

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

N. Sundharraj¹, P. Subhashini², R. Suganya³, S. Ananthi^{4*}

N. Sundharraj¹, P. Subhashini², R. Suganya³ Student, Department of Pharmacology, Sri Vijay Vidyalaya College of Pharmacy, Dharmapuri, Tamil Nadu, India

S. Ananthi⁴ *Mentor, Department of Pharmacology, Sri Vijay Vidyalaya College of Pharmacy Dharmapuri, TamilNadu, India

Abstract - Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance and progressive β -cell dysfunction, and its management increasingly targets carbohydrate-digesting and incretin-degrading enzymes. The present study evaluated the in vitro antidiabetic potential of an ethanolic leaf extract of *Bergera koenigii* (curry leaf) through inhibition of α -amylase, α -glucosidase and dipeptidyl peptidase-4 (DPP-4). Leaves were shade-dried, powdered and extracted by Soxhlet extraction using ethanol. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, phenolics, terpenoids and steroids. Enzyme inhibition assays were performed at extract concentrations of 25–400 μ g/mL, using acarbose and sitagliptin as reference standards. The extract exhibited concentration-dependent inhibition of all three enzymes: α -amylase inhibition increased from 5.91% to 39.18%, α -glucosidase inhibition from 6.20% to 40.02%, and DPP-4 inhibition from 18% to 78% across the tested range, compared with 85% inhibition for sitagliptin at the highest concentration. The pronounced DPP-4 inhibitory response, together with a broad phytochemical profile rich in flavonoids and carbazole alkaloids, suggests that *Bergera koenigii* leaf extract is a promising natural candidate for further development as an antidiabetic agent, warranting IC_{50} determination and in vivo validation.

Key Words: *Bergera koenigii*; Type 2 Diabetes Mellitus; α -amylase; α -glucosidase; DPP-4 inhibition; phytochemical screening; natural antidiabetic agent.

1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent elevation of blood glucose levels resulting from defects in insulin secretion, insulin action, or both. Insulin, secreted by pancreatic β -cells, maintains glucose homeostasis by facilitating glucose uptake into peripheral tissues and regulating hepatic glucose output. When insulin production is insufficient or tissues become resistant to its action, glucose accumulates in the bloodstream, producing hyperglycemia that progressively damages multiple organ systems [2,3,20].

Diabetes has become one of the most significant global health challenges of the twenty-first century. Rapid urbanization, sedentary lifestyles, unhealthy dietary patterns, rising obesity rates and an ageing population have driven a steady increase in disease prevalence, placing substantial pressure on healthcare systems worldwide [17,20,39]. The disease is broadly classified into Type 1, Type 2, gestational and other specific forms of diabetes, of which Type 2 Diabetes Mellitus (T2DM) accounts for approximately 90–95% of all cases [21].

T2DM develops through a combination of insulin resistance in skeletal muscle, liver and adipose tissue, together with progressive pancreatic β -cell dysfunction. Compensatory hyperinsulinemia eventually fails under chronic metabolic stress, resulting in declining insulin secretion, increased hepatic glucose output and sustained hyperglycemia [10,21,36]. Current pharmacological management includes oral antidiabetic agents such as metformin, sulfonylureas, thiazolidinediones, sodium-glucose cotransporter-2 (SGLT2) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors, with insulin therapy reserved for advanced disease [9,12,28].

A key therapeutic strategy for controlling postprandial hyperglycemia is inhibition of the carbohydrate-digesting enzymes α -amylase and α -glucosidase, which delays starch hydrolysis and glucose absorption in the small intestine [33,38]. In parallel, DPP-4 inhibitors (“gliptins”) prolong the activity of the incretin hormones GLP-1 and glucose-dependent insulinotropic polypeptide (GIP), enhancing glucose-dependent insulin secretion and suppressing glucagon

release [1,18,27]. Although synthetic inhibitors of these enzymes are clinically effective, they are associated with gastrointestinal side effects and cost considerations, prompting growing interest in plant-derived enzyme inhibitors that may offer comparable efficacy with fewer adverse effects [5,24,25].

Bergera koenigii (*Murraya koenigii*; curry leaf), a member of the family Rutaceae, is a medicinal plant long used in traditional systems of medicine for digestive, antidiabetic and antioxidant purposes. Its leaves are rich in carbazole alkaloids (mahanimbine, koenigine, murrayacine), flavonoids and phenolic compounds, which underlie its reported antidiabetic, antioxidant, antimicrobial, anti-inflammatory, hepatoprotective and anticancer activities. The present study was undertaken to evaluate the *in vitro* antidiabetic potential of an ethanolic *Bergera koenigii* leaf extract through inhibition of α -amylase, α -glucosidase and DPP-4, key enzymatic targets in the management of T2DM.

2. AIM AND OBJECTIVES

Aim: To evaluate the anti-diabetic potential of *Bergera koenigii* (*Murraya koenigii*) leaf extract against Type 2 Diabetes Mellitus through *in vitro* enzyme inhibition studies.

Objectives

- To collect and authenticate *Bergera koenigii* leaves.
- To prepare an ethanolic leaf extract by Soxhlet extraction.
- To perform preliminary qualitative phytochemical screening of the extract.
- To estimate the total phenolic and flavonoid contents.
- To evaluate *in vitro* α -amylase inhibitory activity.
- To evaluate *in vitro* α -glucosidase inhibitory activity.
- To evaluate *in vitro* DPP-4 inhibitory activity.
- To determine the IC₅₀ values of the extract for each enzyme.
- To compare the antidiabetic activity with the standard drugs acarbose and sitagliptin.
- To assess the potential of *Bergera koenigii* as a natural antidiabetic agent.

3. LITERATURE REVIEW

A number of recent studies have examined the phytochemical composition and pharmacological activities of *Bergera koenigii*. Reviews by Asha Jyothi et al. (2025), Swami et al. (2025) and Bhalerao et al. (2025) have consistently identified carbazole alkaloids, flavonoids, tannins and phenolic compounds as the principal bioactive constituents responsible for the plant's antioxidant, antidiabetic, antimicrobial, anti-inflammatory and hepatoprotective properties. Mehta et al. (2025) and Sharma et al. (2024) emphasized the role of these constituents in inhibiting carbohydrate-metabolizing enzymes and improving glucose metabolism, while Franyoto et al. (2024) additionally reported neuroprotective and wound-healing activities together with a favourable toxicity profile at therapeutic doses.

Directly relevant to the present work, Ramesh et al. (2023) demonstrated dose-dependent inhibition of α -amylase and α -glucosidase by *Bergera koenigii* leaf extract, concluding that the plant may help reduce postprandial blood glucose levels. Patel et al. (2021) reported a significant reduction in blood glucose and improved insulin sensitivity following administration of the leaf extract, attributing the effect mainly to carbazole alkaloids and phenolic compounds. Singh et al. (2023) and Kesari et al. (2020) provided comprehensive phyto-pharmacological reviews supporting the plant's traditional use in diabetes and inflammation, while Kumar et al. (2022) confirmed strong free-radical scavenging activity associated with its flavonoid and polyphenol content. Collectively, these studies establish a consistent evidence base for the antidiabetic potential of *Bergera koenigii* and support its evaluation against additional enzymatic targets such as DPP-4, as undertaken in the present study.

4. MATERIALS AND METHODS

4.1 Collection and Authentication of Plant Material

Fresh leaves of *Bergera koenigii* (curry leaf) were collected, washed thoroughly with distilled water to remove surface impurities, and authenticated by a qualified botanist. A voucher specimen was preserved for future reference.

4.2 Preparation of Plant Material and Extraction

The washed leaves were shade-dried at room temperature and powdered using a mechanical grinder. About 50 g of the powdered material was extracted with ethanol (70–95%) using a Soxhlet apparatus for 6–8 hours. The extract was filtered through Whatman No. 1 filter paper, concentrated under reduced pressure using a rotary evaporator, dried to a semisolid mass, and stored in an airtight container at refrigerated conditions until further analysis.



Fig. 1: *Bergera koenigii* (curry leaf)

4.3 Preliminary Phytochemical Screening

Qualitative phytochemical screening was carried out using standard procedures to detect major classes of secondary metabolites, including alkaloids (Mayer's, Wagner's and Dragendorff's tests), flavonoids (alkaline reagent test), tannins and phenolics (ferric chloride and lead acetate tests), saponins (foam test), cardiac glycosides (Keller–Killiani test), and terpenoids/steroids (Salkowski's and Liebermann–Burchard tests).

4.4 Quantitative Phytochemical Analysis

Total phenolic content (TPC) was estimated by the Folin–Ciocalteu method using gallic acid as standard, with absorbance measured at 765 nm and results expressed as mg gallic acid equivalents (GAE)/g extract. Total flavonoid content (TFC) was estimated by the aluminium chloride colorimetric method using quercetin as standard, with absorbance measured at 415 nm and results expressed as mg quercetin equivalents (QE)/g extract. All determinations were performed in triplicate.

4.5 In Vitro α -Amylase Inhibition Assay

Extract concentrations of 25, 50, 100, 200 and 400 μ g/mL were incubated with α -amylase enzyme in sodium phosphate buffer for 10 minutes, followed by addition of starch substrate. The reaction was terminated with dinitrosalicylic acid (DNS) reagent, developed by heating in a boiling water bath, cooled, diluted, and read spectrophotometrically at 540 nm. Acarbose served as the reference standard.

4.6 In Vitro α -Glucosidase Inhibition Assay

The same range of extract concentrations was incubated with Baker's yeast α -glucosidase in phosphate buffer (pH 6.8) at 30 °C for 5 minutes, using p-nitrophenyl- α -D-glucopyranoside (pNPG) as substrate. The reaction was stopped with sodium hydroxide and absorbance measured at 410 nm, with acarbose as reference standard.

4.7 In Vitro DPP-4 Inhibition Assay

DPP-4 inhibitory activity was assessed using Gly-Pro-p-nitroanilide (Gly-Pro-pNA) as substrate in a 96-well microplate format. Extract concentrations of 25–400 μ g/mL were incubated with DPP-4 enzyme in assay buffer, and absorbance was measured at approximately 405 nm following blank correction. Sitagliptin was used as the reference DPP-4 inhibitor.

4.8 Calculation and Statistical Analysis

Percentage enzyme inhibition was calculated as: % Inhibition = [(Absorbance of control – Absorbance of sample) / Absorbance of control] $\times$ 100. IC₅₀ values were to be derived from concentration–response curves by non-linear regression. All assays were performed in triplicate and results expressed as mean $\pm$ standard deviation (SD).

5. RESULTS

Preliminary phytochemical screening of the ethanolic *Bergera koenigii* leaf extract gave positive results for alkaloids, flavonoids, tannins, saponins, glycosides, phenolics, terpenoids and steroids (Table 1), indicating a broad phytochemical profile consistent with the plant's reported pharmacological activities.

S.No.	Phytoconstituent	Test Used	Result
1	Alkaloids	Mayer's / Wagner's / Dragendorff's test	+
2	Flavonoids	Alkaline reagent test	+
3	Tannins	Ferric chloride test	+
4	Saponins	Foam test	+
5	Glycosides	Keller–Killiani test	+
6	Phenolics	Lead acetate test	+
7	Terpenoids	Salkowski's test	+
8	Steroids	Liebermann–Burchard test	+

Table 1: Preliminary phytochemical screening of *Bergera koenigii* leaf extract

Enzyme inhibition assays showed a clear concentration-dependent increase in activity against all three targets (Table 2, Fig. 2). α -Amylase inhibition rose from $5.91 \pm 0.42\%$ at $25 \mu\text{g/mL}$ to $39.18 \pm 0.69\%$ at $400 \mu\text{g/mL}$, while α -glucosidase inhibition increased from $6.20 \pm 0.45\%$ to $40.02 \pm 0.71\%$ over the same range, the two enzymes showing closely comparable responses throughout. DPP-4 inhibition showed the strongest response among the three assays, rising from 18% at $25 \mu\text{g/mL}$ to 78% at $400 \mu\text{g/mL}$, approaching the 85% inhibition produced by sitagliptin at the equivalent concentration.

Concentration ($\mu\text{g/mL}$)	α -Amylase Inhibition (%)	α -Glucosidase Inhibition (%)	DPP-4 Inhibition (%)	Sitagliptin (%)
25	5.91 ± 0.42	6.20 ± 0.45	18	—
50	9.84 ± 0.46	10.31 ± 0.48	30	—
100	15.72 ± 0.51	16.48 ± 0.53	44	—
200	23.64 ± 0.58	24.77 ± 0.62	62	—
400	39.18 ± 0.69	40.02 ± 0.71	78	85

Table 2: Percentage enzyme inhibition by *Bergera koenigii* leaf extract at increasing concentrations

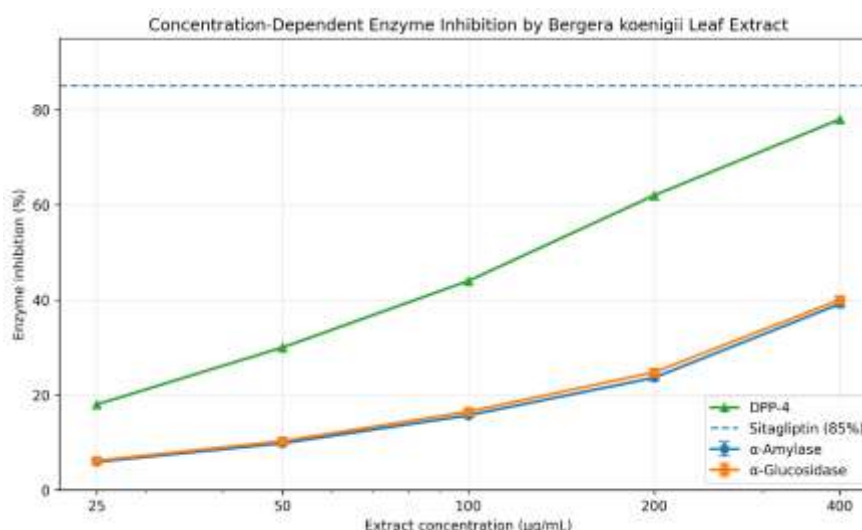


Fig. 2: Concentration-dependent inhibition of α -amylase, α -glucosidase and DPP-4 by *Bergera koenigii* leaf extract, with sitagliptin (85%) shown for reference

Numerical IC_{50} values were not derived within the scope of the reported concentration range and require concentration–response regression using the original replicate absorbance data; a numerical acarbose control result was likewise not obtained for direct comparison with the α -amylase and α -glucosidase assays.

6. DISCUSSION

The present study demonstrates that ethanolic *Bergera koenigii* leaf extract inhibits α -amylase, α -glucosidase and DPP-4 in a concentration-dependent manner. The comparable responses observed for α -amylase and α -glucosidase suggest a shared mechanism of interaction, potentially through binding of phenolic and flavonoid constituents at or near the enzyme active sites, consistent with previous reports of plant-derived carbohydrate-hydrolysing enzyme inhibitors [24,30,33]. The markedly stronger DPP-4 inhibitory response indicates a greater apparent affinity of the extract's constituents for this target under the assay conditions tested.

The diverse phytochemical profile of the extract — comprising alkaloids, flavonoids, tannins, saponins, glycosides, phenolics, terpenoids and steroids — provides a plausible chemical basis for the observed enzyme inhibition, particularly given the established antioxidant and antidiabetic properties of carbazole alkaloids and flavonoids reported for this plant. However, the present data do not identify which individual constituent is responsible for the activity, and this relationship should be regarded as a plausible interpretation rather than a confirmed mechanism.

At 400 $\mu\text{g/mL}$, DPP-4 inhibition (78%) approached, but did not equal, the inhibition produced by sitagliptin (85%); this finding is encouraging but does not establish equivalence to the standard drug in the absence of matched dosing and statistical comparison. Similarly, α -amylase and α -glucosidase inhibition remained below 50% at the highest concentration tested, indicating comparatively weaker activity against these two targets over the tested range. Further work should include complete concentration–response curves, replicate-level data, IC_{50} calculation by non-linear regression, statistical comparison with acarbose and sitagliptin, quantitative TPC/TFC measurement, and identification of the specific active constituents, ideally supported by *in vivo* validation.

7. CONCLUSION

The ethanolic leaf extract of *Bergera koenigii* showed a positive and broad phytochemical profile and exhibited concentration-dependent inhibitory activity against α -amylase, α -glucosidase and DPP-4, three key enzymatic targets in the management of Type 2 Diabetes Mellitus. α -Amylase inhibition increased from 5.91% to 39.18%, α -glucosidase inhibition from 6.20% to 40.02%, and DPP-4 inhibition from 18% to 78% across the 25–400 $\mu\text{g/mL}$ concentration range, with the extract's DPP-4 inhibition at 400 $\mu\text{g/mL}$ (78%) approaching that of sitagliptin (85%). These findings support *Bergera koenigii* as a promising natural source of antidiabetic enzyme inhibitors and justify further investigation, including IC_{50} determination, active-constituent isolation, and *in vivo* evaluation, before its potential therapeutic application can be established.

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