

In Vitro Inhibitory Effect of Methanol Leaf and Stem Extract of *Leptadenia hastata* on α -Amylase

Ananias Amen ¹; Yusufu Dawoye ²; Asif Bakari Bala ³; Faisal Mohammed Gambo ⁴; and Bako Jaben Peter ⁵

^{1,2,4}Department of Biochemistry/Chemistry, Federal Polytechnic Bali, Nigeria. ³Department of Biological Sciences, Federal Polytechnic Bali, Nigeria. ⁵Department of Agricultural Technology, Taraba State Polytechnic, Suntai.

Corresponding author: amenananas@gmail.com

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Abstract

Medicinal plants play a significant role in the treatment of Non-Communicable Diseases such as diabetes mellitus, being a serious metabolic disorder. *Leptadenia hastata* is one of those plants used traditionally in Northern Nigeria and around the world for treatment and management of diabetes mellitus. This research aims to determine the invitro inhibitory effect of methanol leaf and steam extract of *Leptadenia hastata* on α -amylase. Plant parts (leaf and stem) were collected, dried under shed. Extraction was done by cool maceration. The enzyme activity assay was determined by DNSA (3, 5-Dinitrosalicylic acid) method and Activity value obtained at $1.75\mu\text{mol}^{-1}\text{min}^{-1}$. Enzyme was characterized, optimum pH and temperature of 5 and 35°C were obtained respectively. The inhibitory effects of methanol stem extract on porcine pancreatic α -amylase was highest at the concentration of 1.0mg/ml and 1.2mg/ml with a percentage inhibition of 25% and 20%. The methanol leaf extract showed inhibition of 20.99% and 22.36%. The synthetic drug (metformin) showed highest percentage of inhibition at 68.75%. *Leptadenia hastata* extract has the potential of controlling blood sugar level. However, the synthetic drug seems to perform better than the leaf and stem extract. The use of both plant parts could show incredible inhibitory activity.

Keywords: α -Amylase, Methanol, Extract, Activity, Plant.

Introduction

Green plants from ancient times have been found useful in the treatment of different ailments even before the advent of modern drugs. These plants, called medicinal plants are so named because of their valuable interventions in the treatment and management of many health challenges traditionally. The scientific proof of traditional claims has assisted in the advancement of modern medicine as it is today (Hazurah *et al.*, 2021). Plant-based drugs has been used in many cultures around the world to treat many diseases including Diabetes Mellitus (DM) (Yedjou *et al.*, 2023). Many plants have been traditionally recommended for the treatment of DM because of the presence of bioactive compounds like alkaloids such as quinine, caffeine, cocaine and other forms of flavonoids with

hypoglycemic potentials (Chikezie *et al.*, 2015). These plants have little, or no side effects and a lot of existing medicines are derived from the plants (Kooti *et al.*, 2016).

Diabetes mellitus is a grave and chronic metabolic disorder, linked with serious complications like cardiovascular diseases and kidney disorders (Yedjou *et al.*, 2023). Diabetes Mellitus was the root cause of 1.5 million deaths of adults and 48% of deaths occurred before the age of 70 years. Approximately 463 million adults globally have diabetes and global prevalence stands at 9.3% and could rise to 10.9% by 2045 (Khadayat *et al.*, 2020; Willcox *et al.*, 2021). It is also becoming more common among children and adolescents (Vivo-barrachina *et al.*, 2022). In diabetes mellitus, the beta cells of the pancreas fail to produce little, or no insulin and diagnosis is done by observing elevated levels of glucose in the blood (Khadayat *et al.*, 2020). The control of postprandial hyperglycemia is could be an effective clinical strategy for the treatment of diabetes mellitus (type-2- diabetes). Carbohydrate-hydrolyzing enzymes like α -amylase (from saliva and pancreas) and α - glucosidase (in epithelium of the small intestine) are inhibited. Both synthetic drugs and plants extracts plays inhibitory role against these enzymes (Ibrahim *et al.*, 2017). Due to sight effect evident in the used of synthetic drugs such as Metformin, acarbose and miglitol and the cost involved, the use of medicinal plants has become a good alternative because of their low cost, safety, likelihood of high compliance and low incidence of undesirable side effects.

Statement of the Problem

Diabetes mellitus is prevalent among younger and older adults. Treatment and control of those conditions has proven difficult and complicated. The use of drugs has proven not so effective in some persons, while affordability of such drugs is another challenge. Therefore, many have resulted in taking herbs to bring the situation under control and one of such herbs basically used in the northern part of Nigeria is *Leptadenia hastata* (L. *hastata*). The veracity of such claim is so important to be proven scientifically.

Aim and Objectives

The aim of this research is to determine the *invitro* inhibitory effect of methanol leaf and stem extract of *Leptadenia hastata* on α -amylase.

Specific Objectives

- i. To characterize the α -amylase (enzyme).
- ii. To determine the inhibitory effect of the steam extract on the enzyme.
- iii. To determine the inhibitory effect of the leaf on the enzyme.

Materials and methods

Chemicals and Materials

Glacial acid, NaOH pellets, Sodium acetate, concentrated by hydrochloric acid, starch (from Kermel), Porcine Pancreatic α -amylase (Sigma), 3, 5 dinitrosalicylic Acid (KEM LEGHT), Metformin 500mg (ZANZA Health care).

Plant Collection and Preparation

The leaves and stem of *leptadenia hastata* was collected at the permanent side of the federal polytechnic Bali. The sample (Leave and stem) of *leptadenia hastata* was collected and dried under shed at room temperature, avoiding exposure to sunlight. Dried sample was grounded into powder using pestle and mortar. The powdered plant was stored in a closed container for use.

Extracts Preparation

The method by (Khadayat *et al.*, 2020) was adopted in extract preparation. The powdered leave 40g and stem 30g of *leptadenia hastata* was soaked for 72 hours (3 days) in 200ml of methanol separately. It was then filtered by muslin cloth and collected in a conical flask. Then the filtrates were again filtered through Whatman filter paper (No. 1) and were concentrated by evaporation evaporated using hot plate.

Porcine Pancreatic α -Amylase Activity Assay

Method used in the activity assay is DNS method according to Anita and Muralikrishna, (2009). One percent (1%) starch was prepared by adding 1g in 100ml distilled water. Nine Hundred and eighty microliters (980 μ l) of the substrate (starch solution) were introduced in a test tube and 20 μ l of the enzyme was added. It was incubated at 35°C for 10min. the reaction was stopped by the addition of one milliliter (1ml) of DNS reagent. The mixture was further heated in boiling water for 10mins, cooled to room temperature and absorbance was taken 540nm. The activity was calculated using the formula below:

Enzyme activity = moles of substrate converted to product per unit time =

$$\frac{\Delta A/min}{\epsilon b} \times \frac{Vt}{Vs} \times 10^6$$

$\Delta A/min$ = Average absorbance change per minutes

ϵ = molar absorptivity of substrate

b = light path

Vt = Total assay of volume in ml

Vs = volume of specimen in ml

10^6 = factor to convert moles to micromoles

Characterization of the Enzyme

The enzyme was characterized as a function of pH and Temperature, since enzymes perform optimally at a given temperature and pH among other factors.

Determination of Optimum pH

Fifty millimole (50mM) of sodium acetate buffer was prepared such that a pH of 3.5-6.0 were obtained. The pH ranges were adjusted by the addition of either 0.1M NaOH or 0.1HCl. Five hundred microliters (500 μ l) of the enzyme in 500 μ l of each buffer was determined after the addition of 1ml of substrate. A graph of absorbance against each pH will be determined (Ritu *et al.*, 2017)

Determination of Optimum Temperature

Activity was determined at Temperature range of 20, 25, 30, 35, 40, 45 50 all in Degree Celsius. Five hundred microliters (500 μ l) of the enzyme in 500 μ l of sodium acetate buffer (pH=4.90) was subjected to different temperature in a water bath. The temperature at which the enzyme exhibits highest activity was taken as the optimum temperature. Absorbance was measured at 540nm (Ritu *et al.*, 2017).

Inhibition Assay**Leaf and Stem Extract**

Point two grams (0.2g) of both leaf and stem extracts of *leptadenia hastata* were dissolved in 100mls of water to obtain a stock of 2.0mg/ml. concentrations of 1.0mg/ml, 1.2mg/ml, 1.3mg/ml, 1.4mg/ml and 1.5mg/ml were obtained from the stock. Two hundred microliters (200 μ l) of the enzyme prepared in the buffer was incubated with different concentrations of the extracts (leaf and stem extracts) at 35°C for 10min. After pre incubation, 500 μ l of 1% starch solution was introduced into the test tubes containing different concentrations of the extracts mixed with the enzymes. After incubation of the mixture at 35°C for 10min, reaction was stopped by the addition of 1ml of the DNS reagents. It was then incubated in boiling water for 5min. Absorbance was measured at 540nm, and the values taken in triplicates (Anita and Muralikrishna, 2009).

Synthetic Drug

Five hundred milligram (500mg) of the synthetic drug (Metformin) was dissolved in 100mls of water to obtain a stock of 5.0mg/ml. concentrations of 1.0mg/ml, 1.2mg/ml, 1.3mg/ml, 1.4mg/ml and 1.5mg/ml were obtained from the stock. Two hundred microliters (200 μ l) of the enzyme prepared in the buffer was incubated with different concentrations of the drug (Metformin) at 35°C for 10min. After pre incubation, 500 μ l of 1% starch solution was introduced into the test tubes containing different concentrations of the drug mixed with the enzymes. After incubation of the mixture at 35°C for 10min, reaction was stopped by the addition of 1ml of the DNS reagents. It was then incubated in boiling water for 5min. Absorbance was measured at 540nm, and the values taken in triplicates (Anita and Muralikrishna, 2009).

Statistical Analysis

The results were analyzed using simple percentage inhibition.

Results and Discussion

Results

Activity value obtained is $1.75\mu\text{mol}^{-1}\text{min}^{-1}$. This means the enzyme converted $1.75\mu\text{mol}^{-1}\text{min}^{-1}$ of the substrate (starch) to product per minutes. This means Porcine Pancreatic α -amylase hydrolyzed the conversion of starch into its simple repeated unit which is glucose. The optimum pH and Temperature of porcine pancreatic α -amylase is provided in figure 1 and figure 2 respectively. The enzyme performed optimally at pH 5 after subjecting it to a range of pH values between 3.5-6. The enzyme also showed high activity at 35°C , among ranges of temperatures between 20°C - 50°C .

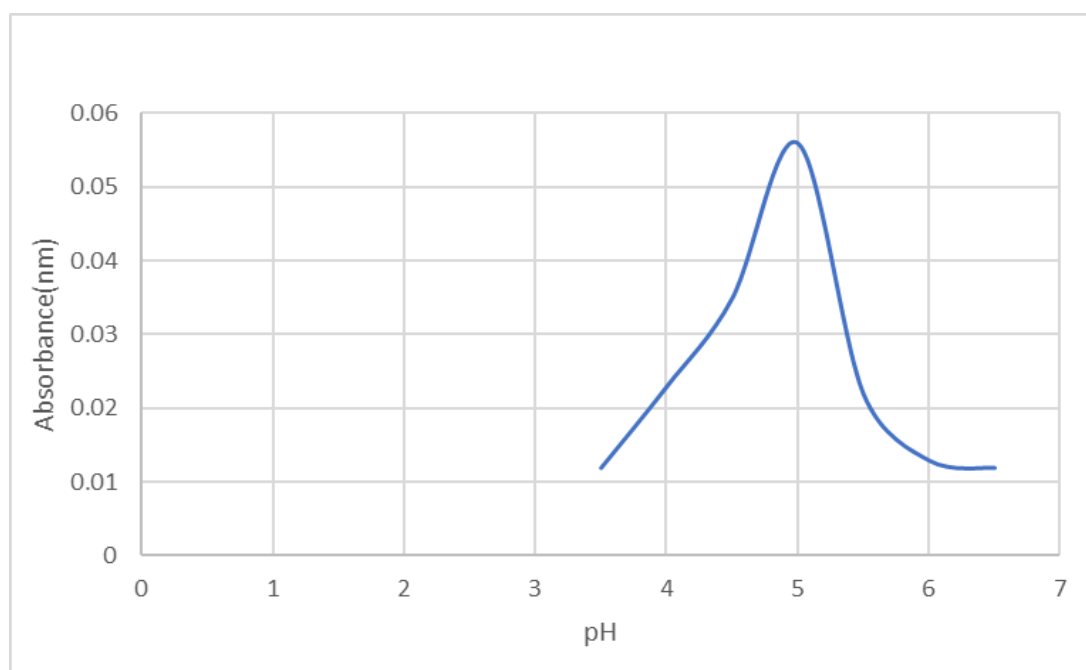


Figure 1: Optimum pH for Porcine Pancreatic α -amylase

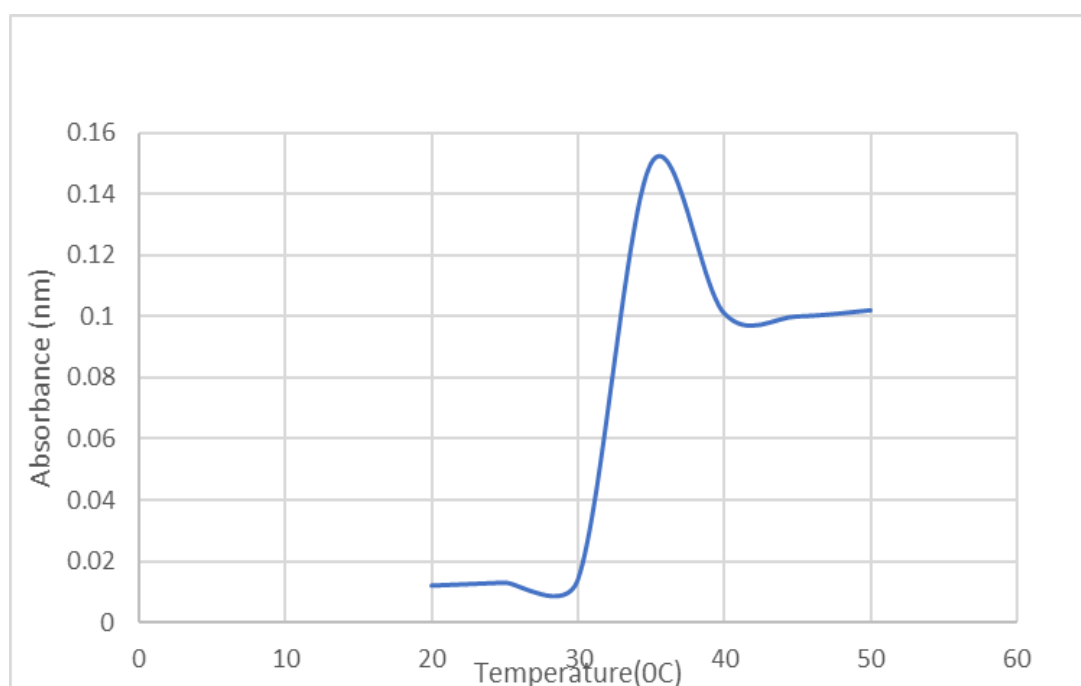


Figure 2: Optimum Temperature for Porcine Pancreatic α -amylase.

The inhibitory activity of the stem extract of *L. hastata* is shown in figure 3. The result indicates that the lowest concentration of the extract (0.1mg/ml) had the highest inhibition of 29.9%. While higher concentration of 1.5mg/ml has percentage inhibition of 17.7%. The result showed decrease percentage inhibition with increase extract concentration.

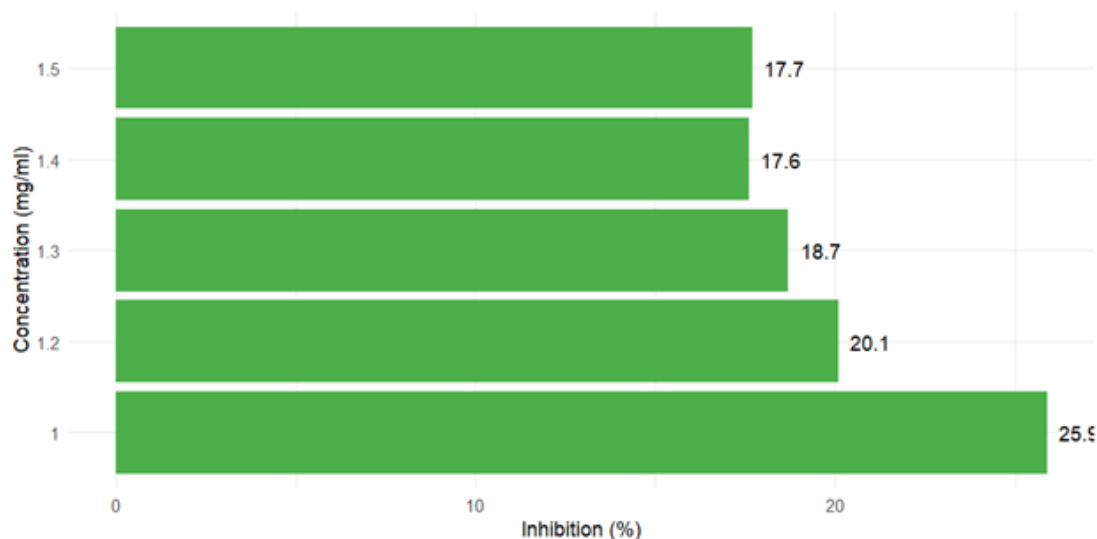


Figure 3: Percentage inhibition of the methanol leaf extract of *L. hastata*

The inhibitory activity of the leaf extract of *L. hastata* is presented in figure 4. The result showed that the concentration of 1.3mg/ml had the highest inhibition (22.36%), slightly

above the concentration of 1.2mg/ml (20.99%). Moderate variation in inhibition is visible across the different concentrations.

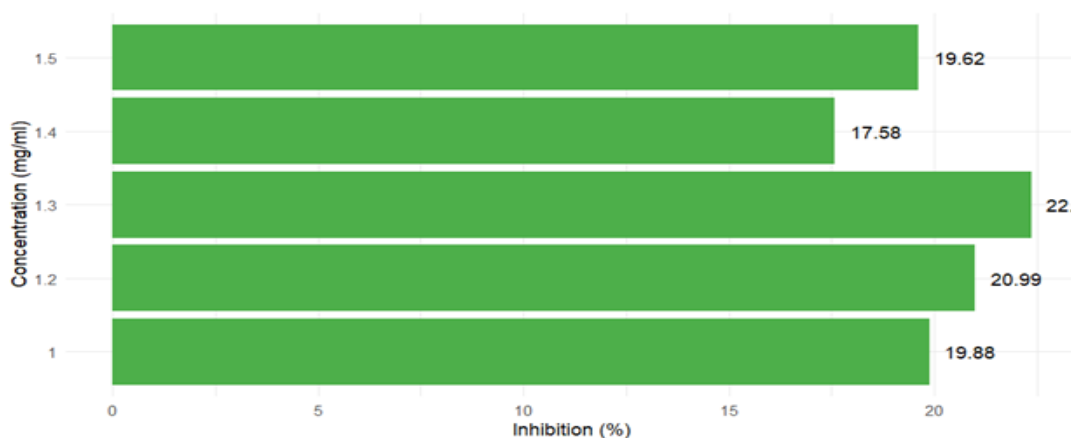


Figure 4: Percentage inhibition of the methanol leaf extract of *L. hastata*

The inhibitory effect of Standard drug (metformin) is displayed in figure 5. The result showed the standard drug exhibiting the highest inhibitory effect at the concentration of 1.4mg/ml (68.75%). There is a noticeable variation across the different concentrations in term of their inhibitory potentials.

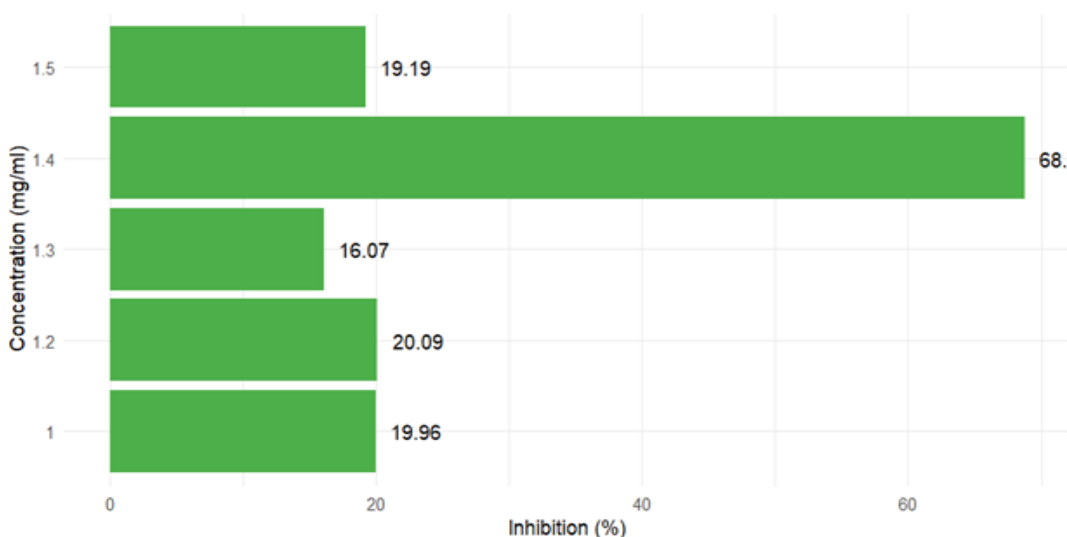


Figure 3: Percentage inhibition of Standard drug (metformin)

Discussion

Whole plants or its parts have been involved right from ancient time to modern time in treatment of different diseases. One of the diseases that have pose a serious challenge to

human existence is diabetes mellitus, induced by impaired pancreatic tissues, drugs, microorganisms and diets. Postprandial hyperglycemic condition without metabolic system in place is unhealthy for the body. The activities of Carbohydrate metabolizing enzymes like α -amylase need to be checked in a dysfunctional insulin production. Chemical compounds, either organic or inorganic, taken to control the actions of metabolizing enzymes acts as inhibitors. In the current study, it was found that the leaf and stem of *L. hastata* has the potential of inhibiting the activity of α -amylase, though slightly lower than the synthetic drug (metformin). The use of *L. hastata* in the treatment of diabetes mellitus agrees with (Kabir *et al.*, 2016) findings who examined the Inhibitory Intestinal Glucose Uptake and Antioxidant *In-Vitro* Potentials of Aqueous Leaf Extract of *Leptadenia hastata* (Pers). The methanol stem extract of *L. hastata* (Figure, 3) has the potential to inhibit α -amylase activity as observed in the trend of inhibition. Although no literature has reported the inhibitory activity of the stem extract. Methanol leaf extracts of *L. hastata* has inhibitory activity against α -amylase (Figure 4). The antidiabetic effects of leaf extract of *L. hastata* has been reported by (Attah *et al.*, 2019; Kabir *et al.*, 2021 & Chukwuma *et al* 2022). The leaf of *L. hastata* has proven to be a promising remedy for managing diabetes mellitus. The use of synthetic drug like metformin and acarbose to control diabetes mellitus is common. They act as inhibitors to carbohydrates metabolizing enzymes. In this research, metformin is used as control (Figure 5). It showed remarkable inhibitory activity against α -amylase. The synthetic drug has showed more inhibitory action against the enzyme than the stem and leaf extract.

Conclusion

According to the result of the study on the methanol leaf and stem extracts of *L. hastata* on α -amylase, the crude methanol extract showed a remarkable inhibitory activity on the enzyme. The leaf and stem of *L. hastata* has the potential to be used in controlling and treatment of diabetes mellitus. Furthermore, research is needed in isolating the compounds responsible for the inhibitory action on the enzyme from both the stem and leaf.

Recommendation

Furthermore, research is needed in isolating the compounds responsible for the inhibitory action on the enzyme from both the stem and leaf.

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