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ORIGINAL ARTICLE

In vitro α-amylase and α-glucosidase Inhibitory and Cytotoxic Activities of Extracts from *Cissus cornifolia* Planch Parts

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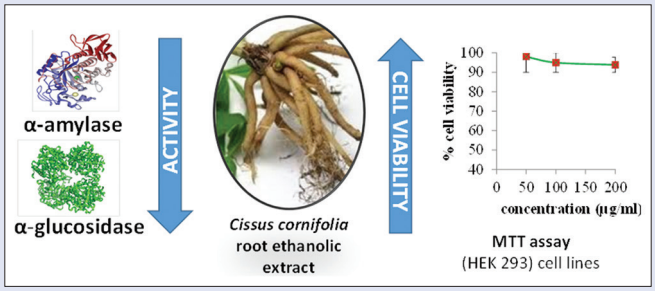
ABSTRACT

Context: Hyperglycemia is the hallmark of type 2 diabetes mellitus, and its prevention will go a long way in managing the disease and its associated complications. Reduction of postprandial hyperglycemia through retarding carbohydrates digesting enzymes is one of the major therapeutic approaches used in the management of diabetes. **Objective:** The aim of the present study was to investigate the antidiabetic and cytotoxic effects of *Cissus cornifolia* extracts *in vitro*. **Materials and Methods:** The α-amylase and α-glucosidase inhibitory activities of ethanolic and aqueous extracts of *C. cornifolia* root and leaves were investigated when the cytotoxic effects of these extracts were analyzed using MTT assay on human embryonic kidney (HEK 293) cell lines. **Results:** The root ethanolic extract showed a mild α-amylase inhibitory activity with IC_{50} value of 22.75 ± 1.23 μg/ml, but strong α-glucosidase inhibitory activity with IC_{50} value 2.81 ± 0.97 μg/ml and the aqueous root extract indicated moderate inhibition for both α-amylase and α-glucosidase with IC_{50} values of 33.70 ± 3.75 and 37.48 ± 2.35 μg/ml, respectively. The ethanolic root extract was found nontoxic at tested concentrations on HEK 293 cell lines as confirmed by the MTT assay with 93% cell viability at the highest concentration (200 μg/ml) tested. However, the aqueous extracts (leaf and root) were found cytotoxic at concentrations above 50 μg/ml. **Conclusion:** Data of this study suggest that the root ethanolic extracts of *C. cornifolia* possesses moderate α-amylase, but strong α-glucosidase inhibitory activity *in vitro* and did not show significant cytotoxicity with the tested concentrations.

Key words: *Cissus cornifolia,* cytotoxicity, diabetes, α-amylase, α-glucosidase

SUMMARY

- Present study was conducted to examine effects of antidiabetic and cytotoxic effects of *Cissus conrnifolia* root and leaves extracts *in vitro*. Data of this study suggest that the root ethanolic extract of *C. cornifolia* possesses mild to moderated antidiabetic activity via inhibiting carbohydrate digesting enzymes when no significant toxicity was observed with tested concentrations.



Abbreviations used: alex: Aqueous leaf extract; arex: Aqueous root extract; CC: *Cissus cornifolia*; DNS: Dinitrosalicylic acid; DMSO: Dimethylsulfoxide; elex: Ethanolic leaf extract; erex: Ethanolic root extract; IDF: International Diabetes Federation; MEM: Minimum essential medium; NIDDM: Noninsulin-dependent diabetes mellitus; pNPG: Para-nitrophenyl glucopyranoside; SD: Standard deviation; T2D: Type 2 diabetes.

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INTRODUCTION

Diabetes is one of the major threats to global public health, and the number of diabetic cases is increasing tremendously in all over the world and expected to be doubled by 2030.[1] According to recent data from the International Diabetes Federation, diabetes is more prevalent in the developing countries where people have adopted high-calorie westernized diets with the lack of physical activities.[1] Noninsulin-dependent diabetes mellitus (NIDDM), commonly known as type 2 diabetes (T2D), contributes approximately 90%–95% of all cases of diabetes.[2] It is a dysfunction of endocrine system cause by low secretion of insulin by the pancreatic β-cells and insulin resistance or insensitivity of cells to insulin action to regulate blood glucose levels which leads to hyperglycemia.[3,4]

Chronic hyperglycemia has been considered as one of the principal causes for several diabetic complications. Since the major source of glucose is dietary carbohydrates, the inhibition of key carbohydrates digesting enzymes such as α-amylase and α-glucosidase, would be vital in preventing postprandial rise in blood glucose, chronic hyperglycemia as well as diabetic complications. This is because inhibitors of these enzymes (α-amylase and α-glucosidase) delays the carbohydrates digestion and reduces the rate of glucose absorption from the gut and finally lowers the postprandial rise in blood glucose level. Therefore, inhibition of α-amylase and α-glucosidase is a key in the management and treatment of NIDDM or T2D.[5,6]

Conventionally, the control of blood glucose level in T2D is achieved using available oral hypoglycemic agents; however, all these have been reported to have limited efficacy and undesirable side effects.[7,8] Numerous plant-derived compounds which mimic the action of oral hypoglycemic agents such as voglibose, miglitol, and acarbose have been isolated[9-12] and proved to be effective in inhibiting carbohydrates

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digesting enzymes. Hence, more searches for plant-derived antidiabetic compounds would be worthwhile because plant-derived products have shown impressive performance in the discovery of some currently available conventional medicines.[12] However, despite the strong α-amylase and α-glucosidase inhibitory activities, some plant-derived compounds may have potential toxic and carcinogenic effects,[13] which make them unsuitable for therapeutic applications. It is, therefore, of utmost importance to intensively investigate the potential cytotoxic activity to validate the safety and continued use of medicinal plant extracts or compounds before their therapeutic applications. It has been documented that some plants' extracts do have bioactivity, but it is counteracted by their cytotoxicity;[14] hence, such scenarios need to be expansively evaluated to assess the overall efficacy of the plant material.

Cissus cornifolia (Baker) Planch (Vitaceae) commonly called the "Ivy grape" is indigenous to Zimbabwe. Locally, it is called "Mudzambiringa'" and "Idebelebe" by the Shona and Ndebele-speaking Zimbabweans, respectively. The plant is traditionally used by the Shona speaking people as a remedy for gonorrhea when taken with native natron while the leaf-sap is used by the Tanganyika of Tanzania as a sedative in cases of mental derangement.[15] The root decoction is also used for malaria, septic tonsil, cardiac problems, pharyngitis, and diabetes.[15] Our survey of the medicinal use of *C. cornifolia* in Mrewa district, Zimbabwe, also revealed that its roots are used to treat diabetes mellitus among other ailments. At present, not much scientific information on *C. cornifolia* exists in the literature apart from preliminary reagent-based phytochemical analysis, which revealed that the plant possesses glycoside, flavonoids, saponins, steroids, terpenoids, and tannins.[16-19] However, in a more recent study, we detected phenolic compounds such as pyrogallol, resorcinol and catechol, vanillin (aldehyde), and long chain fatty acids were identified as phytochemical components of the plant and possibly responsible for the observed antioxidant activity.[20]

The present study was, therefore, undertaken to further probe the *in vitro* antidiabetic and cytotoxicity activities of the *C. cornifolia* ethanol and aqueous root and leaf extracts as potential sources of nontoxic therapeutic agents, which can be useful in achieving normoglycemia in diabetic patients.

MATERIALS AND METHODS

Chemicals

Acarbose, α-amylase, α-glucosidase, dinitrosalicylic acid (DNS), human embryonic kidney cells (HEK 293), monosodium and disodium phosphate, minimum essential medium, glutamax, 3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), 10% fetal bovine serum (FBS), 4-nitrophenyl-d-glucopyranoside (pNPG), and porcine pancreatic amylase were procured from Sigma-Aldrich through Capital Lab Supplies, New Germany, South Africa. Dimethyl sulfoxide (DMSO) was purchased from Merck Chemical Company, Durban, South Africa. Starch was purchased from Associated Chemicals Enterprises, South Africa.

Plant sample

The plants parts (leaf and root) of *C. cornifolia* were collected during the period of February 2013 from Mrewa, Mashonaland East province, Zimbabwe. The plant samples were identified and authenticated by the Harare Botanical Garden and Herbarium, Harare, Zimbabwe, and voucher specimens were deposited with a number CC082. The plant samples were immediately washed with distilled water, cut into small pieces, and shade-dried until a constant weight was attained. The dried samples were ground to fine powder using a blender, stored individually in air-tight Ziploc bags, and transported to the University of KwaZulu-Natal, Westville Campus, Durban, South Africa, for further analysis.

Preparation of plant extract

Forty grams of the finely powdered plant part was separately defatted with hexane. The defatted materials were sequentially extracted with ethanol and water by soaking for 48 h in 200 ml of the respective solvent. For ethanol extracts, after filtration through Whatman filter paper (No. 1), the ethanol was evaporated under reduced pressure using a rotary evaporator (Buchi Rotavapor II, Buchi Germany) at 40°C, and the remaining ethanol was allowed to evaporate freely at room temperature. Aqueous extracts were dried using a freeze dryer. The solvent extracts in each case were weighed, transferred to microtubes, and stored in a refrigerator at 4–8°C until required.

α-amylase inhibitory activity of plant extracts

The α-amylase inhibitory activity of plant extracts was determined according to the method described by Shai *et al.*[21] with slight modifications. A 250 µL of each extract or acarbose at different concentrations (30–240 µg/mL) was incubated with 500 µL of porcine pancreatic amylase (2 U/mL) in 100 mM phosphate buffer (pH 6.8) at 37°C for 20 min. A 250 µL of 1% starch dissolved in 100 mM phosphate buffer (pH 6.8) was then added to the reaction mixture and incubated at 37°C for 1 h. Then, 1 mL of DNS color forming reagent was added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm, and the inhibitory activity was expressed as a percentage of a control sample without the inhibitors.

$$\text{Inhibitory activity}(\%) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$

α-glucosidase inhibitory activity of plant extracts

The α-glucosidase inhibitory activity was determined according to the method described by Ademiluyi and Oboh[22] with slight modifications. Briefly, a 250 µL solution of the compound or acarbose at different concentrations (30–240 µg/mL) was incubated with 500 µL of 1.0 U/mL α-glucosidase solution in 100 mM phosphate buffer (pH 6.8) at 37°C for 15 min. Thereafter, a 250 µL of pNPG solution (5 mM) in 100 mM phosphate buffer (pH 6.8) was added, and the mixture was further incubated at 37°C for 20 min. The absorbance of the released p-nitrophenol was measured at 405 nm, and the inhibitory activity was expressed as a percentage of a control sample without the inhibitors.

$$\text{Inhibitory activity}(\%) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$

In vitro cytotoxic activity of plant extracts (MTT assay)

The plant extracts were prepared (reconstituted in 10% DMSO), vortexed, filtered through Whatman filter paper (No. 1), and left for 15 min before further dilution with the respective growth medium and tested on the HEK 293 cell lines. On the other hand, routine cell culture maintenance of the HEK 293 was done by incubating the cells at 37°C in a humidified atmosphere supplemented with 5% CO_2. Cells were replenished with fresh growth medium every 2–3 days, consisting of media (Minimum Essential Medium + Glutmax™ + antibiotics [100 U/ml penicillin, 100 µg/ml streptomycin, 0.25 µg/ml amphotericin B] +10% FBS]).

To investigate the cytotoxic effects, the aqueous and ethanol extracts were tested using the HEK 293 kidney cells by MTT assay.[23,24] Confluent monolayer culture suspensions of the cells were trypsinized and plated into 96-well plates at a seeding density of 2.5×10^3 cells per well and incubated for 24 h at 37°C in a 5% CO_2 incubator in a culture medium containing 10% FBS. Following 24-h incubation and attachment, the cell culture medium was replaced with fresh

medium. Thereafter, varied concentrations of plant extracts (50–200 µg/mL) were added in triplicate to the cells, and the plate was incubated for 48 h as previously. Two controls, one containing only cells (positive control) and one containing DMSO (negative control) were also used. After 48 h, all culture media were removed from the plates, the cells were washed with phosphate-buffered saline (PBS) and 100 µL of the cell media and 100 µL of MTT solution (5 mg/mL in PBS) was added to each well. The plates were then incubated for 4 h at 37°C. Thereafter, the MTT infused medium was removed, and 100 µL DMSO solution was added to each well to dissolve the insoluble formazan crystals. Absorbance was measured at 570 nm using a Mindray MR-96A microplate reader. The assessment of cytotoxicity was based on a comparison with untreated cells and expressed as IC_{50} (the concentration of the sample required to inhibit 50% of cell proliferation), calculated from the dose–response curve (curve fit-nonlinear regression, four parameters).

Statistical analysis

All data are presented as the mean ± standard deviation of triplicate determination. Data were analyzed by SPSS statistical software (version 19, Windows IBM Inc., New York, USA) using Tukey's-HSD significant difference multiple range *post hoc* tests. Values were considered significantly different at $P < 0.05$.

RESULTS

Figure 1 shows the inhibitory activity of *C. cornifolia* ethanol and aqueous extracts on α-amylase and α-glucosidase, respectively. Figure 1a demonstrates that the ethanol and aqueous root extracts had significantly higher ($P < 0.05$) α-amylase inhibitory activity than acarbose while the leaf (ethanol and aqueous) extracts had lower α-amylase inhibitory potentials than acarbose. Furthermore, the root extracts showed better α-glucosidase inhibitory activity than acarbose [Figure 1b] with the ethanol root extract showing a significantly higher ($P < 0.05$) activity than all other extracts and acarbose. However, the leaf extracts (ethanol and aqueous) had lower activities than acarbose. This is also further demonstrated in Table 1 where the IC_{50} values of the root (ethanol and aqueous) extracts for inhibiting α-amylase and α-glucosidase were significantly lower than acarbose and leaf (ethanol and aqueous) extracts.

Figure 2 displays the cytotoxic activity of *C. cornifolia* extracts on HEK 293 kidney cell lines as confirmed by MTT assay. As indicated in the figure, *C. cornifolia* ethanol (leaf and root) extracts did not cause any significant decrease in cell viability across all tested concentrations (50–200 µg/ml). However, the aqueous (leaf and root) extracts displayed a notable increase in cell death as the concentration of extracts increases. This is also consistent with the high IC_{50} [Table 2] values obtained for *C. cornifolia* ethanol root and leaf extracts (2.67 ± 75.44 and 1.63 ± 120.11 mg/ml), respectively. All (leaf and root) aqueous extracts showed a significant cytotoxic activity as shown in Figure 2 by the reduction in cell viability at 200 µg/ml by approximately 50 and 30%, respectively. The leaf extract was the most toxic as indicated by the lowest IC_{50} value of 241.29 µg/ml [Table 2].

Table 1: IC_{50} values for α-amylase and α-glucosidase inhibition activity of *Cissus cornifolia* extracts

Name of extract	IC_{50} values (µg/mL)	
	α-amylase	α-glucosidase
CC elex	90.78±3.20^{c}	85.62±5.54^{c}
CC alex	145.24±17.90^{d}	75.31±9.34^{d}
CC erex	22.75±1.23^{a}	2.81±0.97^{a}
CC arex	33.70±3.75^{a}	37.48±2.35^{b}
Acarbose	52.11±0.56^{b}	57.18±3.54^{c}

Data are presented as mean±SD values of triplicate determinations. $^{a-c}$Different superscripts letters within a column for a given enzyme are significantly different from each other extracts or standard (Tukey's-HSD multiple range *post hoc* test, $P<0.05$). CC: *Cissus cornifolia*; elex: Ethanolic leaf extract; alex: Aqueous leaf extract; erex: Ethanolic root extract; arex: Aqueous root extract; SD: Standard deviation; HSD: Honestly significant difference

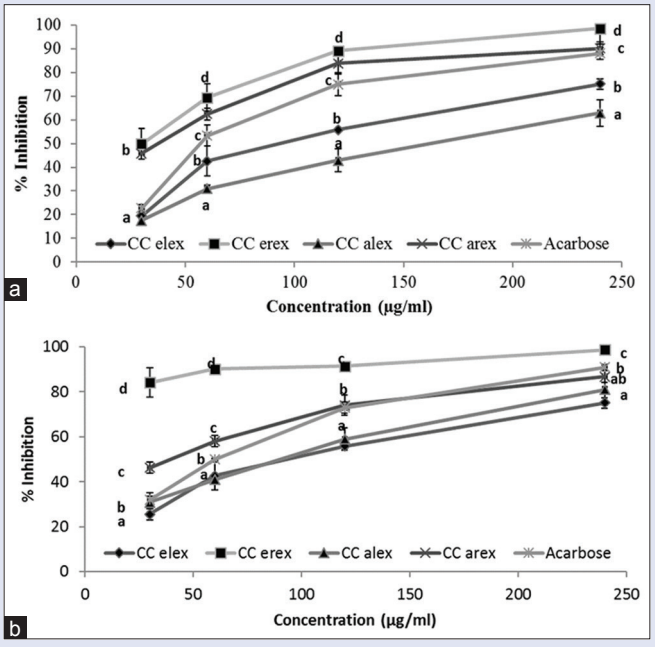

Figure 1: The α-amylase (a) and α-glucosidase (b) inhibitory activities of *Cissus cornifolia* extracts. Data are presented as mean ± standard deviation values of triplicate determinations. $^{a-d}$Different superscripts letters for a given value within the figure are significantly different from each other (Tukey's - HSD multiple range *post hoc* test, $P < 0.05$). CC: *Cissus cornifolia*; elex: Ethanolic leaf extract; alex: Aqueous leaf extract; erex: Ethanolic root extract; arex: Aqueous root extract

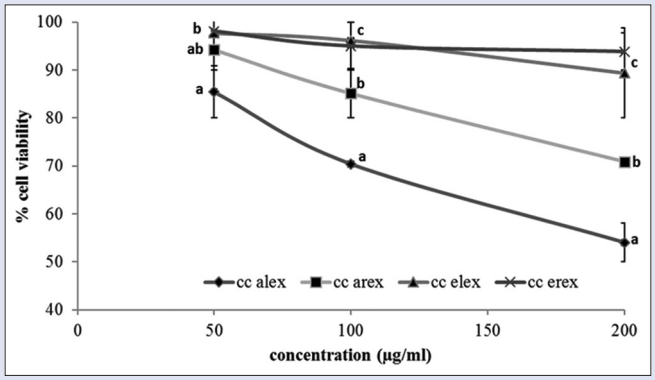

Figure 2: Cytotoxicity activity *Cissus cornifolia* extracts on human embryonic kidney 293 kidney cells lines as confirmed by MTT cell proliferation assay. Data are presented as mean ± standard deviation values of triplicate determinations. $^{a-c}$Different superscripts letters for a given concentration are significantly different from each other extracts (Tukey's - HSD multiple range *post hoc* test, $P < 0.05$). CC: *Cissus cornifolia*; elex: Ethanolic leaf extract; alex: Aqueous leaf extract; erex: Ethanolic root extract; arex: Aqueous root extract

Table 2: IC_{50} values for cytotoxic activity of *Cissus cornifolia* extracts on human embryonic kidney cells

Name of extract	Cytotoxicity IC_{50} (µg/ml)
CC elex	1.63±120.11[c]
CC alex	241.29±21.41[a]
CC erex	2.67±75.44[d]
CC arex	726.67±35.45[b]

Data are presented as mean±SD values of triplicate determinations. [a-d]Different superscripts letters within a column are significantly different from each other extracts (Tukey's-HSD multiple range *post hoc* test, $P<0.05$). elex: Ethanolic leaf extract; alex: Aqueous leaf extract; erex: Ethanolic root extract; arex: Aqueous root extract; CC: *Cissus cornifolia*; SD: Standard deviation; HSD: Honestly significant difference

DISCUSSION

One therapeutic approach for preventing diabetes mellitus is to retard the absorption of glucose through inhibition of key carbohydrates digesting enzymes, α-amylase and α-glucosidase, which are located in the brush borders of the small intestine. Although a number of studies reported the α-amylase and α-glucosidase inhibitory activities of various medicinal plant-originated compounds,[9-12] the similar activity of *C. cornifolia*, a plant commonly used for the traditional treatment of diabetes in Zimbabwe, has not been investigated yet, with or without its cytotoxic effects.

The present study showed that *C. cornifolia* root (ethanol and aqueous) extracts moderately inhibit α-amylase and ethanol root extract strongly inhibits α-glucosidase activity while the aqueous root extract moderately inhibits α-glucosidase activity. According to Krentz and Baile,[25] better clinical outcome could be derived from specific α-amylase and α-glucosidase inhibitors with a mild inhibitory activity against the α-amylase and strong inhibitory activity against α-glucosidase and also not cytotoxic to the target cells. Indeed, these are the features of potential α-glucosidase inhibitors that usually attract pharmaceutical companies. Interestingly, in our study, the root ethanol extract of *C. cornifolia* had demonstrated these properties and therefore could be considered for therapeutic application to delay postprandial hyperglycemia.

In a previous study, we have reported that some phenolic compounds are the major constituents of *C. cornifolia* root extracts, which include isomers of benzenediol (resorcinol, catechol, and hydroquinone), pyrogallol and vanillin (phenolic aldehyde)[20] and were implicated as the possible bioactive antioxidant components of the extracts. Interestingly, apart from being effective antioxidants, phenolic compounds, and phenol derivatives have been reported to be potent α-glucosidase and α-amylase inhibitors in several previous studies.[26-28] Indeed, some isolated phenolics have been reported to be the main bioactive antidiabetic agents of *Brickellia cavanillesii*[29] and *Garcinia mangostana*,[30] and the activity was mediated through the inhibition of α-glucosidase activity. Based on the above, the observed α-amylase and α-glucosidase inhibitory activities of the root extracts might be directly proportional to the amount of phenolic constituents of the extracts. This can be further supported by the fact that the leaves might have a lower amount of phenolics to inhibit α-amylase and α-glucosidase activity, whereas the root might have a higher concentration of phenolics which significantly inhibited the α-amylase and α-glucosidase activities.

However, despite the moderate α-amylase and strong α-glucosidase inhibitory activities observed for the *C. cornifolia* root extracts, most plant-derived compounds have been reported to be potentially toxic and carcinogenic, which make them unsuitable for therapeutic applications.[13] Natural products are generally considered safer than pharmaceuticals. However, there have been several reports pointing out that plant extracts and compounds of plant origin are neither completely safe nor poisonous. Our results point out that both the activity and toxicity need to be taken into account to evaluate the total antidiabetic activity of plant extracts. Furthermore, we observed that some extracts are toxic above certain concentrations; hence, further investigation is warranted to determine a safer dose for consumption. Toxicity in plant extract can be as a result of a number of factors among them the presence of toxic compounds in the extract.[14] Individual compounds from crude extracts need to be isolated and investigated as individual or combined activity and toxicity.

CONCLUSION

Data obtained from this study suggest that ethanolic root extract of *C. cornifolia* exerts an inhibitory effect on α-glucosidase and α-amylase and was also nontoxic at tested concentrations. The results displayed by the ethanol root extract are interesting enough to stimulate further *in vivo* experiments. In addition, these results support the traditional use of *C. cornifolia* in the management of diabetes. Further studies on experimental animals and humans are warranted along with the identification of pure bioactive compounds.

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Conflicts of interest

There are no conflicts of interest.

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