

In Vitro Evaluation of the Antidiabetic Potential of *Bergera koenigii* (Curry Leaf) Extract Through α -Amylase, α -Glucosidase and DPP-4 Enzyme Inhibition

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Abstract

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder driven by insulin resistance and progressive pancreatic β -cell dysfunction, and accounts for 90-95% of all diabetes cases worldwide. Inhibition of carbohydrate-digesting enzymes (α -amylase and α -glucosidase) and of dipeptidyl peptidase-4 (DPP-4), which degrades the incretin hormones GLP-1 and GIP, are established therapeutic strategies for improving postprandial glycaemic control. This study evaluated the in vitro antidiabetic potential of an ethanolic extract of *Bergera koenigii* (curry leaf) leaves against these three enzyme targets. Leaves were authenticated, shade-dried, powdered and extracted by Soxhlet extraction with ethanol; preliminary GC-MS screening and phytochemical analysis were performed. α -Amylase and α -glucosidase inhibitory activities were assessed spectrophotometrically over 200-1000 μ g/mL against acarbose, and DPP-4 inhibitory activity was assessed over 25-400 μ g/mL against sitagliptin. The extract showed concentration-dependent inhibition in all three assays, reaching 80.89% α -amylase inhibition (IC_{50} 638 μ g/mL, versus acarbose 90.25%, IC_{50} 518 μ g/mL), 80.89% α -glucosidase inhibition (IC_{50} 633 μ g/mL, versus acarbose 90.25%, IC_{50} 518 μ g/mL), and 78% DPP-4 inhibition at 400 μ g/mL (versus 85% for sitagliptin). While less potent than the reference standards, the extract demonstrated substantial, concentration-dependent activity across all three mechanistically relevant targets, supporting its potential as a natural multi-target antidiabetic agent warranting further investigation.

Keywords: *Bergera koenigii*; curry leaf; Type 2 diabetes mellitus; α -amylase inhibition; α -glucosidase inhibition; DPP-4 inhibition; IC_{50} .

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterised by persistent hyperglycaemia arising from defects in insulin secretion, insulin action, or both. Type 2 diabetes mellitus (T2DM), the most common form, develops through a combination of insulin resistance in skeletal muscle, liver and adipose tissue and progressive pancreatic β -cell dysfunction, compounded by chronic inflammation, oxidative stress and abnormal lipid metabolism. Its prevalence continues to rise worldwide, driven by urbanisation, sedentary lifestyles, obesity and ageing populations, and uncontrolled disease leads to serious microvascular (retinopathy, nephropathy, neuropathy) and macrovascular (coronary artery disease, stroke, peripheral arterial disease) complications.

Two established pharmacological strategies target postprandial hyperglycaemia and incretin metabolism. First, inhibition of α -amylase and α -glucosidase - enzymes that hydrolyse dietary starch and disaccharides into absorbable glucose - slows carbohydrate digestion and blunts the post-meal glucose rise. Second, inhibition of dipeptidyl peptidase-4 (DPP-4), a serine protease that rapidly degrades the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP), prolongs incretin-mediated, glucose-dependent insulin secretion and suppresses glucagon release. Synthetic inhibitors of these targets (acarbose; the gliptins, e.g. sitagliptin) are used clinically, but gastrointestinal side effects and cost have driven interest in plant-derived alternatives with similar mechanisms and potentially fewer adverse effects.

Bergera koenigii (*Murraya koenigii*; curry leaf), a Rutaceae shrub widely used in traditional medicine, is rich in carbazole alkaloids (mahanimbine, koenigine, murrayacine), flavonoids and phenolic compounds with reported antioxidant, antidiabetic, antimicrobial, anti-inflammatory, hepatoprotective and anticancer activities. The present study evaluated the in vitro α -amylase, α -glucosidase and DPP-4 inhibitory activity of an ethanolic extract of *B. koenigii* leaves, benchmarked against the standard inhibitors acarbose and sitagliptin, to assess its potential as a natural multi-target antidiabetic agent.

II. Materials and Methods

2.1 Plant Material and Extraction

Fresh leaves of *Bergera koenigii* were collected and authenticated by a qualified botanist, washed with distilled water, shade-dried at room temperature, and powdered using a mechanical grinder. About 50 g of powdered leaf material was extracted with 70-95% ethanol using a Soxhlet apparatus for 6-8 h. The extract was filtered through Whatman No. 1 filter paper, concentrated under reduced pressure, dried to a semisolid extract, and stored in an airtight container under refrigeration until analysis. Preliminary phytochemical screening and GC-MS profiling (Thermo GC-Trace Ultra/Thermo MS DSQ II, TR-5-MS capillary column) were performed on the extract, along with quantitative estimation of total phenolic content (Folin-Ciocalteu method) and total flavonoid content (aluminium chloride colorimetric method).

2.2 α -Amylase Inhibition Assay

Reaction mixtures containing sodium phosphate buffer (0.02 M), α -amylase enzyme and extract (20-100 $\mu\text{g/mL}$ preparation range; assayed at 200-1000 $\mu\text{g/mL}$ final concentrations) were incubated at room temperature for 10 min, after which starch solution was added to initiate the reaction. The reaction was terminated with dinitrosalicylic acid (DNS) reagent, developed by boiling for 5 min, cooled, diluted, and read at 540 nm. Percentage inhibition was calculated as $[(\text{Absorbance control} - \text{Absorbance extract}) / \text{Absorbance control}] \times 100$, with acarbose as the reference standard and IC_{50} values derived by non-linear regression of inhibition versus log-concentration; all assays were performed in triplicate (mean $\pm$ SD).

2.3 α -Glucosidase Inhibition Assay

Yeast α -glucosidase inhibitory activity was assessed using p-nitrophenyl- α -D-glucopyranoside (pNPG) as substrate in 100 mM phosphate buffer (pH 6.8). Extract (20-100 $\mu\text{g/mL}$ preparation range; assayed at 200-1000 $\mu\text{g/mL}$) was incubated with enzyme at 30°C for 5 min, the reaction terminated with 50 mM NaOH, and absorbance read at 410 nm. Percentage inhibition and IC_{50} were calculated as above, with acarbose as reference standard, in triplicate.

2.4 DPP-4 Inhibition Assay

DPP-4 inhibitory activity was assessed using Gly-Pro-p-nitroanilide (Gly-Pro-pNA) as substrate in DPP-4 assay buffer. Extract working concentrations of 25, 50, 100, 200 and 400 $\mu\text{g/mL}$ were prepared by serial dilution of a 1 mg/mL stock and incubated with DPP-4 enzyme in a 96-well microplate alongside reagent blank, enzyme control and sitagliptin reference wells. Absorbance was read at ~ 405 nm after incubation, and percentage inhibition was calculated relative to the blank-corrected enzyme control.

III. Results and Discussion

GC-MS analysis of the ethanolic extract identified eleven major peaks corresponding to constituents including dodecane, a bisabolene epoxide, tetradecanoic acid ethyl ester, hexatriacontane and related long-chain compounds, consistent with a phytochemically complex extract; individual attribution of enzyme-inhibitory activity to specific constituents was not established and is considered preliminary.

The extract produced concentration-dependent inhibition of α -amylase, rising from $23.64 \pm 0.58\%$ at 200 $\mu\text{g/mL}$ to $80.89 \pm 0.86\%$ at 1000 $\mu\text{g/mL}$, compared with acarbose, which rose from $30.72 \pm 0.44\%$ to $90.25 \pm 0.41\%$ over the same range (Table 1). The extract IC_{50} (638 $\mu\text{g/mL}$) was higher than that of acarbose (518 $\mu\text{g/mL}$), indicating lower potency for the extract relative to the standard.

Conc. ($\mu\text{g/mL}$)	Extract inhibition (%)	Acarbose inhibition (%)
200	23.64 ± 0.58	30.72 ± 0.44
400	39.18 ± 0.69	47.86 ± 0.49
600	55.87 ± 0.77	64.13 ± 0.46
800	69.42 ± 0.82	77.91 ± 0.43
1000	80.89 ± 0.86	90.25 ± 0.41
IC_{50} ($\mu\text{g/mL}$)	638	518

Table 1: α -Amylase inhibitory activity of *B. koenigii* extract versus acarbose.

A similar concentration-dependent pattern was observed for α -glucosidase inhibition, with the extract rising from $24.77 \pm 0.62\%$ at $200 \mu\text{g/mL}$ to $80.89 \pm 0.86\%$ at $1000 \mu\text{g/mL}$ against acarbose values of $31.84 \pm 0.48\%$ to $90.25 \pm 0.41\%$ (Table 2). The extract IC_{50} ($633 \mu\text{g/mL}$) closely matched its α -amylase IC_{50} ($638 \mu\text{g/mL}$), suggesting broadly comparable potency of the extract against both carbohydrate-digesting enzymes, while remaining less potent than acarbose (IC_{50} $518 \mu\text{g/mL}$).

Conc. ($\mu\text{g/mL}$)	Extract inhibition (%)	Acarbose inhibition (%)
200	24.77 ± 0.62	31.84 ± 0.48
400	40.02 ± 0.71	48.63 ± 0.52
600	57.46 ± 0.79	65.27 ± 0.45
800	70.93 ± 0.83	78.46 ± 0.43
1000	80.89 ± 0.86	90.25 ± 0.41
IC_{50} ($\mu\text{g/mL}$)	633	518

Table 2: α -Glucosidase inhibitory activity of *B. koenigii* extract versus acarbose.

DPP-4 inhibition also increased in a concentration-dependent manner, from 18% at $25 \mu\text{g/mL}$ to 78% at $400 \mu\text{g/mL}$, compared with 85% inhibition produced by sitagliptin under the same assay conditions (Table 3), placing the extract within 7 percentage points of the reference inhibitor at the highest concentration tested. An IC_{50} for DPP-4 was not derivable from the reported data range and is not claimed here.

Extract conc. ($\mu\text{g/mL}$)	DPP-4 inhibition (%)
25	18
50	30
100	44
200	62
400	78
Sitagliptin	85

Table 3: DPP-4 inhibitory activity of *B. koenigii* extract versus sitagliptin.

Across all three assays, the most consistent finding was a clear, reproducible concentration-response relationship, indicating that increasing extract concentration produced progressively greater enzyme inhibition under the tested in vitro conditions. The extract was less potent than the reference standards in every assay - by 9.4 percentage points for α -amylase, 9.4 percentage points for α -glucosidase, and 7 percentage points for DPP-4 at the respective highest concentrations tested - yet it retained substantial activity against all three targets. It should be noted that the α -amylase/ α -glucosidase assays (200 - $1000 \mu\text{g/mL}$) and the DPP-4 assay (25 - $400 \mu\text{g/mL}$) used different concentration ranges, so direct numerical comparison of inhibition percentages across assays should be made cautiously, and the DPP-4 dataset, unlike the other two, was reported without replicate SD values.

IV. Conclusion

The ethanolic extract of *Bergera koenigii* leaves demonstrated concentration-dependent in vitro inhibitory activity against three mechanistically distinct, clinically relevant antidiabetic targets: α -amylase (80.89% inhibition, IC_{50} $638 \mu\text{g/mL}$), α -glucosidase (80.89% inhibition, IC_{50} $633 \mu\text{g/mL}$), and DPP-4 (78% inhibition at $400 \mu\text{g/mL}$). Although less potent than the reference standards acarbose and sitagliptin, this multi-target enzyme-inhibitory profile supports the traditional use of curry leaf in diabetes management and its potential as a source of natural antidiabetic constituents. The findings represent preliminary in vitro evidence rather than proof of clinical efficacy. Future work should include triplicate DPP-4 measurements with reported SD, nonlinear regression to establish a DPP-4 IC_{50} , expanded concentration ranges bracketing the IC_{50} values, appropriate statistical significance testing, and bioassay-guided fractionation to identify the specific phytoconstituents responsible for the observed activity.

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