II. Further in vitro- ant diabetic activity be evaluated

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