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Research Article

In-Vitro Antidiabetic Effect of *Ziziphus mucronata* Leave Extracts

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Abstract

Background: Diabetes is a metabolic disorder characterized by hyperglycemia due to the body's inability to produce insulin or inaction of the produced insulin or a combination of both. One antidiabetic therapeutic approach is to reduce gastrointestinal glucose production and absorption through the inhibition of carbohydrate digesting enzymes such as alpha-amylase as well as through the inhibition of hemoglobin glycosylation.

Objective: This study sets out to evaluate the *in vitro* antidiabetic activity of *Ziziphus mucronata* extracts for their effect on alpha-amylase and glycosylation of hemoglobin.

Methods: Successive gradient maceration of *Z. mucronata* leaves were carried out using Hexane, Acetone, Methanol and separately with water to obtain four (4) extracts labelled HE, AE, ME, and WE respectively. These were subjected to *in vitro* studies for their inhibitory effect on alpha-amylase and hemoglobin glycosylation, Standard laboratory methods were used to screen for phytochemicals of the most potent extract.

Results: The result showed that AE, ME and WE extract exhibited a dose-dependent increase in percentage inhibition of both alpha-amylase and hemoglobin glycosylation. However, on a striking note, the AE showed a more potent data result with percentage (%) potency of 71.02 at 1mg/ml, the lowest glucose concentration (of 25mg/ml) at 0.242nm as well as the highest hemoglobin glycosylation inhibitory mean concentration of 3.663nm after 72 hours. The AE of *Z. mucronata* (the most potent) revealed the presence of alkaloids, anthraquinone, glycosides, flavonoids, phenols, saponin, tannins and terpenoids.

Conclusion: Thus, the Acetone extract is more likely to give a lead antidiabetic drug molecule of drug when further explored; which somewhat justify the folkloric claims of *Z. mucronata* leave as an antidiabetic.

Keywords: Hemoglobin glycosylation, Alpha-amylase enzymes, Inhibition, Glucose.

1.0 INTRODUCTION

A global prevalence of diabetes mellitus and impaired glucose tolerance in adults has been on the rise in recent years. The rate at which the prevalence of diabetes mellitus changes in many countries and regions has been enhanced by rapid urbanization and the increasing tendencies for sedentary lifestyle. According to the world health organization, about 108 million people were estimated to be diabetic in 1980. This estimate rose to 422 million individuals in 2014 (Cho *et al.*, 2018). In Nigeria, the total pooled prevalence of diabetes mellitus was 5.77%. In the six geopolitical zones of Nigeria, the pooled prevalence of diabetes mellitus indicates that the north-west zone is placed at 3.0% (95% CI 4.3-7.7), the north-east zone at 5.9% (95% CI 2.9-9.4), the north-central zone at 3.8% (95% CI 4.0-7.1), the south-west zone at 5.5% (95% CI 2.4- 9.4), the south-east zone at 4.6% (95% CI 3.4-5.9), and the south-south zone at 9.8% (95% CI 7.2-12.4) (Uloko *et al.*, 2018). There is an absence of effective cure for diabetes mellitus at the moment and available drugs and insulin therapy used for

the management of the disease are associated with several adverse effects (Chikezie *et al.*, 2015). Thus, the adverse effects and high cost of anti-diabetic drugs has led to the search for medicinal plants that exhibit hypoglycaemic property, with a view to applying them for the management of diabetes mellitus (Ali *et al.*, 2006).

Z. mucronata is an important multi-purpose plant. The genus *Ziziphus* comprise of approximately 135 plant species, appearing as a spiny shrubs or trees, mostly found in Indo-Malayan arid region, while few others are found in Africa, Australia, America and the subcontinent of South Asia (Mhaskar *et al.*, 2000). Biologically, *Ziziphus* species are known to possess various important pharmacological activities including antimicrobial (Najafi *et al.*, 2013), antioxidant and anti-inflammatory properties, antidiabetic, anti-malarial and anthelmintic properties, anticancer, antiulcer, analgesic, sedative and antipyretic effects amongst other recorded activities (Madara *et al.*, 2010). The species are generally not toxic and safe for both human and animal consumption (Al-Saedi *et al.*, 2017). Members of the genus

are known to possess a large number of cyclopeptide alkaloids, flavonoids, tannins, saponins, terpenoids, fatty acids, sterols and a wide variety of phenolic compounds (Modi *et al*, 2014). Thus, the long list of bioactivities reported for this plant as well as the folklore claims of antidiabetic properties in the leaves, prompted this study. Hence the need to further scientifically validates and establish it antidiabetic potency.

2.0 MATERIALS AND METHODS

2.1 Drug, Chemicals and Reagents

Gentamycin (Merck, Germany) was purchased from pharmaceutical store. Organic solvents used for extraction of plant material included n-hexane (C₆H₁₄), Acetone (C₄H₈O₂), Methanol (CH₃OH), all are of analar grade (Sigma Aldrich, Germany). Enzyme (α-amylase) and substrates (Potato starch and p-nitro phenyl α-D glucopyranoside) were purchased from Sigma Chemical Co St. Louis M.O., (USA).

2.2 Apparatus and Equipment

General laboratory glass ware including volumetric flask, measuring cylinder, beakers, pestle and mortar, filter papers, test tubes, micro pipette, weighing balances and water bath, were used in the study.

2.3 Plant Material

Fresh leave of *Z. mucronata* (Kurna) were collected from a farmland around Kwatarkwashi village in Zamfara state in January, 2020. The plant sample was authenticated at the Herbarium, Botany unit of Department of Biological science of the Usmanu Danfodiyo University, Sokoto, Nigeria. A voucher number, UDUSH/ANS/0831 was used and the plant specimen was deposited for referencing.

2.4 Successive extraction of *Z. mucronata* leaves.

Fresh leave of *Z. mucronata* were rinsed in tap water, air-dried at room temperature, and were crushed into small pieces, these were then grounded and homogenized into a fine powder using pestle and mortar. The finely ground air-dried leave (200 g) was successively extracted using solvents of increasing polarity (1500mls each) (Hexane, Acetone, Methanol and separately with Water). Thus, 200g of the fine powder was firstly extracted twice with hexane for 48 hours. The combine extracts were filtered and the filtrate concentrated using rotary evaporator. The mac obtained from this was then dried and further extracted twice (72 hours each) with acetone and again the combined extracts was filtered and concentrated. The mac obtained from filtration of the acetone was then extracted twice (72 hours each) with methanol and the combine extracts was filtered and concentrated (John *et al.*, 2018). The water extract was similarly prepared in the same way but using fresh powdered plant material of 200g. Consequently, four resulting extract residues were obtained and labelled as HE, AE, ME and WE for hexane extract, acetone extract, methanol extract and water extract respectively. These were kept separately at 4°C until when needed.

2.5 In vitro Antidiabetic effects of *Z. mucronata* leaves.

2.5.1 Alpha-amylase inhibitory activity (Enzymatic)

The effect of the HE, AE, ME, and WE of *Z. mucronata* extracts on alpha amylase activity (EC 3.2.1.1) was determined according to the method described by Rammohan *et al*, 2008. The enzyme solution was prepared by dissolving α-amylase in 20mM phosphate buffer (6.9) at the concentration of 5mg/ml. 1ml of the extract of various concentrations (5, 10, 15, 20 and 25) mg/ml and 1ml of

enzyme solutions were mixed and incubated at 25°C for 10min. After incubation, 1ml of starch (0.5%) solution was added to the mixture and further incubated at 25°C for 10min. The reaction was stopped by adding 2ml of dinitrosalicylic acid (DNS, color reagent), and heating the reaction mixture in a boiling water bath for 5min. After cooling, the absorbance was measured calorimetrically at 565 nm and the inhibition percentage was calculated using the formula, below;

$$\text{Percentage inhibition} = \frac{1 - \text{absT}}{\text{abs C}} \times 100.$$

2.5.2 Inhibitory Concentration (IC₅₀) of alpha-amylase by AE (most potent).

The concentration of the plant extracts required to inhibit 50% of enzyme (IC₅₀) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by

$$I \% = \frac{(A_c - A_s)}{A_c} \times 100, \text{ (Shai } et al, 2010).$$

where **Ac** is the absorbance of the control and **As** is the absorbance of the sample.

2.5.3 Hemoglobin glycosylation (Non-enzymatic)

To measure hemoglobin glycosylation, 1ml each of Glucose (2%) hemoglobin (0.06%) and Gentamycin (0.02%) in phosphate buffer 0.01M at pH 7.4. were taken and mixed in a test tube. The methanol extract was weighed and dissolved in DMSO to obtain stock solutions of 5 µg/ml. Thereafter 1 ml of each concentration was added to the above mixture. The Mixture was incubated in dark at room temperature for 72 hrs. The degree of glycosylation of hemoglobin was measured calorimetrically at 520 nm (Adisa *et al*, 2004). Metformin was used as a standard drug for assay and % inhibition was calculated using the formula:

$$\% \text{ inhibition of glycosylation} = \left(\frac{Abs_{sample} - Abs_{control}}{Abs_{sample}} \right) \times 100$$

Where Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample implies absorbance of the test sample.

2.5.4 Inhibitory Concentration (IC₅₀) of hemoglobin glycosylation.

The concentration of the plant extracts required to inhibit 50% of glycosylation (IC₅₀) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated using the equation;

$$I \% = \frac{(A_c - A_s)}{A_c} \times 100 \text{ (Shai } et al, 2010).$$

where **Ac** is the absorbance of the control and **As** is the absorbance of the sample.

3.0 RESULTS AND DISCUSSION

The percentage yield of the extracts was found to be 1.3, 4.9, 8.8 and 6.4grams for HE, AE, ME and WE respectively. The AE of *Z. mucronata* (most potent) revealed the presence of phytochemicals such as flavonoids, tannins, terpenoids, phenols, anthraquinone and alkaloids.

3.1 Hemoglobin glycosylation effect of *Ziziphus mucronata* leave extracts.

The plant extracts except n-hexane significantly inhibited the haemoglobin glycosylation (P<0.05) which is indicated

by the presence of increasing concentration of haemoglobin (Table 1). Acetone extract of *Ziziphus mucronata* (AEZM) exhibited higher inhibition of glycosylation as compared with the standard Metformin. The plant extracts also displayed the inhibition of haemoglobin glycosylation at different physiological concentrations of the glucose over the period of 72 hours, indicating that the plant extracts decrease the formation of the glucose- haemoglobin complex and thus amount of free haemoglobin increases.

3.2 Alpha-amylase inhibitory Effect of *Ziziphus mucronata* leave extracts.

The administration of four extracts of *Ziziphus mucronata* indicated that; all four extracts except n-hexane exhibited a dose dependent increase in alpha-amylase inhibitory activity. At concentration of 1.0 mg/ml, AEZM (most potent extract), is 71.02% while Voglibose (standard drug), is 83.47 (Table 2). And also, the IC₅₀ of AEZM is 0.62 while that of voglibose is 0.42 (figure 1 and 2).

Table 1. Effect of EAZM on hemoglobin glycosylation.

Period	Conc. (mg/ml)	Metformin	nEZM	AEZM	MEZM	WEZM
24hours	5	0.712±0.51 ^{ab}	0.533±0.02 ^{ab}	0.670±0.51 ^{bc}	0.548±0.23 ^b	0.701±0.41 ^{bc}
	10	1.124±1.25 ^{ab}	0.487±2.34 ^b	1.664±2.42 ^b	1.336±3.47 ^{bc}	0.704±1.23 ^{bc}
	15	1.291±0.16 ^{ab}	0.566±2.42 ^{bc}	1.924±1.52 ^{ab}	1.356±2.34 ^{ab}	0.712±0.18 ^b
	20	1.637±1.84 ^{ab}	0.655±2.54 ^{ab}	2.703±3.61 ^b	2.043±3.61 ^{ab}	0.841±2.31 ^{ab}
	25	1.830±4.21 ^b	0.767±3.46 ^{bc}	3.35±2.42 ^{bc}	3.221±0.81 ^{ab}	0.871±1.56 ^{bc}
48hours	5	0.771±1.28 ^c	0.533±2.48 ^{bc}	1.035±3.12 ^b	0.443±2.31 ^{bc}	0.697±3.12 ^{ab}
	10	1.059±2.41 ^{ab}	0.487±4.21 ^c	1.674±3.14 ^{bc}	1.385±3.14 ^{ab}	0.811±2.54 ^b
	15	1.364±4.23 ^{bc}	0.566±3.24 ^{ab}	2.037±2.15 ^{ab}	1.769±2.34 ^{bc}	0.812±3.12 ^{ab}
	20	1.635±0.61 ^b	0.655±2.42 ^{ab}	2.706±1.84 ^b	2.241±3.24 ^{ab}	0.861±2.41 ^{bc}
	25	1.935±1.26 ^{ab}	0.767±1.54 ^{bc}	3.951±2.81 ^{bc}	2.520±2.48 ^b	0.791±1024 ^{ab}
72hours	5	0.742±0.84 ^{ab}	0.451±0.94 ^{bc}	0.845±1.35 ^{ab}	0.500±0.69 ^{ab}	0.855±0.97 ^b
	10	1.092±2.42 ^{bc}	0.408±3.21 ^{bc}	1.669±2.45 ^b	1.360±0.96 ^{bc}	0.821±1.58 ^{ab}
	15	1.328±3.15 ^{ab}	0.495±2.45 ^{bc}	1.974±3.15 ^{ab}	1.563±3.15 ^{bc}	0.799±2.34 ^{ab}
	20	1.636±1.65 ^{ab}	0.653±2.34 ^{bc}	2.705±2.35 ^{ab}	2.142±3.15 ^{ab}	0.787±2.47 ^b
	25	1.883±0.95 ^a	0.720±1.58 ^{ab}	3.663±3.15 ^{bc}	2.617±1.85 ^{ab}	0.881±3.51 ^{bc}

Values are expressed as mean ± SEM, values having the same superscript are not significantly different at (P<0.05). analyzed using one-way anova of n=3

nEZM= n-hexane extract of *Ziziphus mucronata*, AEZM= acetone extract of *Ziziphus mucronata*, MEZM= methanol extract of *Ziziphus mucronata* and WEZM= water extract of *Ziziphus mucronata*

Table 2: Effect of AEZM on alpha-amylase inhibition % inhibition mean ± SEM

Concentration	Voglibose	nEZM	AEZM	MEZM	WEZM
0.2	37.25±0.15 ^a	05.12±0.23 ^{ab}	23.32±0.23 ^{ab}	20.41±0.54 ^{ab}	19.85±0.45 ^{ab}
0.4	48.26±0.35 ^{bc}	08.35±1.25 ^{bc}	36.53±0.38 ^a	31.58±3.45 ^{ab}	29.12±2.15 ^{ab}
0.6	63.59±0.13 ^b	06.58±3.64 ^{ab}	49.00±0.12 ^b	42.34±2.67 ^{ab}	38.75±1.85 ^{ab}
0.8	70.54±0.34 ^b	05.98±2.87 ^{bc}	62.75±0.38 ^{bc}	50.95±1.82 ^{ab}	45.12±2.15 ^{ab}
1.0	83.45±0.25 ^{ab}	11.58±3.54 ^{ab}	71.02±0.15 ^b	65.43±2.84 ^{ab}	58.75±1.85 ^{ab}

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM. The IC₅₀ value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions. AEZM= Acetone extract of *Ziziphus mucronata*, nEZM= n-hexane extract of *Ziziphus mucronate*, MEZM= Methanol extract of *Ziziphus mucronate*, WEZM= Water extract of *Ziziphus mucronata*.

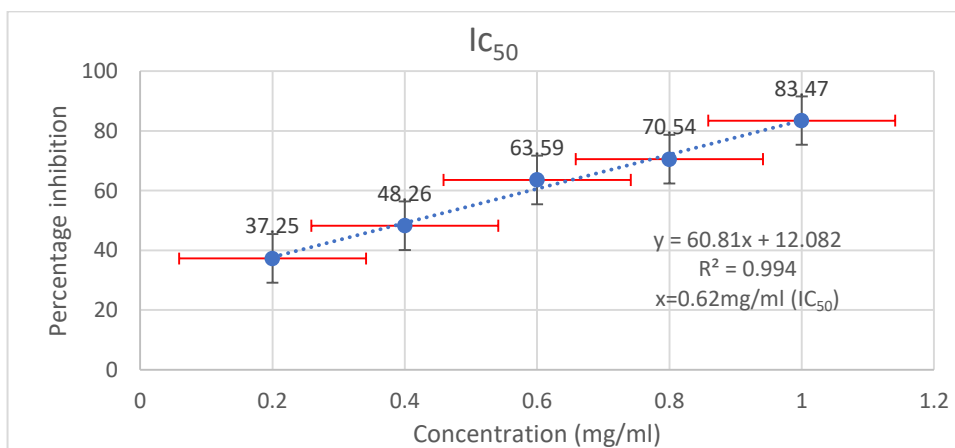


Figure 1: Alpha-amylase Inhibitory concentration (IC₅₀) of AEZM.

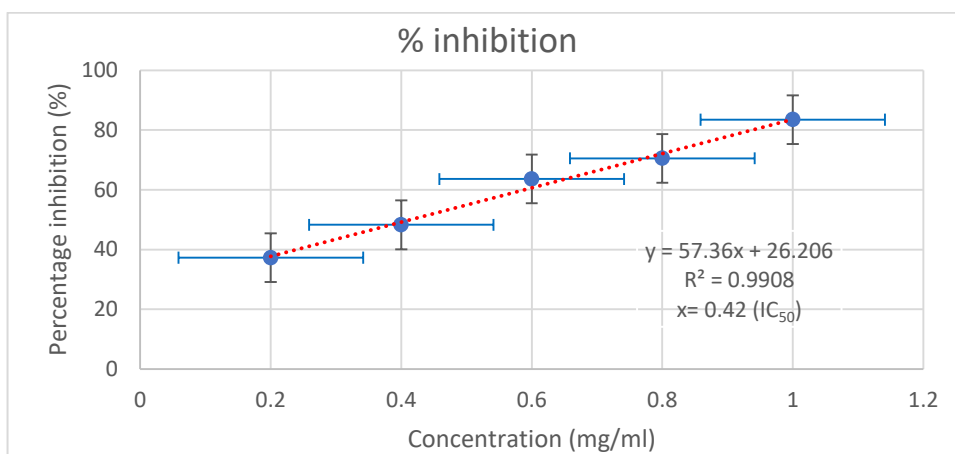


Figure 2: Alpha-amylase Inhibitory concentration (IC₅₀) of standard drug (Voglibose).

Table 3: Phytochemical constituents of Acetone leave extract of *Ziziphus mucronata*.

TEST	RESULTS
Phenols	++
Anthraquinone	+
Tannins	++
Flavonoids	++
Carbohydrate	ND
Terpinoids	+
Saponin	++
Alkaloids	++

Keys: + = Present, ND = Not detected

DISCUSSION

Metabolism of carbohydrates including fat and protein are affected when insulin is lacking, which can lead to homeostatic imbalance (Frier *et al*, 2006). Recent attempts to understand the activity of intestinal enzymes such as alpha-amylase which is important in digestion of carbohydrate and also in the absorption of glucose resulted to the development of recent pharmacological agents. Alpha-amylase inhibitors stop the digestion of carbohydrates and slow down the absorption. Acarbose is competitive inhibitor of alpha-amylase and slows down the absorption of starch and disaccharides (Hengameh *et al*, 2016). therefore, one of the therapeutic approaches for reducing blood glucose levels in diabetic patient is to inhibit the absorption of carbohydrate after meal. Inhibition of this enzyme (alpha-amylase) reduced the high blood glucose in diabetes (Conforti *et al*, 2005). Acarbose is a complex oligosaccharide that delays the digestion of carbohydrates. It inhibits the action of

pancreatic amylase in breakdown of starch. The reaction mechanisms involved in inhibition of alpha-amylase enzymes by plant protein inhibitors are not clearly understood. However, there are some suggestions that the plant protein (flavanols) might cause conformational changes in structure of the enzyme (Hengameh *et al*, 2016). The maintenance of plasma glucose concentration for a long term under a variety of dietary conditions is one of the most important and closely regulated processes observed in human (Srividya *et al*, 2012). Increased concentration of plasma glucose leads to its binding to hemoglobin which may result in the formation of glycated haemoglobin. The *in vitro* assays of the present study indicated that all the plant extracts of *Z. mucronata* except hexane exhibited higher inhibition of alpha-amylase and glycosylation of haemoglobin as compared with the control. The acetone extract of *Z. mucronata* (AEZM) exhibited significantly higher activity than other plant extracts. And haemoglobin glycosylation at different concentrations of the glucose over the period of 72 hours, indicating that the plant extracts decrease the formation of the glucose- haemoglobin complex and therefore the concentration of free haemoglobin increases. Tetracyclic triterpenoids, such as dammarane, cucurbitane, and protostane groups, happens to be class of triterpenoids commonly found in several medicinal plants, especially those commonly used for the treatment of diabetes and its complications, such as *Panax quinquefolium*, *Panax notoginseng*, *Gynostemma pentaphyllum*, and *Ganoderma lucidum* (Kaiser *et al*, 2015). Flavonoids can inhibit some of the complication of diabetes mellitus (Havsteen *et al.*, 2002). Several researches have been carried out to understand the potential role of flavonoid in the

treatment of diabetes (Jung *et al.*, 2006; Matsui *et al.*, 2006; Qi *et al.*, 2010). Flavonoid has been demonstrated using different experimental models of treatments and the drug candidates have been shown to possess beneficial effects against the disease, either through their ability to prevent glucose absorption or to promote glucose tolerance (Zhou *et al.*, 2009). Phenolic compounds are commonly found in plants, and they have been reported to have several biological activities, including antioxidant activity. Several studies have showed that the phenolic content in plants can be linked to their antioxidant activity (Kujala, 2004; Lavelli, 2000). Any of these secondary metabolites either singly or in combination is capable of causing antidiabetic activity of the extract, and many of such compounds are known to possess potent antioxidant activity (Lee *et al.*, 2004).

CONCLUSIONS

In conclusion, *Z. mucronata* possesses alpha-amylase inhibitory potential, preventing the digestion of starch and also inhibited glycosylation of hemoglobin and thereby prevented the formation of glycated end products. Such activities validates the use of *Z. mucronata* in traditional medicine.

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