**Effect of *Justicia Insularis* Leaf Extract on Alpha Glucosidase and Alpha-Amylase Activities in Rats**

**Abstract**

Diabetes mellitus is a global health challenge, necessitating the need for alternative treatments. *Justicia insularis*, a medicinal plant in African traditional medicine, known for its anti-diabetic potential was investigated for its inhibitory potential on alpha-amylase and alpha-glucosidase enzymes of rats.The leaf extract (150,300, and 450 mg/kg) of *Justicia insularis*,was investigated *in vivo* for inhibitory effect on alpha amylase and alpha glucosidase enzymes using starch, sucrose and maltose as substrates. Acarbose was used as reference drug. Blood glucose levels (BGL) of rats, post administration of the substrate and extract concurrently, were monitored over 3 hours as a parameter to measure the inhibitory potentials of the extract. The leaf extract non dose-dependently caused significant (p<0.05) reduction in blood glucose levels of treated rats with the various substrates used. The results suggest that the leaf extract of *Justicia insularis* has the potentials to inhibit alpha amylase and alpha glucosidase in rats. The phytochemicals present in the leaf of *Juticia insularis* may have been responsible for the observed effects.

**K eywords:** *Justicia insularis*, Anti-diabetic, Enzyme inhibition, Alpha-amylase, Alpha-glucosidase, Phytochemicals

**Introduction**

Diabetes mellitus is a complex metabolic disorder characterized by chronic hyperglycemia resulting from insulin deficiency, impaired insulin action, or a combination of both (ADA, 2018). It is a significant global health concern, with increasing prevalence and serious complications such as cardiovascular disease, neuropathy, nephropathy and retinopathy (IDF, 2017). The search for effective and safe anti-diabetic treatments has led researchers to explore medicinal plants and natural compounds as potential therapeutic agents. Inhibition of alpha glucosidase and alpha amylase enzymes remains one of the many significant approaches towards achieving euglycemia in diabetic subjects especially in preventing post- prandial glucose excursions linked to both macro and micro vascular complications.

Alpha-amylase and alpha-glucosidase are major enzymes in carbohydrate digestion and absorption, respectively (Akwador *et al.,* 2021). Alpha-amylase catalyzes the hydrolysis of starch into smaller polysaccharides and maltose in the oral cavity and small intestine (Verma *et al.,* 2018). On the other hand, alpha-glucosidase enzymes, localized at the brush border of the small intestine, further hydrolyze disaccharides to glucose, which can then be absorbed (Ajiboye, 2022). Inhibiting these enzymes can delay carbohydrate digestion and reduce the postprandial rise in blood glucose levels, offering a potential strategy for managing diabetes (Mony, 2023).

*Justicia insularis* T. Anderson (Acanthaceae family) is a vegetable used for both nutritional and medicinal purposes as digestive, weaning agent and laxative (Telefo *et al.,* 2004; Ajibeson *et al.,* 2008; Telefo *et al.,* 2012; Adeyemi and Babtunde, 2014) as well as local malaria remedy in Nigeria and across Africa (Enyiekere *et al.,* 2024a). Extracts of *J. insularis* leaves have been shown to produce estradiol *in vitro* (Telefo *et al.*, 2004), promote ovarian folliculogenesis and fertility in female rats (Telefo *et al.,* 2012), possess anti-oxidant(Adeyemi and Babtunde, 2014), antianaemia(Enyiekere *et al.,* 2024b), anticonvulsant(Elkana *et al.,* 2024), antimalarial activity against rodent malarial parasites, *Plasmodium berghei* (Enyiekere *et al.,* 2024a)and hepatoprotective activities(Wood *et al.,* 2020). Phytochemical compounds such as saponins, alkaloids, tannins, flavonoids, anthraquinones, cardiac glycosides (Telefo *et al.,* 2004; Oyomah *et al.,* 2019 ), and clerodane diterpenoids; 16(α/β)-hydroxy-cleroda-3,13 (14)Z-dien-15,16-olide and 2, 16-oxo-cleroda-3,13(14)E-dien-15-oic acid have been isolated and characterised from the leaf extract (Fadayomi *et al.,* 2021). GC-MS analysis of dichloromethane fraction revealed presence of glyceraldehyde; hexanoic acid; 1,1-dimethylethyl ester; hexanoic acid, butyl ester; hexanoic acid, 2,4-dimethyl-, methyl ester; E-2-tetradecen- 1-ol, oxirane, tetradecyl-; trans-β-ocimene; α-pinene among others, while unsaturated fatty acids such as hexanoic acid; pentanoic acid, 3-methyl-; hexanoic acid, 1,1-dimethylethyl ester; hexadec-9-enoic acid; 7-tert-butyldimethylsilyloxy-, methyl ester; heneicosanoic acid, methyl ester; octa-2,4,6-triene; 1,3,6-heptatriene, 5-methyl-, (E)-; phytol, acetate; octadecanoic acid, 2-hydroxy-1,3-propanediyl ester; octadecanoic acid, docosyl ester and others were found to be present in ethyl acetate fraction(Anyiekere *et al.,* 2024a).

Considering the traditional use of *Justicia insularis* in diabetes management and its documented ethnopharmacological applications, this study aims to explore the anti-diabetic potential of *J. insularis* leaf extract. We specifically focus on evaluating its inhibitory effects on alpha-amylase and alpha-glucosidase enzymes, elucidating the role of its phytochemical composition, and assessing its impact on postprandial blood glucose levels.

**Materials and Methods**

***Collection and identification of plant material***

The plant material *Justicia insularis* (leaves) was collected from the University of Uyo premises, Akwa Ibom State, Nigeria, in March 2023. The plant was identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. A voucher specimen (FPH 83b) of the plant was deposited in the Department of Pharmacognosy and Natural Medicine herbarium at the University of Uyo.

 ***Experimental Animals***

The albino Wistar rats (weighing between 120 - 135 g) both male and female, used for this study were sourced from the Animal House at the Faculty of Pharmacy, University of Uyo, Uyo. The animals were housed in standard cages and were maintained on standard pelleted feed (Guinea Feed) and water *ad libitum.* Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo. All animal experiments complied with the National Institute of Health Guide for Care and Laboratory Animals (pub. No. 85-23, revised 1985).

***Preparation of Extract***

The leaves were washed and shade-dried for two weeks. The dried plant materials were cut into smaller pieces and pulverized using an electrical pulverizer. The 1.5 kg of powdered leaves powder was soaked in 50% ethanol for a duration of 72 hours. Subsequently, the mixture underwent filtration, and the resulting liquid filtrate was then subjected to concentration and evaporation to remove all moisture / solvent under vacuum conditions at a temperature of 40˚C using a rotary evaporator. The sample was preserved in a refrigerator at a temperature of -4˚C until it was used for the intended tests.

 ***In vivo alpha-amylase and glucosidase inhibition study***

***Alpha-Amylase inhibitory study***

Thirty-six Wistar rats were divided into 6 groups of 6 rats each. The rats in all groups were fasted for 18 h and fasting blood glucose concentration was first taken at 0 min before administration. Group I, as the normal control, received distilled water (10 mL/kg). Group II rats were orally administered starch at 2 g/kg body weight) and distilled water (10 mL/kg) simultaneously (with distilled water as vehicle). Rats in group III were administered starch (2 g/kg) and the standard drug (acarbose) at 100 mg/kg simultaneously. Based on previously determined LD50 and doses, Groups IV, V, and VI were administered with starch (2 g/kg) and *J. insularis* leaf extract at 150, 300 and 450 mg/kg respectively. All administrations were done orally and blood glucose concentration was monitored at 30, 60, 90, 120 and 180 min (Gidado *et al.,* 2019; Okokon *et al.,* 2023).

***Alpha Glucosidase inhibitory study***

The procedure as described above was used for this study but with sucrose and maltose used as substrates (Gidado *et al.,* 2019; Okokon *et al.,* 2022).

 ***Blood Glucose Determination***

Drops of blood from tip of rats’ tails were dropped on stripes and glucose concentration was measured using a glucometer according to manufacturer’s specifications (Accu-chek, Indiana). The glucometer works using an electrochemical detection system with the following principle; the biosensor system makes use of disposable dry reagent strip based on glucose oxidase method. Each strip has an electrode impregnated with the enzyme glucose oxidase, which react with glucose in the blood sample when dropped on the membrane covering the reagent pad (strip), to produce gluconic acid. During the reaction, in which electric current is generated, an electrochemical mediator transfers electrons to the electrode surface. This electrode sensor measures the current produced when the enzyme converts glucose to gluconic acid. The magnitude of the generated current is proportional to the amount of glucose present in the drop of blood sample, thus giving an accurate reading of the blood glucose concentration (WHO, 2011).

***Statistical Analysis***

Data obtained from this work were analysed statistically using one –way ANOVA followed by Tukey-Kramer multiple comparison test using Instat Graphpad software, (San Diego, USA). Differences between means were considered significant at 5% level of significance *ie* *p≤ 0.05*.

**Results**

***In vivo Alpha-amylase and glucosidase inhibition assay***

Administration of starch (2 g/kg) to fasted rats caused varying degrees of elevation of blood glucose concentrations of the treated animals after 30 min. The proportions were as follows: starch (62.62%), extract-treated groups (ranging from 4.41% to 14.21%), and acarbose-treated groups (17.97%). The increases were diminished after 60 minutes, with only the groups administered with higher dosages of the extract (300 and 450 mg/kg) having percentage increases of 8.82 and 22.16%, respectively. The average blood glucose levels of all the groups treated with the extract were lowered to normal level after 120 minutes. Nevertheless, after 180 minutes, the groups administered with higher doses of the extract (300 and 450 mg/kg) had percentage increments of 7.65 and 11.37%, respectively. In addition, the concurrent administration of starch and acarbose effectively suppressed the increase in blood glucose levels (Table 1).

Administration of sucrose (2 g/kg) produced a 41.14% increase in blood glucose level 30 minutes post-administration of the sucrose in the control group. BGL increments of 30.58-70.35 % were also recorded in groups treated with 150,300 and 450 mg/kg of extract. At 60 min, percentage increases in BGL of groups treated with 150, 300, and 450 mg/kg of extract were 25.58, 0.41 and 6.38 %, respectively. There was no increment in BGL of all the extract-treated groups from 120 -180 min (Table 2).

There was a 90.94% increase in blood glucose level 30 min following maltose administration in the control group. However, 22.39 - 74.65 % increases in BGL were observed in the extract-treated groups. At 60 min, groups treated with 150,300 and 450 mg/kg extract had percentage increments of 9.64, 41.64, and 64.97%, respectively, while percentage increases of 31.87 and 61.28% were recorded for 300 and 450 mg/kg treated groups at 120 min respectively. At 180 min, only the group treated with the highest dose (450 mg/kg) had a percentage increase of 44.24% (Table 3).

**Table 1.** Effect of ethanol leaf extract of *Justicia insularis* on blood glucose level of rat after oral administration of starch load

|  |  |  |
| --- | --- | --- |
| TREATMENT  | DOSE  | BLOOD GLUCOSE LEVEL mg/dL IN MIN |
|  | mg/kg | 0 min | 30 min | 60 min | 120 min | 180 min |
| Control (normal saline) | - | 86.00±11.53 | 87.66±7.12(1.93) | 87.66±7.62(1.93) | 91.0±7.50(5.81) | 80.00±6.02 |
| Starch |  | 66.0±3.60 | 107.33±6.36a(62.62) | 91.66±2.02(38.87) | 77.66±3.71(17.66) | 70.66±2.72(6.59) |
| Acarbose | 100 | 72.33±2.69 | 85.33±12.97(17.97) | 80.33±7.21(11.06) | 74.0±1.00(2.30) | 72.33±8.68(0) |
| Extract | 150 | 68.0±3.00 | 71.0±2.51(4.41) | 58.66±3.28a() | 59.66±5.23a() | 55.66±0.88a() |
|  | 300 | 56.66±7.68 | 64.0±1.52(12.95) | 61.66±3.33(8.82) | 52.0±4.50b() | 61.0±5.19a(7.65) |
|  | 450 | 58.66±2.72 | 67.0±4.58(14.21) | 71.66±6.93(22.16) | 57.66±6.38a() | 65.33±2.96(11.37) |

Data are expressed as MEAN ± SEM, Significant at ap<0.05, bp< 0.01, compared to control (n=6). Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

**Table 2.** Effect of ethanol leaf extract of *Justicia insularis* on blood glucose level of rat after oral administration of sucrose load

|  |  |  |
| --- | --- | --- |
| TREATMENT  | DOSE  | BLOOD GLUCOSE LEVEL mg/dL IN MIN |
|  | mg/kg | 0 min | 30 min | 60 min | 120 min | 180 min |
| Control (normal saline) | - | 100.00±4.25 | 88.33±1.85 | 92.33±4.25 | 89.0±4.35 | 87.33±3.84 |
| Sucrose | 2000 | 81.0±4.50 | 114.33±5.50b(41.14) | 112.66±1.45a(39.08) | 97.33±1.63(20.16) | 94.15±4.81(16.23) |
| Acarbose | 100 | 90.33±2.48 | 86.66±2.90 | 82.0±6.00 | 71.66±3.75 | 78.0±3.78 |
| Extract | 150 | 75.33±3.28 | 128.33±2.17c(70.35) | 94.60±3.19(25.58) | 54.66±6.88() | 65.00±8.88 |
|  | 300 | 9.33±4.33 | 115.66±7.35b(45.79) | 79.66±9.20(0.41) | 66.00±3.05() | 60.66±8.33() |
|  | 450 | 73.0±4.04 | 95.33±1.45(30.58) | 77.66±6.88(6.38) | 64.66±1.20() | 56.66±2.72() |

Data are expressed as MEAN ± SEM. Significant at ap<0.05, bp< 0.01, compared to control (n=6). Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

**Table 3.** Effect of ethanol leaf extract of *Justicia insularis* on blood glucose level of rat after oral administration of maltose load

|  |  |  |
| --- | --- | --- |
| TREATMENT | DOSE  | BLOOD GLUCOSE LEVEL mg/dL IN MIN |
|  | mg/kg | 0 min | 30 min | 60 min | 120 min | 180 min |
| Normal Control  | - | 100.00±4.25 | 88.33±1.85 | 92.33±4.25(1.80) | 89.0±4.35(1.55) | 87.33±3.84(3.98) |
| Maltose | 2000 | 70.00±11.67 | 133.66±15.44c(90.94) | 128.66±8.78a(83.80) | 99.36±5.36(41.94) | 84.0±7.21(20.0) |
| Acarbose | 100 | 85.34±1.36 | 88.22±1.10(3.37) | 86.0±2.20(0.77) | 84.26±1.14a() | 82.28±2.26() |
| Extract  | 150 | 86.33±7.66 | 105.66±8.83a(22.39) | 94.66±12.38a(9.64) | 60.33±6.88a() | 70.33±8.76a() |
|  | 300 | 83.66±3.71 | 134.0±9.85b(60.17) | 118.5±12.71a(41.64) | 110.33±15.52a(31.87) | 67.0±13.01() |
|  | 450 | 72.33±2.33 | 126.33±8.41b(74.65) | 119.33±15.33b(64.97) | 116.66±9.25b(61.28) | 104.33±7.88a(44.24) |

Data are expressed as MEAN ± SEM, Significant at ap<0.05, bp< 0.01, compared to control. (n=6). Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

**Discussion**

The plant parts of *Justicia insularis* are used in traditional Ibibio medicine to treat many ailments, including diabetes. This study examined the impact of *Justicia insularis* leaf extract on alpha-amylase and alpha-glucosidase activities in rats. The extract was observed to prevent the rise in blood glucose levels after administration of starch, and it was noted that the lowest dosage had the most prominent inhibitory effect. The extract maybe acting as a partial agonist thereby exhibiting antagonistic effect at higher doses. There may be other mechanistic interplay beyond the effect on the digestive ennzymes. For instance, activation of intestinal sodium – glucose co – transporter (SGLT-1) and / or activation of renal glucose reabsorption (SGLT-2) may contribute to the observed effects (Osigwe *et al.,* 2015). The observed effect of the extract on these digestive enzymes could also be substrate specific. The thorough breakdown of complex carbohydrates in the diet, such as starch, is accomplished by the collaborative activity of α-amylases and α-glucosidase enzymes. The α-amylase enzyme breaks down the α-bonds in α-linked polysaccharides, producing disaccharides such as maltose. These disaccharides are then further broken down into monosaccharides by α-glucosidase enzymes that are attached to the cell membrane (Adeyemi and Babatunde, 2014). The suppression of these enzymes activities hinders the breakdown of consumed carbohydrates, thus inhibiting their absorption leading to a little or no increase in blood glucose levels after consuming meals rich in carbohydrates, as seen in this research. Various medicinal plants have been shown to have the ability to inhibit α-amylase and α-glucosidase, making them viable agents for controlling Type 2 diabetes mellitus (Hasan *et al.,* 2023).

In the same way, the leaf extract effectively prevented increases in blood glucose levels when given along with maltose and sucrose. The lowest dosage (150 mg/kg) showed the greatest inhibition. This may serve as a guide in the traditional use of *Justicia insularis* in the management of Type 2 diabetes especially in minimizing post – prandial glucose excursions. The administration of acarbose, the reference medication used in this investigation, effectively suppressed the elevation of blood glucose levels when co-administered with starch, maltose, and sucrose. This investigation supports the findings published in previous studies on other species of *Justicia*, such as *Justicia carnea* (Ani *et al.,* 2020; Anigboro *et al.,* 2021). These studies also detected considerable suppression of alpha-amylase and alpha-glucosidase activities. The inhibitory actions of this species maybe associated with their phytochemical constituents, particularly polyphenols. The leaf of *Justicia insularis* has been reported to contain saponins, alkaloids, tannins, flavonoids, anthraquinones, and cardiac glycosides, polyunsaturated fatty acids, acyclic monoterpenoids (Oyomah *et al.,* 2019; Anyiekere *et al.,* 2024a) and two specific compounds, 16(α/β)-hydroxy-cleroda-3,13 (14)Z-dien-15,16-olide and 2,16-oxo-cleroda-3,13(14)E-dien-15-oic acid(16). Polyunsaturated fatty acids such as oleic acid, palmitic acid, stearic acid, linoleic acid among others are reported to exert inhibitory effect on alpha amylase and alpha glucosidase enzymes in vitro and in silico (Oliveira *et al.,* 2016; Chelladurai and Chinnacharny, 2018; Daou *et al.,* 2022) . The existence of these phytochemicals in the extract may have contributed to the observed activities in this study, thereby elucidating one mode of anti-diabetic activity of *J. insularis* leaves.

 The presence of phytochemical substances such as flavonoids, saponins, tannins, and terpenoids in plant extracts have been repeatedly shown to contribute to inhibition of alpha-amylase and α-glucosidase (Ishnava and Motisariya, 2018). Furthermore, plant polyphenolic chemicals have been identified to have various effects on biological systems, such as inhibiting enzymes (Kalita *et al.,* 2018). Phenolic compounds are recognized for their strong ability to bind metal ions and precipitate proteins, forming insoluble protein complexes (Anigboro *et al.,* 2021). Additionally, they function as biological oxidants. The existence of polyphenolic chemicals in the leaf extract, together with the presence of terpenes, indicates that they may have the ability to inhibit α-amylase and the membrane-bound intestinal α-glucosidase enzymes.

**Conclusion**

The findings of this research suggest that the leaf extract of *Justicia insularis* may exhibit anti-diabetic effects by inhibiting the alpha-amylase and alpha-glucosidase enzymes. This activity may be linked to the presence of phytochemical ingredients in the plant.

**Ethical Approval**

Permission and approval for animal studies were obtained from the College of Health Sciences Animal -Ethics Committee, University of Uyo. All animal experiments complied with the National Institute of Health Guide for Care and Laboratory Animals (pub. No. 85-23, revised 1985).

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