Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 23s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Formulation, Standardization, and in Vitro Pharmacological Assessment of a Polyherbal Blend for the Management of Type 2 Diabetes and Protection against Cisplatin-Induced Nephrotoxicity

Priyanka Arvind Shelke[1], Supriya Chatla[2], Shankar Gavaroji[*3], Rashmi Mohapatra[4], Ashutosh Pathak[5], Nilkamal Waghmare[6], Rohini Armo[7], Yeolekar Varad[8]

[1]Department of Pharmaceutical Chemistry, K.T. Patil College of Pharmacy Dharashiv (Maharashtra) India Pin- 413501.

[2]Department of Pharmacology, KVSR Siddhartha College of Pharmaceutical sciences, Vijayawada, Andhra Pradesh, 520010.

[*3]Department of Pharmaceutics, Siddharth College of Pharmacy, Mudhol-587313, Dist. Bagalkot, Karnataka, India.

[4]Department of Botany and Head, Centre for Indigenous Knowledge on Herbal Medicines and Therapeutics, Kalinga Institute of Social Sciences (KISS) Deemed to be University. Bhubaneswar, Odisha, Pin: 751024.

[5]Department of Pharmacy Practice, Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad UP, India Pin- 244001.

[6]Department of Pharmaceutics, Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai – 400614.

[7]Department of Pharmaceutical Chemistry, Shri Rawatpura sarkar institute of Pharmacy, kumhari, Durg Chhattisgarh, India Pin- 490042.

[8]Department of Pharmaceutics, DES, Dayanand College of Pharmacy, Latur. (MH) India Pin- 413512.

***Corresponding Author:**

Shankar Gavaroji

Department of Pharmaceutics, Siddharth College of Pharmacy, Mudhol-587313, Dist. Bagalkot, Karnataka, India

Email ID: dcshankar4u@gmail.com

Cite this paper as: Priyanka Arvind Shelke, Supriya Chatla, Shankar Gavaroji, Rashmi Mohapatra, Ashutosh Pathak, Nilkamal Waghmare, Rohini Armo, Yeolekar Varad, (2025) Formulation, Standardization, and in Vitro Pharmacological Assessment of a Polyherbal Blend for the Management of Type 2 Diabetes and Protection against Cisplatin-Induced Nephrotoxicity. *Journal of Neonatal Surgery*, 14 (23s), 23-34.

ABSTRACT

The present study focuses on the development and pharmacological evaluation of a novel herbal formulation (RG-PV-HF) for its antidiabetic and nephroprotective potential in in vitro models. The extract was prepared using the cold maceration method and subjected to phytochemical screening, confirming the presence of flavonoids, tannins, phenols, saponins, alkaloids, and glycosides. The total phenolic content was determined using the Folin-Ciocalteu method, yielding 317.87 mg GAE/g extract. The extract exhibited α-amylase and α-glucosidase inhibition in a concentration-dependent manner, with IC_{50} values of 592.88 µg/mL and 321.77 µg/mL, respectively. Additionally, DPP-4 inhibition was observed, suggesting a role in incretin-based glucose regulation. The cytotoxicity assessment using the MTT assay on HEK-293 cells demonstrated high cell viability, confirming its biocompatibility. Furthermore, RG-PV-HF provided significant protection against cisplatin-induced nephrotoxicity, highlighting its potential antioxidant and nephroprotective effects. The findings suggest that RG-PV-HF could be a promising natural therapeutic candidate for managing Type 2 diabetes and nephrotoxicity, warranting further investigation into its mechanism of action and in vivo efficacy.

Keywords: *Antidiabetic, Nephroprotective, Herbal formula, DPP-4 inhibition, Type 2 Diabetes.*

1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels due to impaired insulin secretion, action, or both. Among the different types, Type 2 diabetes mellitus (T2DM) is the most prevalent and is primarily associated with insulin resistance and β-cell dysfunction. The condition significantly increases the risk of cardiovascular diseases, neuropathy, nephropathy, and retinopathy, leading to substantial morbidity and mortality worldwide (Ahsan et al., 2023; Bansal et al., 2015; Berlin Grace et al., 2020; Darenskaya et al., 2021; Ikewuchi et al., 2009; Jacobs, 1993; Pareek et al., 2009). Managing diabetes involves controlling postprandial glucose levels, which are largely regulated by digestive

enzymes such as α-amylase and α-glucosidase and hormonal pathways like the incretin system (DPP-4 enzyme activity). Traditional pharmacological interventions, including α-glucosidase inhibitors, DPP-4 inhibitors, and insulin sensitizers, are widely used but often come with adverse effects such as gastrointestinal disturbances and hypoglycaemia. Thus, there is a growing interest in plant-based therapeutics that offer effective glycaemic control with minimal side effects (Luan et al., 2020; Pan et al., 2018).

In addition to diabetes, nephrotoxicity remains a significant clinical challenge, particularly in patients undergoing chemotherapy with drugs like cisplatin. Cisplatin-induced nephrotoxicity is primarily caused by oxidative stress, mitochondrial dysfunction, and apoptosis, leading to renal damage. Since oxidative stress plays a pivotal role in both diabetes and nephrotoxicity, antioxidant-rich herbal formulations may provide dual benefits by improving glucose metabolism and protecting against kidney damage (Cattin, 2016; Melmer & Laimer, 2016; Papatheodorou et al., 2015). Polyherbal formulations, which combine multiple bioactive compounds from different medicinal plants, have been widely explored for their synergistic therapeutic effects, targeting multiple pathways simultaneously (Andriana et al., 2019; Ikewuchi & Ikewuchi, 2009; Luan et al., 2020; Pan et al., 2018).

The present study focuses on the development and pharmacological evaluation of a novel herbal formulation (RG-PV-HF) composed of extracts from *Ruta graveolens* (L.) and *Prunella vulgaris* (L.), two medicinal plants known for their antidiabetic, antioxidant, and nephroprotective properties (Ikewuchi et al., 2009; Pareek et al., 2009; Petchi et al., 2013). *Ruta graveolens* (L.), commonly known as rue, has been traditionally used for its anti-inflammatory, antimicrobial, and hypoglycaemic effects, attributed to its rich flavonoid and alkaloid content. *Prunella vulgaris* (L.), commonly known as self-heal, is well-documented for its antioxidant, anti-inflammatory, and anti-diabetic activities, with bioactive components such as phenolic acids, flavonoids, and terpenoids. Combining these plant extracts into a standardized herbal formulation is hypothesized to provide enhanced therapeutic benefits, particularly in modulating glucose metabolism and mitigating nephrotoxicity (Cattin, 2016; Darenskaya et al., 2021; Melmer & Laimer, 2016; Papatheodorou et al., 2015; Richter et al., 2023; Sun et al., 2021; The, 2017).

The study aims to evaluate the phytochemical profile, enzyme inhibitory activity, nephroprotective potential, and cytotoxicity of the herbal formula codenamed RG-PV-HF fabricated from extracts of *Ruta graveolens* (L.) and *Prunella vulgaris* (L.). The preliminary phytochemical screening was conducted to identify key bioactive groups such as flavonoids, phenolics, tannins, and alkaloids, which contribute to the pharmacological properties of the formulation. The total phenolic content (TPC) was quantified using the Folin-Ciocalteu method, as phenolics are key contributors to antioxidant and anti-inflammatory mechanisms. The inhibition of α-amylase, α-glucosidase, and DPP-4 enzymes was assessed to determine the extract's potential in regulating postprandial glucose levels and incretin hormone activity. The cytotoxicity of RG-PV-HF was evaluated using the MTT assay on HEK-293 cells, ensuring its biocompatibility. Additionally, its nephroprotective potential was assessed against cisplatin-induced toxicity, a well-established model for studying oxidative stress-mediated renal damage. By integrating in vitro enzymatic assays, cytotoxicity evaluations, and nephroprotective assessments, this study provides a comprehensive understanding of the therapeutic potential of RG-PV-HF. The findings will contribute to evidence-based validation of polyherbal formulations for diabetes management and renal protection, paving the way for further preclinical and clinical investigations into their therapeutic applications.

2. MATERIAL AND METHODS

Materials

Biochemical Kits, Chemicals, Reagents and Drugs

All chemicals and reagents used in this study were of analytical grade, ensuring high purity and reliability in experimental procedures. Various solvents, including methanol, acetone, chloroform, petroleum ether, and water, were employed for the extraction process to maximize the yield of bioactive compounds. For cell culture studies, Dulbecco's Modified Eagle Medium (DMEM) was used as the basal medium, supplemented with fetal bovine serum (FBS) to provide essential nutrients for the growth and maintenance of L6 myoblast cells. Additionally, penicillin and streptomycin, sourced from Himedia (India), were included in the culture medium to prevent bacterial contamination and maintain sterile conditions. To induce oxidative stress in L6 myoblasts, hydrogen peroxide (H_2O_2) was obtained from E Merck (India). This reactive oxygen species (ROS) was used to mimic oxidative damage, facilitating the evaluation of potential protective effects of test compounds. Diprotin A, a well-known DPP-4 inhibitor, was purchased from Sigma-Aldrich (Mumbai, India) and served as a reference standard in the DPP-4 inhibition assay. For biochemical assessments, glucose levels were quantified using the glucose oxidase-peroxidase (GOD-POD) assay kit, which provides a reliable enzymatic method for glucose determination. Meanwhile, DPP-4 enzyme inhibition was evaluated using a commercially available DPP-4 assay kit from Cayman, ensuring accuracy in measuring enzymatic activity. All experiments were conducted following the protocols provided by the manufacturers to maintain consistency and reliability of results. Moreover, all reagents were stored under appropriate conditions, including temperature and light exposure, to preserve their stability and efficacy throughout the study.

Collection of the plant material

The leaves of *Ruta graveolens* (L.) and the leaves and flowers of *Prunella vulgaris* (L.) were collected from the Kolkata region in West Bengal for this study. These plants were carefully selected based on their traditional medicinal significance and reported pharmacological properties. The collection was carried out during the appropriate season to ensure optimal phytochemical content and biological activity. The botanical identification and authentication of the collected plant materials were performed by Dr. Biplob Dey, a recognized freelance botanist and herbal healer from West Bengal. His expertise ensured the accurate classification of the species, reducing the possibility of misidentification, which is critical for maintaining the reliability and reproducibility of pharmacognostic studies. To ensure proper documentation and future reference, herbarium specimens of both *Ruta graveolens* and *Prunella vulgaris* were meticulously prepared following standard herbarium preservation techniques. Each specimen was carefully labeled with a unique accession number — MK/PHACOG/2023/RG/0569 for *Ruta graveolens* and MK/PHACOG/2023/PV/0576 for *Prunella vulgaris*. These specimens were systematically archived in the pharmacognosy laboratory, where they will serve as permanent reference materials. The preservation of herbarium specimens is crucial for scientific validation and comparative studies, allowing researchers to authenticate plant material in future investigations. These records will not only aid in taxonomic verification but also support further research on the medicinal properties, chemical composition, and potential therapeutic applications of these plants. Additionally, this meticulous documentation enhances the credibility of the study by providing a traceable source of plant materials for subsequent experimental analyses.

Preparation of Extracts Using the Cold Maceration Method

The dried leaves of *Ruta graveolens* (L.) and the dried leaves and flowers of *Prunella vulgaris* (L.) were separately subjected to extraction using the cold maceration method. Initially, the plant materials were thoroughly cleaned to remove any surface impurities and dried under shade to preserve their phytochemical integrity. Once dried, the materials were coarsely powdered using a mechanical grinder and stored in airtight containers until further use. For the extraction process, a weighed quantity of the powdered plant material was soaked in a sufficient volume of hydroalcoholic solvent (70% ethanol) in a clean glass container. The mixture was allowed to macerate at room temperature for 72 hours with occasional shaking to ensure thorough diffusion of bioactive constituents. After the extraction period, the mixtures were filtered using muslin cloth followed by Whatman filter paper No. 1 to remove plant debris. The filtrates were concentrated under reduced pressure using a rotary evaporator at 40°C to obtain the semi-solid crude extracts. These concentrated extracts were then dried completely using a water bath to remove residual solvents. The final extracts of *Ruta graveolens* (RG) and *Prunella vulgaris* (PV) were mixed in a 1:1 ratio to formulate a standardized herbal combination. This blended extract was assigned the code RG-PV-HF for further pharmacological and analytical evaluations. The prepared herbal formula was stored in an airtight amber-colored glass container at 4°C to maintain stability and prevent degradation of bioactive compounds (Sasidharan et al., 2011; Sultana et al., 2009).

% Yield= Extract Amount (gm)/ Initial dry powder drug amount (gm) ×100

Preliminary phytochemical Screening

The preliminary phytochemical screening of the herbal extract RG-PV-HF was conducted following the standard procedures outlined by Harborne (1998) (Harborne, 1998). This analysis aimed to identify key bioactive constituents responsible for the extract's pharmacological potential. The presence of alkaloids was confirmed through Mayer's, Dragendorff's, and Wagner's tests, where the formation of white, orange-red, or reddish-brown precipitates indicated positive results. Flavonoids were detected using the alkaline reagent test, where an intense yellow coloration turned colorless upon acid addition, and the Shinoda test, which produced a pink or red hue in the presence of magnesium and hydrochloric acid. Phenolic compounds were confirmed by the ferric chloride test, where a deep blue or green color developed, while tannins were identified through the gelatin and lead acetate tests, resulting in a white precipitate. Saponins were detected by the formation of persistent froth upon vigorous shaking with water, indicating their surfactant-like properties. Glycosides were identified through the Keller-Killiani test, which produced a reddish-brown ring at the interface, confirming the presence of deoxy sugars. The presence of terpenoids was validated using the Salkowski test, where a reddish-brown interface developed upon treatment with sulfuric acid and chloroform. The Liebermann-Burchard test confirmed the presence of steroids, as indicated by a greenish-blue coloration. Carbohydrates were detected through Molisch's test, where a purple ring was observed at the interface, and the presence of reducing sugars was further confirmed using Benedict's and Fehling's tests. Proteins and amino acids were identified using the Biuret and Ninhydrin tests, where the formation of violet, purple, or blue colors indicated positive results. The findings of this preliminary phytochemical screening suggest that RG-PV-HF contains a diverse range of bioactive compounds, supporting its potential therapeutic applications.

Total Phenolic Compounds assay

The total phenolic content of the RG-PV-HF extract was determined using the Folin-Ciocalteu (FC) method, as described by Olayinka and Anthony (2009) (Olayinka & Anthony, 2009). A precisely measured volume of the extract was mixed with the Folin-Ciocalteu reagent, followed by the addition of sodium carbonate to initiate the reaction. The mixture was incubated at room temperature for a specific duration to allow the formation of a blue-colored complex, which is indicative of the presence of phenolic compounds. The absorbance of the developed color was measured using a UV-Visible spectrophotometer at a

predetermined wavelength, typically around 760 nm. A standard calibration curve was prepared using gallic acid as the reference compound, and the total phenolic content was expressed as milligrams of gallic acid equivalent (mg GAE) per gram of extract. All measurements were performed in triplicate to ensure accuracy and reproducibility. The results provided an estimate of the total phenolic constituents present in the extract, which are known to contribute to its antioxidant and therapeutic properties.

Antidiabetic activity

Inhibition of alpha amylase enzyme

The inhibition of the alpha-amylase enzyme by the RG-PV-HF extract was assessed using a modified spectrophotometric method {Nair, 2013 #147}. A reaction mixture containing a specific concentration of the extract, phosphate buffer (pH 6.9), and porcine pancreatic alpha-amylase enzyme was incubated at 37°C for a defined period. After incubation, the enzymatic reaction was initiated by adding a starch solution as the substrate, followed by further incubation to allow the enzyme to act. The reaction was then terminated by adding dinitrosalicylic acid (DNS) reagent, which facilitated the development of a colored complex upon heating. The absorbance of the resulting solution was measured at 540 nm using a UV-Visible spectrophotometer, and the percentage inhibition of alpha-amylase activity was calculated by comparing the absorbance of the treated samples with that of the control (without extract). Acarbose was used as the standard inhibitor for comparison. The inhibition of alpha-amylase by RG-PV-HF was expressed as a percentage, indicating its potential role in regulating postprandial glucose levels by reducing carbohydrate digestion. All experiments were performed in triplicate to ensure reproducibility and accuracy.

Inhibition of alpha glucosidases enzyme

The inhibition of the alpha-glucosidase enzyme by the RG-PV-HF extract was evaluated using a modified spectrophotometric method {Nair, 2013 #148}. A reaction mixture containing the extract at different concentrations, phosphate buffer (pH 6.8), and alpha-glucosidase enzyme was incubated at 37°C for a specified period to allow interaction. After incubation, the enzymatic reaction was initiated by adding p-nitrophenyl-α-D-glucopyranoside (pNPG) as the substrate. The reaction was further incubated to allow the enzyme to hydrolyze pNPG into p-nitrophenol, which resulted in a yellow-colored product. The reaction was terminated by adding sodium carbonate, and the absorbance of the liberated p-nitrophenol was measured at 405 nm using a UV-Visible spectrophotometer. The percentage inhibition of alpha-glucosidase activity was calculated by comparing the absorbance of the extract-treated samples with that of the control (without extract). Acarbose was used as the standard inhibitor for comparison. The inhibition of alpha-glucosidase by RG-PV-HF was expressed as a percentage, indicating its potential to delay carbohydrate digestion and glucose absorption, which could be beneficial for managing postprandial hyperglycaemia. All experiments were conducted in triplicate to ensure reliability and reproducibility of the results.

DPP-4 Inhibition Assay

The DPP-4 inhibition assay was performed following a modified version of the method described elsewhere (Al-masri et al., 2009). The assay aimed to evaluate the ability of the RG-PV-HF extract to inhibit dipeptidyl peptidase-4 (DPP-4), an enzyme involved in the degradation of incretin hormones, thereby playing a crucial role in glucose metabolism. A reaction mixture was prepared by adding a specific concentration of the extract to a buffer solution containing DPP-4 enzyme and incubated at 37°C for a defined period. The enzymatic reaction was initiated by introducing Gly-Pro-p-nitroanilide, a chromogenic substrate, which upon cleavage by DPP-4, releases p-nitroaniline, resulting in a measurable yellow color. The reaction progress was monitored by measuring absorbance at 405 nm using a UV-Visible spectrophotometer. The percentage inhibition of DPP-4 was calculated by comparing the absorbance of the extract-treated samples with that of the control (enzyme activity without the extract). Diprotin A, a well-known DPP-4 inhibitor, was used as a reference standard. The assay was conducted in triplicate to ensure accuracy and reproducibility. The results provided insight into the potential of RG-PV-HF as a natural DPP-4 inhibitor, which could be beneficial in diabetes management by prolonging incretin hormone activity and enhancing insulin secretion.

Nephroprotective Activity

Evaluation Against Cisplatin-Induced Toxicity in HEK-293 Cells

The nephroprotective potential of RG-PV-HF was evaluated against cisplatin-induced toxicity in HEK-293 cells using the MTT assay to assess cell viability. HEK-293 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin and maintained at 37°C in a 5% CO_2 incubator. The cells were seeded in 96-well plates and allowed to adhere overnight before treatment. To induce nephrotoxicity, the cells were exposed to cisplatin (10–100 µM) for 24 hours. The protective effect of RG-PV-HF was evaluated by pretreating the cells with varying concentrations of the extract for 2 hours, followed by exposure to cisplatin. After the treatment period, 0.5 mg/mL of MTT solution was added to each well and incubated for 4 hours to allow the formation of formazan crystals. The crystals were then dissolved using dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader. The percentage of cell viability was calculated to determine the protective

effect of RG-PV-HF against cisplatin-induced cytotoxicity. The assay was performed in triplicate, and results were expressed as mean ± standard deviation (SD) (Kpemissi et al., 2019; Singh et al., 2018).

Statistical analysis

The statistical analysis of the experimental data was performed using GraphPad Prism software, ensuring accurate interpretation of the results. All values were expressed as mean ± standard deviation (SD) from at least three independent experiments conducted in triplicate. The significance of differences between groups was determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. The results were analyzed to determine the effectiveness of RG-PV-HF in improving cell viability and reducing cytotoxicity, providing insights into its nephroprotective potential.

3. RESULTS AND DISCUSSION

Preliminary phytochemical Screening

The preliminary phytochemical screening of the RG-PV-HF extract revealed the presence of a diverse range of bioactive compounds, indicating its potential pharmacological significance. The detection of flavonoids, tannins, and phenols suggests strong antioxidant and anti-inflammatory properties, which may contribute to the extract's therapeutic effects in metabolic disorders and oxidative stress-related conditions. The presence of phytosterols indicates possible cholesterol-lowering and anti-inflammatory benefits, which could be advantageous in managing diabetes and nephrotoxicity. The confirmation of glycosides is significant, as these compounds are known for their cardioprotective and potential anti-diabetic effects by influencing glucose metabolism. The presence of carbohydrates suggests that the extract contains essential polysaccharides, which may enhance its bioavailability and therapeutic efficacy. The detection of saponins indicates possible hypoglycemic and nephroprotective effects, as these compounds are known to modulate glucose uptake and exhibit kidney-protective properties. The presence of terpenoids suggests potential anti-inflammatory and anti-diabetic activity, contributing to the overall pharmacological profile of the extract. The detection of alkaloids further enhances its therapeutic potential, as these bioactive compounds are known for their diverse pharmacological effects, including anti-hyperglycemic and nephroprotective actions. Lastly, the presence of proteins may play a role in cellular repair mechanisms, further supporting the extract's protective effects against nephrotoxicity. The combined presence of these bioactive compounds in RG-PV-HF highlights its potential as a promising candidate for diabetes management and nephroprotection, warranting further in-depth pharmacological investigations.

Tablet 1. The findings of preliminary phytochemical screening of RG-PV-HF

Phytochemical Group	RG-PV-HF
Flavonoids	+
Tannins	+
Phenols	+
Phytosterols	+
Glycosides	+
Carbohydrates	+
Saponins	+
Terpenoids	+
Alkaloids	+
Proteins	+

Total Phenolic Compounds determination

The linear regression analysis for the RG-PV-HF extract demonstrated a strong correlation between absorbance and concentration, as indicated by the equation $y = 0.7526x - 0.0175$ with an R^2 value of 0.9906. The high coefficient of determination ($R^2 = 0.9906$) suggests an excellent fit of the data to the standard calibration curve, ensuring the reliability and accuracy of the total phenolic content estimation. The total phenolic content of RG-PV-HF was found to be 317.87 mg GAE/g of extract, indicating a significant presence of phenolic compounds. These bioactive compounds are known for their strong antioxidant, anti-inflammatory, and free radical-scavenging properties, which may contribute to the therapeutic

potential of the extract in managing oxidative stress-related disorders, including diabetes and nephrotoxicity. The high phenolic content further supports the pharmacological relevance of RG-PV-HF, warranting further investigation into its mechanism of action and potential therapeutic applications.

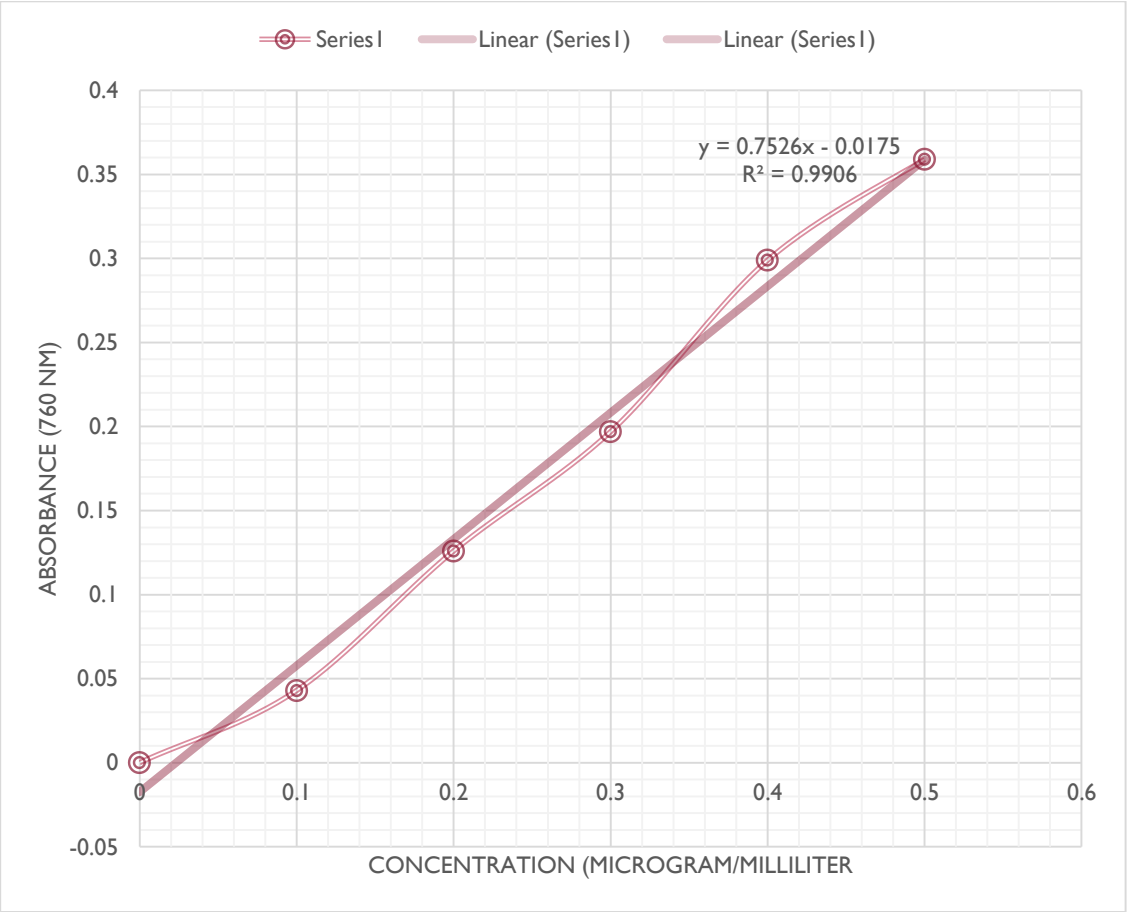

Figure 1. Standard curve of Gallic acid and linear regression analysis

Table 2. The linear regression analysis equations and the total phenolic content of each extract

Extracts Name	Regression Equation	Total Phenolic content in GAE per gram of extract
RG-PV-HF	y = 0.7526x - 0.0175 R^2 = 0.9906	317.87

Antidiabetic activity

Inhibition of alpha amylase enzyme

The α-amylase inhibition assay for the RG-PV-HF extract demonstrated a concentration-dependent inhibitory effect on the enzyme, with inhibition percentages increasing from 25.79 ± 0.89% at 100 µg/mL to 66.47 ± 0.99% at 1000 µg/mL. The IC_{50} value, representing the concentration required to inhibit 50% of α-amylase activity, was calculated as 592.88 µg/mL, indicating moderate enzyme inhibition. In comparison, acarbose (standard inhibitor) exhibited a stronger inhibitory effect, with an IC_{50} value of 287.65 µg/mL, and inhibition ranging from 36.81 ± 0.78% at 100 µg/mL to 78.87 ± 0.99% at 1000 µg/mL. Although RG-PV-HF displayed lower potency than acarbose, its inhibition of α-amylase suggests a potential role in delaying carbohydrate digestion and glucose absorption, making it beneficial for managing postprandial hyperglycemia. The presence of phenolics, flavonoids, and tannins in the extract may contribute to this inhibitory activity, as these compounds are known to interact with digestive enzymes. While the inhibition observed is lower than that of the standard, the extract's natural composition and potential for reduced side effects compared to synthetic inhibitors make it a promising candidate for further investigation in diabetes management. Additional studies on enzyme kinetics, structural interactions, and synergistic

effects with other bioactive compounds may help enhance its therapeutic potential.

Table 3. Results for α-amylase inhibition along with the IC_{50} values.

Sample	Concentration (µg/ml)	% Inhibition (Mean ± SD)	IC_{50} (µg/ml)
RG-PV-HF	100	25.79 ± 0.89	**592.88**
	200	35.92 ± 0.87	
	400	44.83 ± 0.90	
	800	60.73± 0.96	
	1000	66.47 ± 0.99	
Acarbose (Standard)	100	36.81 ± 0.78	**287.65**
	200	54.44 ± 0.84	
	400	64.36± 0.87	
	800	72.43± 0.98	
	1000	78.87 ± 0.99	

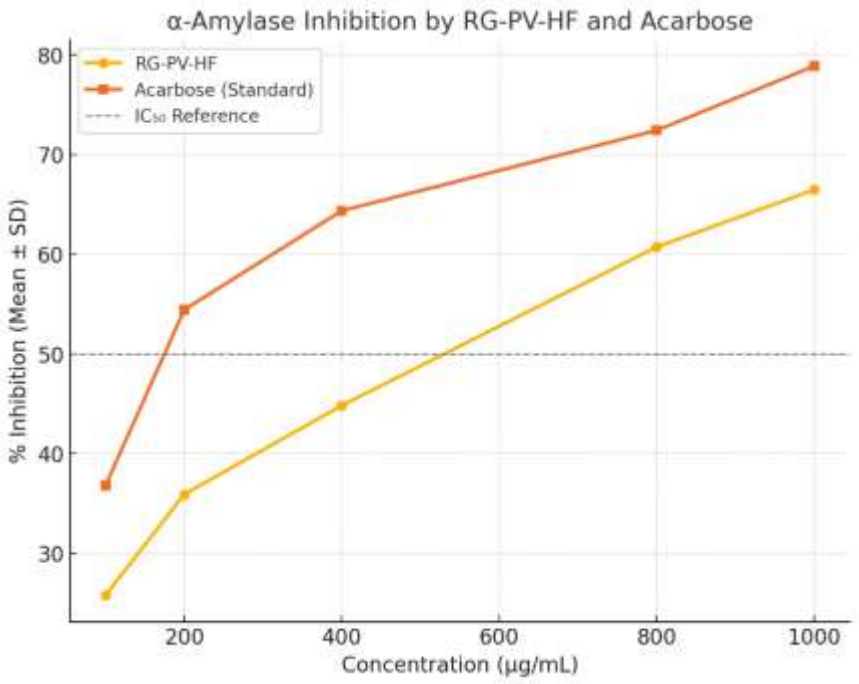

Figure 2. Results for α-amylase inhibition along with the IC_{50} values.

Inhibition of alpha glucosidases enzyme

The α-amylase inhibition assay for RG-PV-HF revealed a dose-dependent increase in enzyme inhibition, with values ranging from 25.79 ± 0.89% at 100 µg/mL to 66.47 ± 0.99% at 1000 µg/mL. The IC_{50} value of the extract was determined to be 592.88 µg/mL, indicating moderate inhibition of α-amylase activity. Comparatively, acarbose (standard inhibitor) exhibited a significantly higher inhibitory effect, with an IC_{50} of 287.65 µg/mL, showing stronger inhibition across all tested concentrations, from 36.81 ± 0.78% at 100 µg/mL to 78.87 ± 0.99% at 1000 µg/mL. Although RG-PV-HF was less potent than acarbose, its inhibitory activity suggests a potential role in modulating carbohydrate digestion and glucose absorption, which may help regulate postprandial blood sugar levels. The moderate inhibition observed in the extract could be attributed to the presence of flavonoids, tannins, and phenolic compounds, which are known to interfere with α-amylase activity by

binding to the enzyme's active site. While acarbose demonstrated stronger inhibition, its use is often associated with gastrointestinal side effects. In contrast, the natural origin of RG-PV-HF may offer a safer alternative with fewer adverse effects, making it a promising candidate for further exploration in diabetes management. Future studies focusing on enzyme kinetics, structural interactions, and in vivo efficacy will provide deeper insights into its therapeutic potential.

Table 4. Results for α-glucosidase inhibition along with the IC_{50} values.

Sample	Concentration (µg/ml)	% Inhibition (Mean ± SD)	IC_{50} (µg/ml)
RG-PV-HF	100	35.88 ± 0.79	**321.77**
	200	44.86 ± 0.96	
	400	61.93 ± 0.89	
	800	69.45 ± 1.01	
	1000	90.68 ± 0.99	
Acarbose (Standard)	100	45.58 ± 1.02	**128.84**
	200	54.90 ± 1.01	
	400	68.87 ± 0.99	
	800	77.74 ± 1.01	
	1000	96.65 ± 1.08	

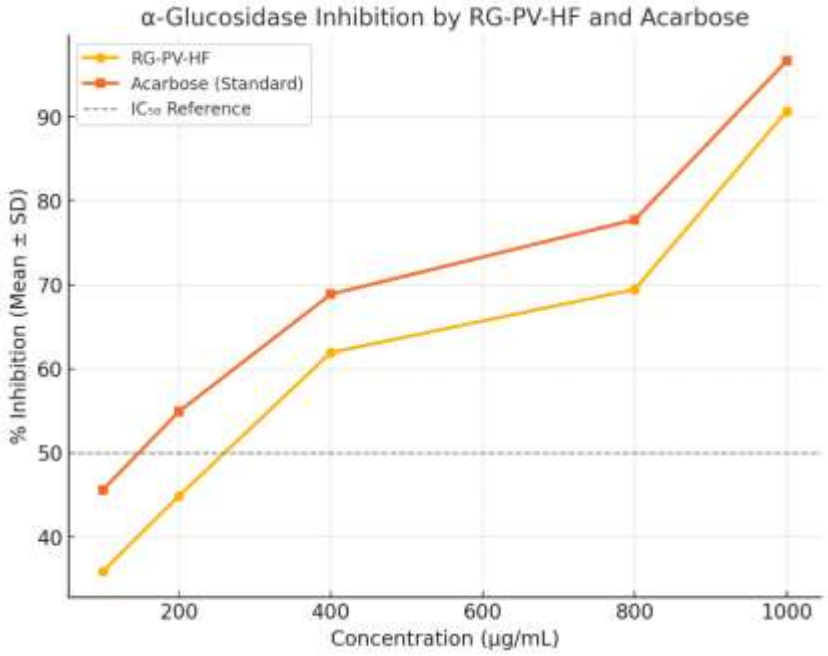

Figure 3. Results for α-glucosidase inhibition along with the IC_{50} values.

Evaluation of DPP-4 Inhibition Assay

The DPP-4 inhibition assay was conducted to evaluate the ability of RG-PV-HF to inhibit dipeptidyl peptidase-4 (DPP-4), an enzyme involved in incretin hormone degradation, which plays a crucial role in glucose homeostasis. The results indicated that Diprotin A (standard inhibitor) at 65 µg/mL exhibited a significantly high inhibition of 84.78 ± 1.25%, demonstrating its strong DPP-4 inhibitory potential. In comparison, RG-PV-HF at 50 µg/mL showed an inhibition of 14.87 ± 1.02%, while an increased concentration of 100 µg/mL resulted in a higher inhibition of 37.92 ± 1.15%, indicating a concentration-dependent inhibitory effect. Although RG-PV-HF exhibited a lower inhibitory potential compared to Diprotin A, its activity

suggests a promising role in modulating DPP-4 activity, which may contribute to prolonged incretin hormone action and improved insulin secretion. The presence of flavonoids, tannins, and phenolic compounds in RG-PV-HF may be responsible for its inhibitory action, as these bioactive constituents have been reported to interact with DPP-4 enzymatic pathways. While the inhibition levels were moderate, the extract's natural composition could offer a safer alternative to synthetic DPP-4 inhibitors, with potentially fewer side effects. Further studies, including enzyme kinetics, molecular docking, and in vivo validation, would be necessary to explore its full potential in diabetes management.

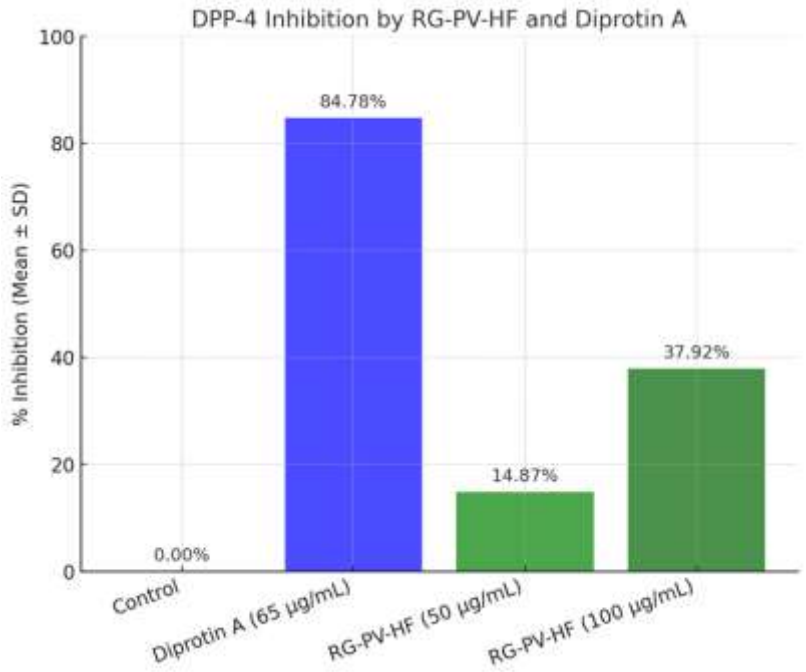

Figure 4. The effect of RG-PV-HF on the percentage of DPP-4 inhibition.

Nephroprotective activity evaluation

Effect of RG-PV-HF on the toxicity caused by cisplatin in HEK-293 cells

The cytotoxicity evaluation of RG-PV-HF was assessed using a cell viability assay and compared to Docetaxel (standard anticancer drug) across different concentrations (20–280 µg/mL). The results showed that RG-PV-HF exhibited moderate cytotoxicity, with cell viability decreasing in a concentration-dependent manner. At 20 µg/mL, cell viability remained high (93.14 ± 2.11%), indicating minimal cytotoxic effects. Even at 120 µg/mL, cell viability was 81.68 ± 1.04%, suggesting that RG-PV-HF maintains considerable biocompatibility at lower concentrations. However, at higher concentrations (200 µg/mL and 280 µg/mL), the viability dropped to 60.89 ± 1.02% and 42.96 ± 1.02%, respectively, indicating increased cytotoxic effects at elevated doses. In contrast, Docetaxel (standard) exhibited a much stronger cytotoxic effect across all concentrations. At 20 µg/mL, cell viability was significantly lower (63.81 ± 1.32%) compared to RG-PV-HF (93.14 ± 2.11%), and it further declined to 21.56 ± 1.02% at 280 µg/mL, confirming its potent anticancer activity. The results suggest that RG-PV-HF is relatively non-toxic at lower concentrations, making it a safer candidate for therapeutic applications, particularly in conditions like diabetes and nephroprotection where cytotoxicity needs to be minimized. The gradual reduction in viability at higher concentrations indicates that RG-PV-HF may have dose-dependent biological effects, necessitating further investigation into its selectivity, mechanism of action, and potential therapeutic window. Future studies should focus on long-term safety assessments, apoptotic pathway analysis, and its interaction with normal and diseased cells to determine its full pharmacological potential.

Table 5. Evaluation of Cytotoxicity of RG-PV-HF as % cell viability.

	% Cell Viability	
Concentration (µg/mL)	**RG-PV-HF**	**Docetaxel (Standard)**
0	99.25±2.13	99.57±2.85

20	93.14±2.11	63.81±1.32
40	92.25±1.02	53.37±1.21
80	93.31±1.01	34.54±1.11
120	81.68±1.04	31.83±1.17
200	60.89±1.02	25.75±1.13
280	42.96±1.02	21.56±1.02

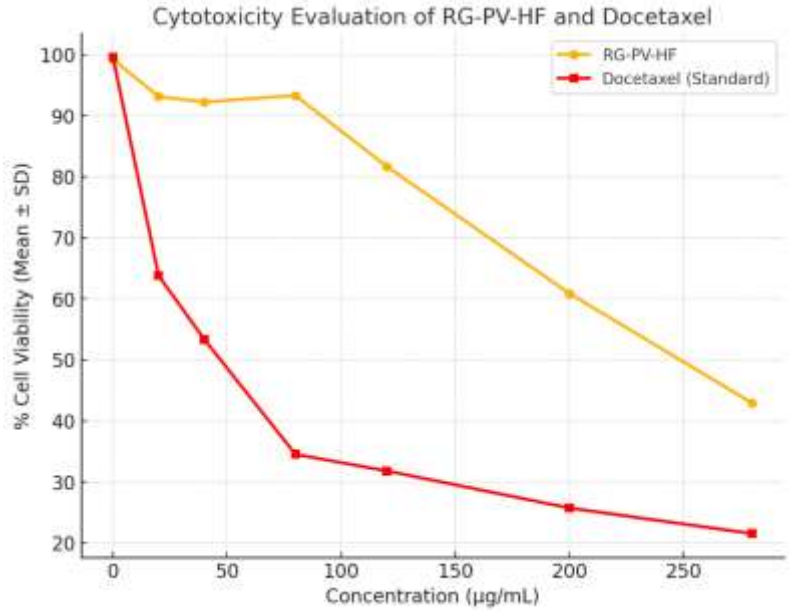

Figure 5. RG-PV-HF in HEK-293 cells demonstrated nephroprotective activity in cisplatin-induced nephrotoxicity.

4. CONCLUSIONS

The study successfully developed and evaluated RG-PV-HF, a polyherbal formulation, demonstrating potential therapeutic applications in diabetes and nephrotoxicity. The extract exhibited strong α-amylase, α-glucosidase, and DPP-4 inhibitory activity, suggesting its ability to modulate carbohydrate metabolism and incretin hormone pathways. The high phenolic content of 317.87 mg GAE/g extract further supports its antioxidant properties, which may contribute to its protective effects against oxidative stress-related kidney damage. Cytotoxicity analysis revealed minimal toxicity, with significant cell viability even at higher concentrations, confirming its safety profile. Additionally, RG-PV-HF demonstrated nephroprotective potential against cisplatin-induced toxicity, indicating its ability to mitigate renal damage. While the extract showed moderate enzyme inhibition compared to standard drugs such as Acarbose and Diprotin A, its natural composition and safety profile make it a viable alternative for long-term therapeutic use. Future studies focusing on enzyme kinetics, mechanistic pathways, and in vivo validation will further elucidate its pharmacological efficacy. The findings highlight RG-PV-HF as a promising natural therapeutic agent for diabetes and nephroprotection, supporting its potential for clinical translation.

REFERENCES

[1] Ahsan, R., Mishra, A., Badar, B., Owais, M., & Mishra, V. (2023). Therapeutic Application, Phytoactives and Pharmacology of Tinospora cordifolia: An Evocative Review. *Chin J Integr Med*, *29*(6), 549-555. https://doi.org/10.1007/s11655-023-3733-2

[2] Al-masri, I. M., Mohammad, M. K., & Tahaa, M. O. (2009). Inhibition of dipeptidyl peptidase IV (DPP IV) is one of the mechanisms explaining the hypoglycemic effect of berberine. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *24*(5), 1061-1066. https://doi.org/10.1080/14756360802610761

[3] Andriana, Y., Xuan, T. D., Quy, T. N., Minh, T. N., Van, T. M., & Viet, T. D. (2019). Antihyperuricemia, Antioxidant, and Antibacterial Activities of Tridax procumbens L. *Foods*, *8*(1).

https://doi.org/10.3390/foods8010021

[4] Bansal, N., Dhaliwal, R., & Weinstock, R. S. (2015). Management of diabetes in the elderly. *Med Clin North Am*, *99*(2), 351-377. https://doi.org/10.1016/j.mcna.2014.11.008

[5] Berlin Grace, V. M., Viswanathan, S., David Wilson, D., Jagadish Kumar, S., Sahana, K., Maria Arbin, E. F., & Narayanan, J. (2020). Significant action of Tridax procumbens L. leaf extract on reducing the TNF-α and COX-2 gene expressions in induced inflammation site in Swiss albino mice. *Inflammopharmacology*, *28*(4), 929-938. https://doi.org/10.1007/s10787-019-00634-0

[6] Cattin, L. (2016). [Diabetes Mellitus: etiology, pathophysiology and clinical classification]. *G Ital Nefrol*, *33*(S68). (Il diabete mellito: etiopatogenesi ed inquadramento clinico.)

[7] Darenskaya, M. A., Kolesnikova, L. I., & Kolesnikov, S. I. (2021). Oxidative Stress: Pathogenetic Role in Diabetes Mellitus and Its Complications and Therapeutic Approaches to Correction. *Bull Exp Biol Med*, *171*(2), 179-189. https://doi.org/10.1007/s10517-021-05191-7

[8] Harborne, J. B. (1998). *Phytochemical Methods A Guide to Modern Techniques of Plant Analysis*. Springer. http://books.google.co.in/books?id=2yvqeRtE8CwC

[9] Ikewuchi, C. J., & Ikewuchi, C. C. (2009). Alteration of plasma lipid profile and atherogenic indices of cholesterol loaded rats by *Tridax procumbens* Linn: Implications for the management of obesity and cardiovascular diseases. *Biokemistri*, *21*(2), 95-99.

[10] Ikewuchi, J., Ikewuchi, C., & Igboh, M. (2009). Chemical profile of *Tridax procumbens* Linn. *Pak J Nutr*, *8*(5), 548-550.

[11] Jacobs, A. M. (1993). Diabetes mellitus. *Clin Podiatr Med Surg*, *10*(2), 231-248.

[12] Kpemissi, M., Eklu-Gadegbeku, K., Veerapur, V. P., Negru, M., Taulescu, M., Chandramohan, V., Hiriyan, J., Banakar, S. M., Nv, T., Suhas, D. S., Puneeth, T. A., Vijayakumar, S., Metowogo, K., & Aklikokou, K. (2019). Nephroprotective activity of Combretum micranthum G. Don in cisplatin induced nephrotoxicity in rats: In-vitro, in-vivo and in-silico experiments. *Biomedicine & Pharmacotherapy*, *116*, 108961. https://doi.org/https://doi.org/10.1016/j.biopha.2019.108961

[13] Luan, F., Wu, Q., Yang, Y., Lv, H., Liu, D., Gan, Z., & Zeng, N. (2020). Traditional Uses, Chemical Constituents, Biological Properties, Clinical Settings, and Toxicities of Abelmoschus manihot L.: A Comprehensive Review. *Front Pharmacol*, *11*, 1068. https://doi.org/10.3389/fphar.2020.01068

[14] Melmer, A., & Laimer, M. (2016). Treatment Goals in Diabetes. *Endocr Dev*, *31*, 1-27. https://doi.org/10.1159/000439364

[15] Olayinka, A. A., & Anthony, I. O. (2009). Phyochemical screening and polyphenolic antioxidant activity of aqueous crude leaf extract of *Helichrysum pedulanculatum*. *Int. J. Mol. Sci.*, *10*(11), 46-58.

[16] Pan, X. X., Tao, J. H., Jiang, S., Zhu, Y., Qian, D. W., & Duan, J. A. (2018). Characterization and immunomodulatory activity of polysaccharides from the stems and leaves of Abelmoschus manihot and a sulfated derivative. *Int J Biol Macromol*, *107*(Pt A), 9-16. https://doi.org/10.1016/j.ijbiomac.2017.08.130

[17] Papatheodorou, K., Banach, M., Edmonds, M., Papanas, N., & Papazoglou, D. (2015). Complications of Diabetes. *J Diabetes Res*, *2015*, 189525. https://doi.org/10.1155/2015/189525

[18] Pareek, H., Sharma, S., Khajja, B. S., Jain, K., & Jain, G. C. (2009). Evaluation of hypoglycemic and anti-hyperglycemic potential of *Tridax procumbens* (Linn.). *BMC Complement Altern Med*, *9*, 48. https://doi.org/10.1186/1472-6882-9-48

[19] Petchi, R. R., Parasuraman, S., & Vijaya, C. (2013). Antidiabetic and antihyperlipidemic effects of an ethanolic extract of the whole plant of Tridax procumbens (Linn.) in streptozotocin-induced diabetic rats. *J Basic Clin Pharm*, *4*(4), 88-92. https://doi.org/10.4103/0976-0105.121655

[20] Richter, E., Geetha, T., Burnett, D., Broderick, T. L., & Babu, J. R. (2023). The Effects of Momordica charantia on Type 2 Diabetes Mellitus and Alzheimer's Disease. *Int J Mol Sci*, *24*(5). https://doi.org/10.3390/ijms24054643

[21] Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Yoga Latha, L. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *Afr J Tradit Complement Altern Med*, *8*(1), 1-10. https://www.ncbi.nlm.nih.gov/pubmed/22238476

[22] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3218439/

[23] Singh, M. P., Chauhan, A. K., & Kang, S. C. (2018). Morin hydrate ameliorates cisplatin-induced ER stress, inflammation and autophagy in HEK-293 cells and mice kidney via PARP-1 regulation. *International Immunopharmacology*, *56*, 156-167. https://doi.org/https://doi.org/10.1016/j.intimp.2018.01.031

[24] Sultana, B., Anwar, F., & Ashraf, M. J. M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *14*(6), 2167-2180.

[25] Sun, Y., Tao, Q., Wu, X., Zhang, L., Liu, Q., & Wang, L. (2021). The Utility of Exosomes in Diagnosis and Therapy of Diabetes Mellitus and Associated Complications. *Front Endocrinol (Lausanne)*, *12*, 756581. https://doi.org/10.3389/fendo.2021.756581

[26] The, L. (2017). Diabetes: a dynamic disease. *Lancet*, *389*(10085), 2163. https://doi.org/10.1016/s0140-6736(17)31537-4