Original research article

**Effect of *Justicia Insularis* T. AndersonLeaf 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* T. Anderson, 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 of rats, post administration of the substrate and extract concurrently, were monitored over 3 h 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 (Shibib *et. al.,* 2024).

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) 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; Enin et al. 2023). “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, were gotten from University of Uyo Animal house at the Faculty of Pharmacy and used for the experiments. The rats were accommodated in standard clean cages and fed on standard pelleted feed (Guinea Feed) and allowed free access to water*.* College of Health Sciences Animal Ethics Committee, University of Uyo gave approval for the use of animal in this study. Full compliance with the National Institute of Health Guide for Care and Laboratory Animals (pub. No. 85-23, revised 1985) was ensured.

***Preparation of Extract***

The collected leaves were thoroughly washed clean and two weeks were used to dry them under shade before being cut into smaller pieces and powdered using an electrical grinder. The 1.5 kg of powdered leaves powder was soaked in 50% ethanol for 72 h and filtered. The resulting liquid filtrate was concentrated under vacuum conditions at a temperature of 40˚C using a rotary evaporator. The sample was stored at -4˚C in a refrigerator until used for the proposed experiments.

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

***Alpha-Amylase inhibitory study***

Thirty-six Wistar rats fasted for 18 h were shared into 6 groups of 6 rats each and their baseline (0 min ) fasting blood glucose concentration were determined before extract/drug administration. The normal control animals in group I, were administered distilled water (10 mL/kg), starch (2 g/kg body weight) was orally administered to group II rats and distilled water (10 mL/kg) simultaneously (with distilled water as vehicle). Rats in group III were orally given starch (2 g/kg) and the standard drug (acarbose) at 100 mg/kg simultaneously. Based on previously determined LD50 and doses (Enyiekere *et al.,* 2024a), starch (2 g/kg) and *J. insularis* leaf extract at 150, 300 and 450 mg/kg were respectively administered orally to groups IV, V, and VI . 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***

Similar procedure as previously described above was used in subsequent experiments where sucrose and maltose were used as substrates (Gidado *et al.,* 2019; Okokon *et al.,* 2022).

***Blood Glucose Determination***

A glucometer was used to measure blood glucose concentration from tail tip blood dropped on stripes according to manufacturer’s instructions (Accu-chek, Indiana). The glucometer operates 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 analyzed statistically using one –way ANOVA followed by Tukey-Kramer multiple comparison test using Instat Graphpad software Version 3.1, (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***

Starch (2 g/kg) administration to fasted rats lead to elevation of blood glucose concentrations of the treated animals in varying proportion after 30 min. The observed proportions were recorded as follows: starch (62.62%), extract-treated groups (ranging from 4.41% to 14.21%), and acarbose-treated groups (17.97%). These elevations were lowered after 60 min, except 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 reduced to normal level after 120 min. Nevertheless, after 180 min, 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).

**Table 1.** Effect of ethanol leaf extract of *Justicia insularis* on blood glucose level of rats 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(-13.74) | 59.66±5.23a(-12.64) | 55.66±0.88a(-18.15) |
|  | 300 | 56.66±7.68 | 64.0±1.52(12.95) | 61.66±3.33(8.82) | 52.0±4.50b(-8.22) | 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(-1.70) | 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.

Increment of 41.14% in blood glucose concentration of rats accompanied oral administration of sucrose (2 g/kg) to rats after 30 min in the control group. BGL increments of 30.58 - 70.35 % were also observed in groups treated with 150, 300 and 450 mg/kg of extract. At 60 min, Percentage increases in BGL of 25.58, 0.41 and 6.38 %,were respectively recorded for groups treated with 150, 300, and 450 mg/kg of extract. There was no increment in BGL of all the extract-treated groups from 120 - 180 min (Table 2).

**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(-4.06) | 82.0±6.00(-9.22) | 71.66±3.75(-20.67) | 78.0±3.78(-13.65) |
| Extract | 150 | 75.33±3.28 | 128.33±2.17c(70.35) | 94.60±3.19(25.58) | 54.66±6.88(-27.44) | 65.00±8.88(-13.71) |
|  | 300 | 79.33±4.33 | 115.66±7.35b(45.79) | 79.66±9.20(0.41) | 66.00±3.05(-16.80) | 60.66±8.33(-23.53) |
|  | 450 | 73.0±4.04 | 95.33±1.45(30.58) | 77.66±6.88(6.38) | 64.66±1.20(-11.42) | 56.66±2.72(-22.38 )  |

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.

An increment of 90.94% in blood glucose level after 30 min following maltose administration in the control group was observed in the normal control group. Similarly, the BGL of the extract-treated groups were observed to have been elevated by 22.39 - 74.65 % after 30 min of maltose administration.. 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 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(-3.89) | 82.28±2.26(-3.59) |
| Extract  | 150 | 86.33±7.66 | 105.66±8.83a(22.39) | 94.66±12.38a(9.64) | 60.33±6.88a(30.12) | 70.33±8.76a(18.53) |
|  | 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(19.91) |
|  | 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 enzymes by higher doses of the extract. For instance, activation of intestinal sodium – glucose co – transporter (SGLT-1) and / or activation of renal glucose reabsorption (SGLT-2) at higher doses of the extract 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 enzyme 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, 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

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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