Evaluation of Inhibitory Activities of Two Common Vegetables (*Heinsia crinata* and *Lasianthera africana*) on Alpha Amylase and Alpha Glucosidase of Rats

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Abstract

Heinsia crinata (Afzel.) G. Taylor (Rubiaceae) and *Lasianthera africana*. P.Beav (Stemonuraceae), which are common vegetables employed in the preparation of soups by the Ibibios and also used as medicine traditionally to treat various diseases including diabetes, were investigated for effect on alpha amylase and alpha glucosidase enzymes. *H. crinata* (450, 900, and 1350 mg/kg) and *L. africana* (250, 500, and 750 mg/kg) leaves extracts were evaluated for their effects on alpha amylase and alpha glucosidase enzymes *in vivo* using starch, sucrose and maltose as substrates. Acarbose was used as reference drug. The leaf extracts caused significant ($p<0.05$) and non dose-dependent reduction in blood glucose levels of treated rats with the various substrates used. The results suggest that the leaf extracts of these vegetables have the potentials to inhibit alpha amylase and alpha glucosidase enzymes in rats.

Keywords: Antidiabetic, Enzymes, Medicinal plants, Vegetables,

Running title: Evaluation of inhibitory activities of two common vegetables (*Heinsia* crinata and *Lasianthera* africana)

Introduction

Vegetables are edible part of plants which can be used as food for both humans and animals. These include; flowers, fruits, stems, leaves, roots, and seeds. They contain vital nutraceutical constituents with health-promoting effects (Zhang *et al.*, 2019). Studies have confirmed that consumption of large quantities of vegetables can be beneficial in disease conditions such as cardiovascular and neurodegenerative diseases, ischemic stroke, arthritis, inflammatory bowel and some forms of cancers (Slavin and Lloyd,

2012). For example, vegetables such as spinach, broccoli and onion are rich sources of health-promoting compounds (Khaman *et al.,* 2012). According to World Health Organization (WHO), above 80% of the rural inhabitants globally depend on traditional plants as a source of nutrients and primary health care (WHO, 2013).

The presence of bioactive compounds confers the vegetables with medicinal values and also affects thousands of physiological functions which promote health (Poobalan *et al.,* 2019). These bioactive compounds present in vegetables include carotenoids, phenolic compounds (flavonoids, phytoestrogens, phenolic acids), phytosterols and phytostanols, tocotrienols, organo-sulfur compounds (allium compounds and glucosinolates) and non-digestible carbohydrates (dietary fibre and prebiotics). These bioactive compounds act as antioxidants, antibacterial compounds, enzyme stimulators, etc. They also enhance health, modulate immunity and thereby prevent and cure gastrointestinal disorders, cardiovascular diseases, cancer, diabetes and other chronic diseases (Poobalan *et al.,* 2019). In Niger Delta region of Nigeria, the Ibibios use either domestic or wild vegetables in the preparation of soup. Examples of such vegetables are *Heinsia crinata* (atama) and *Lasianthera africana* (editan).

Heinsia crinata (Afzel.) G. Taylor (Rubiaceae) and *Lasianthera africana*. P.Beav (Stemonuraceae), are common vegetables primarily used in the preparation of soups by the Ibibios. In addition to their nutritional values, these vegetables are also utilized in Ibibio traditional medicine for the treatment of a number of diseases such as malaria, diabetes, inflammation, pains, ulcer among others (Okokon *et al.,* 2009a; Okokon *et al.,* 2009b). Reports of their biological activities as antimalarial (Okokon *et al.,* 2007; Okokon *et al.,*2009b) antimicrobial (Andy *et al.,* 2008; Morah and Ashipu, 2017), antiulcer (Okokon *et al.,* 2009a; Okokon *et al.,* 2010), anticonvulsant (Okokon *et al.,* 2021), antidiabetic and hypoglycaemic (Okokon *et al.,* 2009b; Ebong *et al.,* 2014; Ekandem *et al.,* 2016; Inyang *et al.,* 2016), analgesic and antipyretic (Andrew *et al.,* 2012; Okokon *et al.,* 2013a), immunomodulatory (Okokon *et al.,* 2013b) are published in literatures. Two triterpenoid saponins have been isolated from the leaves of *H. crinata* (Babady-Billa *et al.,* 1994) and two iridoids; lamalbide 6, 7, 8- triacetate and aglycone lamiridosin 6, 7, 8-triacetate have also been isolated from the stembark (Tshisekedi *et al.,* 2017). Quercetin, quercetin 3-methyl ether, luteolin, rutin, quercetin-3-O-rutinoside (rutin), α-L-rhamnopyranosyl-(1–6)-β-D-glucopyranose) have been isolated and identified from the leaves of *L. africana* (Ekpo *et al.,* 2020). However, there is paucity of information on the effect of these vegetables on alpha-amylase and

alpha-glucosidase activities in rats. Therefore, this present study was designed to evaluate the leaves extracts of *H. crinata* and *L. africana* for alpha-amylase and alpha-glucosidase inhibitory activities in rats.

Materials and methods

Plant materials

Fresh leaves of *H. crinata* and *Lasianthera africana* were procured from Itam market in Uyo, Akwa Ibom State, Nigeria, in June, 2022. The plants were previously identified and authenticated by a taxonomist in the Department of Botany, University of Uyo, Uyo, Nigeria. Herbarium specimens were deposited at Department of Pharmacognosy and Natural Medicine Herbarium (voucher nos. FPHUU 225 and 312). The fresh leaves (2 kg) of each plant were dried on laboratory table for 2 weeks and reduced to powder. The leaves powder (500 g) from each plant was macerated in 50% ethanol (5000 mL) for 72 hours. The liquid filtrate obtained was concentrated in vacuo at 40˚C and all the ethanol was completely removed. The extracts were stored in a refrigerator at 4˚C until used for experiment reported in this study.

Animals

Wistar rats (125-160 g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics committee, University of Uyo.

In vivo alpha-amylase and glucosidase inhibition study

Alpha-Amylase inhibitory study

Forty-five Wistar rats were divided into 9 groups of 5 rats each. The rats in all groups were fasted for 18 h and baseline fasting blood glucose concentration was 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 (orally with distilled water as vehicle) and distilled water (10 mL/kg) simultaneously. Rats in group III were administered starch (2 g/kg) and the standard drug (acarbose) at 100 mg/kg simultaneously. Based on previously determined LD_{50} value for *H. crinata* leaf extract (Okokon *et al.,* 2009b), groups IV, V and VI were administered simultaneously, starch (2 g/kg) and *H. crinata* leaf extract at 450, 900 and 1350 mg/kg respectively. Groups VII, VIII and IX were also administered simultaneously, starch (2 g/kg) and *L. africana* leaf extract at 250, 500 and 750 mg/kg respectively based on a previously established LD_{50} value for the leaf extract (Okokon *et al.,* 2007). All administrations were done orally and blood glucose concentration was monitored at 30, 60, 90, 120 and 180 min (Gidado *et al.,* 2019). The blood glucose level was used to assess the effect of extract on the enzyme activity.

Glucosidase inhibitory study

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

Blood Glucose Determination

Blood from the tip of rats' tails were dropped on stripes and glucose concentration was measured using a glucometer according to manufacturer's specifications (fine test). The glucometer works with the following principle: the blood sample is exposed to a membrane covering the reagent pad (strip), which is coated with an enzyme (glucose oxidase, glucose dehydrogenase). The reaction causes a colour change and the intensity of this change is directly proportional to the amount of glucose in the blood sample. Light from an LED strikes the pad surface and is reflected to a photodiode, which measures the light intensity and converts it to electrical signals. An electrode sensor measures the current produced when the enzyme converts glucose to gluconic acid. The resulting current is directly proportional to the amount of glucose in the sample (WHO, 2011). Increment percentage was calculated from the equation below;

$$\%\ increment = \frac{Time\ zero\ or\ Baseline\ BGL - Time\ (t) BGL}{Time\ zero\ or\ Baseline\ BGL} \times 100$$

Statistical Analysis

Data obtained from this work were analysed statistically using one –way ANOVA followed by Tukey-Kramer multiple comparison test using GraphPad Prism 6, (San Diego, USA). Differences between means were considered significant at $p \leq 0.05$ and very significant at $p \leq 0.001$.

Results

In vivo alpha amylase and glucosidase inhibition assay

Administration of starch (2 g/kg) to fasted rats caused considerable percentages of increase in blood glucose concentrations of the treated animals after 30 mins. The percentages were starch (63.18%) and acarbose-treated group (17.97%). *Heinsia crinata* leaf extract-treated groups had blood glucose level (BGL) increment range of 22.78-36.49%. These increases were reduced after 60 min in groups treated with 450 mg/kg (23.05 %), 900 mg/kg (17.22%), and 1350 mg/kg (13.24%) of the leaf extract respectively. At 120 min, BGL increments of 0%, 0.92% and 9.65% were recorded for 1350, 900 and 450 mg/kg of the extract respectively. The groups treated with higher doses of the extract (900 and 1350 mg/kg) had their BGL reduced to normal level without any further increase at 180 min. *Lasianthera africana* leaf extract-treated groups had BGL increments of 3.81 - 7.72 % after 30 mins, These increases were reduced after 60 min with

only the group treated with the lowest dose (250 mg/kg) having percentage increase of 8.82%. All the extract-treated groups had their BGL reduced to normal level without any further increase at 120 min. Also, co-administration of the starch with acarbose prominently inhibited the rise in the blood glucose concentrations (Table 1).

Administration of sucrose (2 g/kg) produced a 46.01% increase in blood glucose concentration 30 minutes post-administration of the sucrose in the control group. However, animals groups treated with 450, 900 and 1350 mg/kg of *H. crinata* leaf extract had BGL increments of 20.58, 15.48 and 11.23 % within 30 mins of administration of sucrose. These increment in blood glucose levels of the leaf extract-treated groups were reduced to 0.27 -16.20% after 60 mins post-administration of sucrose. There was little or no increment in BGL of all the *H. crinata* leaf extract-treated groups from 120 -180 min (Table 2). In the groups treated with *L. africana* leaf extract, BGL increments of 4.04-22.22 % were recorded in groups treated with 250, 500 and 750 mg/kg of the leaf extract. At 60 min only the group treated with 500 mg/kg of the leaf extract had 14.81% increase in BGL. However, there was no increment in BGL of all the extract-treated groups from 120 - 180 min (Table 2).

There was 60.78% increase in blood glucose concentration 30 min following maltose administration in the control group. However, 13.20 - 44.18 % increases were observed in the *H. crinata* leaf extract-treated groups. At 60 min, the BGL levels of the leaf extract-treated groups were significantly reduced with groups treated with 450, 900, and 1350 mg/kg having percentage increments of 22.11, 16.03 and 7.17% respectively. These reductions were sustained and significant ($p<0.05$) throughout the duration of the study with the groups treated with higher doses of the extract (900 and 1350 mg/kg) recording 0 % increment in BGL at 120 -180 min (Table 3). Similarly, 5.82 - 16.08 % increases in BGL of rats were recorded in animals groups treated with 250 and 500 mg/kg of *L. africana* leaf extract 30 mins post-administration of sucrose, while the group treated with the highest dose (750 mg/kg) had no increment. There was no increment in BGL of all the leaf extract-treated groups from 120 -180 min (Table 3).

TABLE 1: Effect of leaf extracts of *Heinsia crinata* and *Lasianthera africana* 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)	85.41±7.50	80.00±6.02
Starch		73.33±8.25	119.66±5.45[a](63.1)	115.66±1.33[a](57.72)	95.66±3.75[a](30.45)	92.0±6.35(25.46)
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)
H. crinata Extract	450	75.22±2.18	102.67±16.81(36.49)	92.56±11.28(23.05)	82.48±2.26(9.65)	75.53±5.17(0.41)
	900	75.35±5.34	93.6±6.67(24.22)	88.33±5.44(17.22)	76.05±2.88(0.92)	75.33±5.46()
	1350	74.66±1.76	91.67±12.43(22.78)	84.55±43(13.24)	73.35±6.39()	73.0±3.14()
Lasianthera africana Extract	250	90.66±8.83	97.66±4.91(7.72)	98.66±2.02(8.82)	88.66±1.66()	81.0±2.05()
	500	104.3±2.84	100.33±1.45()	98.66±3.33()	74.66±5.23()	72.66±4.84()
	750	96.0±6.50	99.66±2.33(3.81)	85.66±7.26()	81.0±4.04()	66.66±7.83()

Data is expressed as MEAN ± SEM, Significant at [a]$p<0.05$, [b]$p< 0.01$, when compared to control (n=5). Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

TABLE 2: Effect of leaf extracts of *Heinsia crinata* and *Lasianthera africana* 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	92.0±4.04	134.33±2.90[b](46.01)	128.66±5.45[a](39.84)	97.66±0.66(6.15)	104.16±2.48(13.21)
Acarbose	100	90.33±2.48	86.66±2.90	82.0±6.00	71.66±3.75	78.0±3.78
Heinsia crinata Extract	450	74.66±3.45	90.03±4.22(20.58)	86.76±4.15(16.20)	75.35±4.14(0.92)	73.05±3.24
	900	75.0±6.23	86.61±6.34(15.48)	82.66±1.46(10.21)	74.24±2.64()	74.00±2.55()
	1350	85.10±4.56	94.66±5.88(11.23)	85.33±4.67(0.27)	76.00±4.04()	72.20±3.56()
Lasianthera africana Extract	250	90.66±1.85	94.33±1.20(4.04)	80.33±8.29()	84.33±2.60()	65.06±13.34
	500	81.0±9.00	99.0±4.04(22.22)	93.0±7.37(14.81)	75.33±2.96()	69.33±10.65()
	750	103.66±6.11	110.66±3.33(6.75)	88.66±4.63()	81.0±4.50()	72.33±13.17()

Data is expressed as MEAN ± SEM. Significant at [a]$p<0.05$, [b]$p< 0.01$, when compared to control (n=5). Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

TABLE 3: Effect of leaf extracts of *Heinsia crinata and Lasianthera africana* 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
Control normal saline	-	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	82.30±2.14	132.33±1.90^{b}(60.78)	130.22±2.45(58.22)	115.0±2.46(39.73)	106.22±4.24(29.06)
Acarbose	100	85.34±1.36	88.22±1.10(3.37)	86.0±2.20^{c}(0.77)	84.26±1.14^{a}()	82.28±2.26^{a}()
Heinsia crinata Extract	450	80.35±6.28	102.65±9.38(27.75)	98.12±5.64^{a}(22.11)	86.33±5.28^{b}(7.44)	80.33±5.36()
	900	83.68±4.28	97.10±3.24^{b}(44.18)	95.35±4.16(16.03)	82.0±9.17^{a}()	80.00±8.23()
	1350	82.33±10.24	93.20±14.18(13.20)	88.24±5.34 ^{b}(7.17)	75.00±9.26^{b}()	70.31±3.18^{c}()
Lasianthera africana Extract	250	105.66±2.90	122.66±6.33(16.08)	98.12±5.64^{a}()	77.66±2.02^{b}()	78.33±9.56()
	500	91.66±4.84	97.0±3.00^{b}(5.82)	92.66±3.66(1.09)	75.0±3.51^{a}()	72.00±1.64()
	750	107.33±11.39	89.0±3.21()	78.33±4.84 ^{b}()	75.33±2.18^{b}()	62.0±3.60^{c}()

Data is expressed as MEAN ± SEM, Significant at $^{a}p<0.05$, $^{b}p< 0.01$, when compared to control. (n=5). Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

Discussion

The leaf extracts of *H. crinata* and *L. africana* were found to reduce and in some cases, inhibit completely any rise in blood glucose concentration after starch administration at the various doses used. Dietary polysaccharides like starch are digested completely through a combined action of α-amylases and α-glucosidase enzymes. Alpha-linked polysaccharides are reduced to disaccharides like maltose by α-amylase enzyme which digests α-bonds of the α-linked polysaccharides. The disaccharides are further reduced to monosaccharides by membrane bound α-glucosidase enzymes (Kalra, 2014; Alongi and Anese, 2018). Inhibitions of the activities of these enzymes cause delay in the digestion of ingested carbohydrates thereby resulting in reduction in blood glucose concentrations elevation following carbohydrate meals as was observed in this study. These enzymes have been targeted for the management of Type 2 diabetes mellitus. There are reports on many medicinal plants with inhibitory potentials on α-amylase and α-glucosidase (Ibrahim *et al.,* 2014; Esimone *et al.,* 2001).

Also, the leaf extracts of these vegetables were found to significantly inhibit blood glucose rise when co-administered with starch, maltose and sucrose. Similarly, acarbose, the standard drug used in this study, significantly inhibited blood glucose rise when co-administered with starch, maltose and sucrose. The results of this study support the antidiabetic activities earlier reported on the leaf extracts of *H. crinata* (Okokon *et al.,* 2009b; Ebong *et al.,* 2014) and *L. africana* (Ekandem *et al.,* 2016) and further suggest this activity to be one of the mechanisms of antidiabetic action of the plants. The results of this study corroborate earlier report of *in vitro* inhibitory activity of *L. africana* leaf extract on alpha amylase and alpha glucosidase (Shodehinde *et al.,* 2017).

The inhibitory activities of plant extracts on enzymes such as alpha amylase and alpha glucosidase are linked to their phytochemical constituents especially polyphenols among others (Ishnava and Metisariya, 2018). Polyphenolic compounds which have been reported in these plants exert several effects on the biological systems which include enzymes inhibitions (Kalita *et al.,* 2018; Funke and Melzig, 2005). Quercetin, quercetin 3-methyl ether, luteolin, rutin, quercetin-3-O-rutinoside (rutin), α-L-rhamnopyranosyl-(1–6)-β-D-glucopyranose) are examples of polyphenols that have been isolated and identified from the leaves of *L. africana* (Ekpo *et al.,* 2020). Diterpenoids have also been reported from the leaves of *H. crinata* (Babady-Billa *et al.,* 1994). Furthermore, monoterpenes which are richly found in the leaf essential oil of *H. crinata* (Morah and Ashipu, 2017), have also been reported to inhibit alpha amylase and alpha glucosidase (Oboh *et al.,* 2017). There are more reports of the inhibition of these enzymes by compounds present in the leaf extracts of these vegetables in literature (Proenca *et al.,* 2017; Su and Tang, 2019; Proenca *et al.,* 2021). Coumarins in particular have been reported to inhibit alpha glucosidase and alpha amylase activities (Zhao *et al.,* 2015; Karakaya *et al.,* 2018). With the evidence available in literature, one can conclude with reasonable degree of certainty that the observed activity is a product of the activities of different constitutuents of the leaves that have inhibitory effect on the target enzymes.

Conclusion

The results of this study suggest that inhibition of alpha amylase and alpha glucosidase enzymes maybe one of the modes of antidiabetic activity of the leaf extracts of *Heinsia crinata* and *Lasianthera africana* which can be attributed to the activities of its phytochemical constituents.

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Conflict of Interest

The authors declare that there are no conflicts of interest related to this article

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