RESEARCH PAPER

Extraction, Fractionation and Evaluation of Antidiabetic Potential of Active Phytofractions from Torilis leptophylla in Streptozotocin-Induced Diabetic Rats

Harish Joshi[1*], Dr. Rupesh Soni[2]

[1*]PhD Research Scholar, Department of Pharmacology, B R Nahata College of Pharmacy, Mandsaur University, Mandsaur, Madhya Pradesh, India. Email: harishjoshi156@gmail.com

[2]Professor and PhD Supervisor, Faculty of Pharmacy, B R Nahata College of Pharmacy, Mandsaur University, Mandsaur, Madhya Pradesh, India. Email: rupeshsoni77@gmail.com

ABSTRACT

Background: Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia and associated oxidative stress, leading to severe microvascular and macrovascular complications. Despite the availability of various antidiabetic drugs, their long-term use is often limited by adverse effects and high treatment costs. Medicinal plants rich in bioactive phytoconstituents have emerged as promising alternatives for the management of diabetes. Torilis leptophylla (Apiaceae) is traditionally known for its diverse pharmacological properties; however, its antidiabetic potential remains inadequately explored.

Objective: The present study aimed to evaluate the antidiabetic activity of Torilis leptophylla through extraction, phytochemical screening, fractionation, and biological assessment in streptozotocin-induced diabetic rats.

Methods: Successive solvent extraction of Torilis leptophylla was performed using petroleum ether, chloroform, ethanol, and water. The extracts were subjected to preliminary phytochemical screening and acute oral toxicity studies. Antidiabetic activity was evaluated in streptozotocin-induced diabetic rats by monitoring fasting blood glucose levels and glycated haemoglobin (HbA1c). Antioxidant activity was assessed through estimation of superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA). The most active extract was further fractionated using silica gel column chromatography, and the obtained fractions were analysed by thin-layer chromatography (TLC).

Results: The ethanolic extract exhibited the highest extraction yield and demonstrated the presence of alkaloids, flavonoids, glycosides, and other bioactive constituents. Treatment with the ethanolic extract produced a significant reduction in blood glucose and HbA1c levels compared with diabetic control animals. The extract also restored antioxidant status by increasing SOD and GSH levels while reducing MDA concentrations. Fractionation of the active extract yielded multiple fractions, among which Fraction F3 exhibited the most pronounced antidiabetic activity. TLC analysis confirmed the presence of distinct phytochemical constituents in the active fraction.

Conclusion: The findings demonstrate that Torilis leptophylla possesses significant antidiabetic and antioxidant activities, which may be attributed to its rich phytochemical composition. The identification of an active fraction further supports its potential as a promising source of natural antidiabetic agents and warrants further phytochemical isolation and clinical investigation.

Keywords: Torilis leptophylla, Diabetes mellitus, Streptozotocin, Antidiabetic activity, Antioxidant activity, Phytochemical screening, Column chromatography, Thin-layer chromatography.

How to cite this article: Joshi H, Soni R. Extraction, Fractionation and Evaluation of Antidiabetic Potential of Active Phytofractions from Torilis leptophylla in Streptozotocin-Induced Diabetic Rats. Int J Drug Deliv Technol. 2026;16(63s):1338-1349. DOI: 10.25258/ijddt.16.63s.133

Source of support: Nil

Conflict of interest: None

1. INTRODUCTION

1.1 Diabetes Mellitus: An Emerging Global Health Concern

Diabetes mellitus (DM) is one of the most prevalent chronic metabolic disorders affecting millions of individuals worldwide. It is characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The disease is associated with disturbances in carbohydrate, protein, and lipid metabolism and has become a major global health concern due to its rapidly increasing prevalence and associated complications. Diabetes not only affects the quality of life of patients but also imposes a substantial economic burden on healthcare systems worldwide. The growing incidence of diabetes has been linked to rapid urbanization, sedentary lifestyles, obesity, unhealthy dietary habits, genetic predisposition, and population aging. Owing to its multifactorial nature and progressive course, diabetes continues to pose significant challenges in clinical management and public health planning. [5,25,51–56]

Diabetes mellitus is broadly classified into Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM), and other specific forms of diabetes. Type 2 diabetes accounts for approximately 90–95% of all reported cases and represents the most common form of the disease worldwide. [5,25,54,55]

1.2 Pathophysiology of Diabetes Mellitus

The pathophysiology of diabetes mellitus is complex and involves multiple metabolic and molecular abnormalities. In Type 1 diabetes, autoimmune-mediated destruction of pancreatic β-cells results in severe insulin deficiency and impaired glucose utilization. In Type 2 diabetes, insulin resistance develops in peripheral tissues such as skeletal muscle, adipose tissue, and liver, resulting in reduced glucose uptake and increased hepatic glucose production. Progressive β-cell dysfunction further exacerbates hyperglycemia and contributes to disease progression.

Persistent hyperglycemia leads to abnormalities in carbohydrate, lipid, and protein metabolism and initiates a series of biochemical events that contribute to cellular dysfunction and tissue injury. These metabolic disturbances are responsible for the development of diabetic complications and increased disease severity. [31,32,61]

Oxidative stress is also closely associated with the development of diabetic complications. Long-term hyperglycemia promotes the formation of advanced glycation end products (AGEs), activation of protein kinase C, increased polyol pathway activity, and enhanced inflammatory responses. These processes collectively contribute to endothelial dysfunction, vascular injury, and tissue damage. Diabetic retinopathy, nephropathy, neuropathy, and cardiovascular diseases are among the most common complications associated with chronic oxidative stress. Therefore, therapeutic strategies capable of reducing oxidative stress and enhancing antioxidant defenses may play a crucial role in preventing or delaying the progression of diabetic complications. [13–15,31–33,57]

1.3 Oxidative Stress and Diabetic Complications

Oxidative stress plays a central role in the development and progression of diabetes mellitus and its associated complications. Hyperglycemia promotes excessive generation of reactive oxygen species (ROS) through several pathways including glucose auto-oxidation, mitochondrial dysfunction, activation of the polyol pathway, and formation of advanced glycation end products (AGEs).

Increased oxidative stress damages cellular proteins, lipids, and nucleic acids, leading to impaired cellular function and apoptosis. Pancreatic β-cells are particularly susceptible to oxidative injury due to their relatively weak antioxidant defense system. Consequently, oxidative stress contributes to β-cell dysfunction, insulin resistance, and worsening of hyperglycemia. [31–33,57]

Long-term oxidative stress is also implicated in diabetic retinopathy, nephropathy, neuropathy, and cardiovascular diseases. Therefore, compounds possessing antioxidant activity may provide significant therapeutic benefits in diabetes management by protecting tissues from oxidative damage and improving metabolic function. [13–15,31–33]

1.4 Current Therapeutic Approaches and Their Limitations

Current treatment strategies for diabetes include lifestyle modifications, insulin therapy, and oral hypoglycemic agents such as metformin, sulfonylureas, thiazolidinediones, DPP-4 inhibitors, GLP-1 receptor agonists, and SGLT-2 inhibitors. These therapeutic approaches have significantly improved glycemic control and reduced disease-associated complications.

However, long-term use of synthetic antidiabetic drugs is often associated with various adverse effects including hypoglycaemia, gastrointestinal disturbances, weight gain, fluid retention, hepatotoxicity, and poor patient compliance. Moreover, these therapies mainly target blood glucose regulation and often fail to adequately address oxidative stress and chronic diabetic complications. [8,25,61]

1.5 Medicinal Plants as Potential Antidiabetic Agents

Medicinal plants have been extensively utilized in traditional healthcare systems for the management of diabetes and related metabolic disorders. Herbal medicines remain popular because of their affordability,

accessibility, lower incidence of adverse effects, and long history of traditional use.

Several medicinal plants have demonstrated significant antihyperglycemic activity through multiple mechanisms such as stimulation of insulin secretion, enhancement of insulin sensitivity, inhibition of carbohydrate digestion and absorption, modulation of glucose metabolism, and protection of pancreatic β-cells. The multi-targeted nature of plant-derived compounds makes them attractive candidates for the development of novel antidiabetic therapies. [28,29,47–49]

1.6 Phytochemicals Associated with Antidiabetic Activity

The therapeutic effects of medicinal plants are primarily attributed to their phytochemical constituents. Flavonoids, alkaloids, tannins, phenolic compounds, terpenoids, glycosides, and saponins have been reported to possess significant antidiabetic and antioxidant activities.

Flavonoids and phenolic compounds act as potent free radical scavengers and enhance insulin sensitivity. Alkaloids have been shown to influence glucose metabolism and insulin secretion, while terpenoids exhibit anti-inflammatory and antioxidant effects. The synergistic action of these phytochemicals contributes significantly to the overall pharmacological activity of medicinal plant extracts. [29,30,34–36,47]

1.7 Torilis leptophylla: Botanical and Pharmacological Profile

Torilis leptophylla (Family: Apiaceae) is an annual medicinal herb distributed in various temperate and subtropical regions. The plant has been traditionally used for the management of several ailments and has attracted scientific attention due to its rich phytochemical composition.

Previous phytochemical investigations have reported the presence of flavonoids, phenolics, alkaloids, glycosides, tannins, and terpenoids in different parts of the plant. These constituents are known to exhibit antioxidant, anti-inflammatory, antimicrobial, cytoprotective, and therapeutic activities. [20–22]

Several pharmacological studies have demonstrated significant antioxidant activity of Torilis leptophylla. Saeed et al. reported strong free radical scavenging activity that correlated positively with total phenolic and flavonoid content. Additional studies have reported anti-ulcer, gastroprotective, neuroprotective, anti-inflammatory, and anti-arthritic activities, indicating broad pharmacological potential. [20–24,26,27]

1.8 Research Gap and Rationale of the Study

Despite the documented pharmacological activities of Torilis leptophylla, scientific evidence regarding its antidiabetic potential remains limited. Most available studies have focused on crude extracts without identifying the specific phytochemical fractions responsible for biological activity. The isolation and characterization of active phytofractions are essential for understanding the mechanisms underlying therapeutic effects and for developing standardized phytopharmaceutical preparations.

Bioactivity-guided fractionation using chromatographic techniques enables the identification of active fractions enriched with biologically important phytoconstituents. Such an approach can facilitate the discovery of novel natural compounds with potential therapeutic applications in diabetes management. [22,50]

1.9 Aim and Objective of the Present Study

The present study was undertaken to evaluate the antidiabetic potential of Torilis leptophylla through systematic extraction, phytochemical screening, fractionation, and biological evaluation in streptozotocin-induced diabetic rats. The study further aimed to assess its effects on blood glucose levels, glycated haemoglobin (HbA1c), antioxidant parameters including superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA), and to identify the most active phytofraction through column chromatographic separation and thin-layer chromatographic analysis.

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

The whole plant of *Torilis leptophylla* (Family: Apiaceae) was collected from the local region of Mandsaur, Madhya Pradesh, India, during its flowering season. The collected plant material was authenticated by a qualified taxonomist, and a voucher specimen was deposited for future reference. The plant material was thoroughly washed with distilled water to remove adhering dust and foreign matter and was subsequently shade-dried at room temperature. The dried material was pulverized using a mechanical grinder and passed through a suitable sieve to obtain a coarse powder, which was stored in airtight containers until further use.

2.2 Preparation of Extracts

The powdered plant material was subjected to successive solvent extraction using solvents of increasing polarity, namely petroleum ether, chloroform, ethanol, and distilled water. Extraction was carried out using a Soxhlet apparatus. Each extraction cycle was continued until complete exhaustion of the plant material. The obtained extracts were concentrated under reduced pressure using a rotary evaporator and further dried to obtain solid residues. The percentage yield of each extract was calculated based on the initial weight of the plant powder used for extraction.

2.3 Preliminary Phytochemical Screening

The different extracts obtained from *Torilis leptophylla* were subjected to preliminary phytochemical screening using standard qualitative procedures. The extracts were tested for the presence of major phytoconstituents including alkaloids, flavonoids, glycosides, tannins, phenolic compounds, saponins, carbohydrates, proteins, and terpenoids. Characteristic color changes and precipitate formation were recorded as indicators of positive reactions.

2.4 Experimental Animals

Healthy adult Wistar albino rats of either sex weighing between 150–200 g were used for the experimental study. The animals were housed in polypropylene cages under standard laboratory conditions maintained at a temperature of 22 ± 2°C, relative humidity of 50–60%, and a 12 h light/dark cycle. Animals were provided with standard pellet diet and water ad libitum. Prior to experimentation, the animals were acclimatized to laboratory conditions for one week. All experimental procedures were conducted in accordance with CPCSEA guidelines and institutional ethical standards.

2.5 Acute Oral Toxicity Study

The acute oral toxicity study of the ethanolic extract of *Torilis leptophylla* was performed according to OECD guideline 423. Experimental animals were fasted overnight prior to dosing and observed continuously during the initial hours following administration and periodically for 14 days. Behavioural changes, signs of toxicity, mortality, food intake, and general health status were monitored throughout the study period. Based on the results, suitable dose levels were selected for subsequent pharmacological evaluation.

2.6 Induction of Experimental Diabetes

Experimental diabetes was induced by a single intraperitoneal administration of streptozotocin (STZ) at a dose of 45 mg/kg body weight. Streptozotocin was freshly prepared in cold citrate buffer (pH 4.5) immediately before administration. Following STZ injection, animals were provided with 5% glucose solution for 24 h to prevent initial hypoglycemic shock. After 72 h, fasting blood glucose levels were measured using a glucometer. Animals exhibiting fasting blood glucose levels above 250 mg/dL were considered diabetic and selected for the study.

2.7 Experimental Design

The diabetic animals were randomly divided into experimental groups consisting of six animals each. Group I served as the normal control and received vehicle only. Group II served as the diabetic control. Group III received metformin (100 mg/kg body weight) as the standard drug. Groups IV, V, and VI received the ethanolic extract of *Torilis leptophylla* at doses of 100, 200, and 400 mg/kg body weight, respectively. Treatments were administered orally once daily for 21 consecutive days.

2.8 Assessment of Blood Glucose and HbA1c

Fasting blood glucose levels were determined at predetermined intervals during the treatment period using a glucose oxidase-peroxidase based glucometer. Blood samples were collected from the tail vein after overnight fasting. At the end of the experimental period, glycated haemoglobin (HbA1c) levels were estimated using standard biochemical procedures to assess long-term glycemic control.

2.9 Evaluation of Antioxidant Parameters

At the completion of the treatment period, animals were sacrificed and tissue homogenates were prepared for biochemical analysis. Oxidative stress and antioxidant status were assessed using established biochemical methods.

2.9.1 Superoxide Dismutase (SOD)

Superoxide dismutase activity was determined according to the method described by Misra and Fridovich. The assay was based on the inhibition of auto-oxidation reactions mediated by superoxide radicals, and enzyme activity was expressed as units per milligram of protein.

2.9.2 Reduced Glutathione (GSH)

Reduced glutathione levels were estimated using Ellman's reagent (DTNB). The intensity of the yellow-colored chromogen formed was measured spectrophotometrically and expressed as µmol/mg protein.

2.9.3 Malondialdehyde (MDA)

Lipid peroxidation was assessed by measuring malondialdehyde levels using the thiobarbituric acid reactive substances (TBARS) method. The concentration of MDA was expressed as nmol/mg protein.

2.10 Fractionation of the Active Extract

The ethanolic extract, which demonstrated the highest antidiabetic activity, was selected for fractionation. Separation of phytoconstituents was performed using silica gel column chromatography. Elution was carried out using solvent systems of gradually increasing polarity. Fractions were collected sequentially and concentrated under reduced pressure. Similar fractions were pooled based on chromatographic characteristics and designated as F1, F2, and F3.

2.11 Thin Layer Chromatographic Analysis

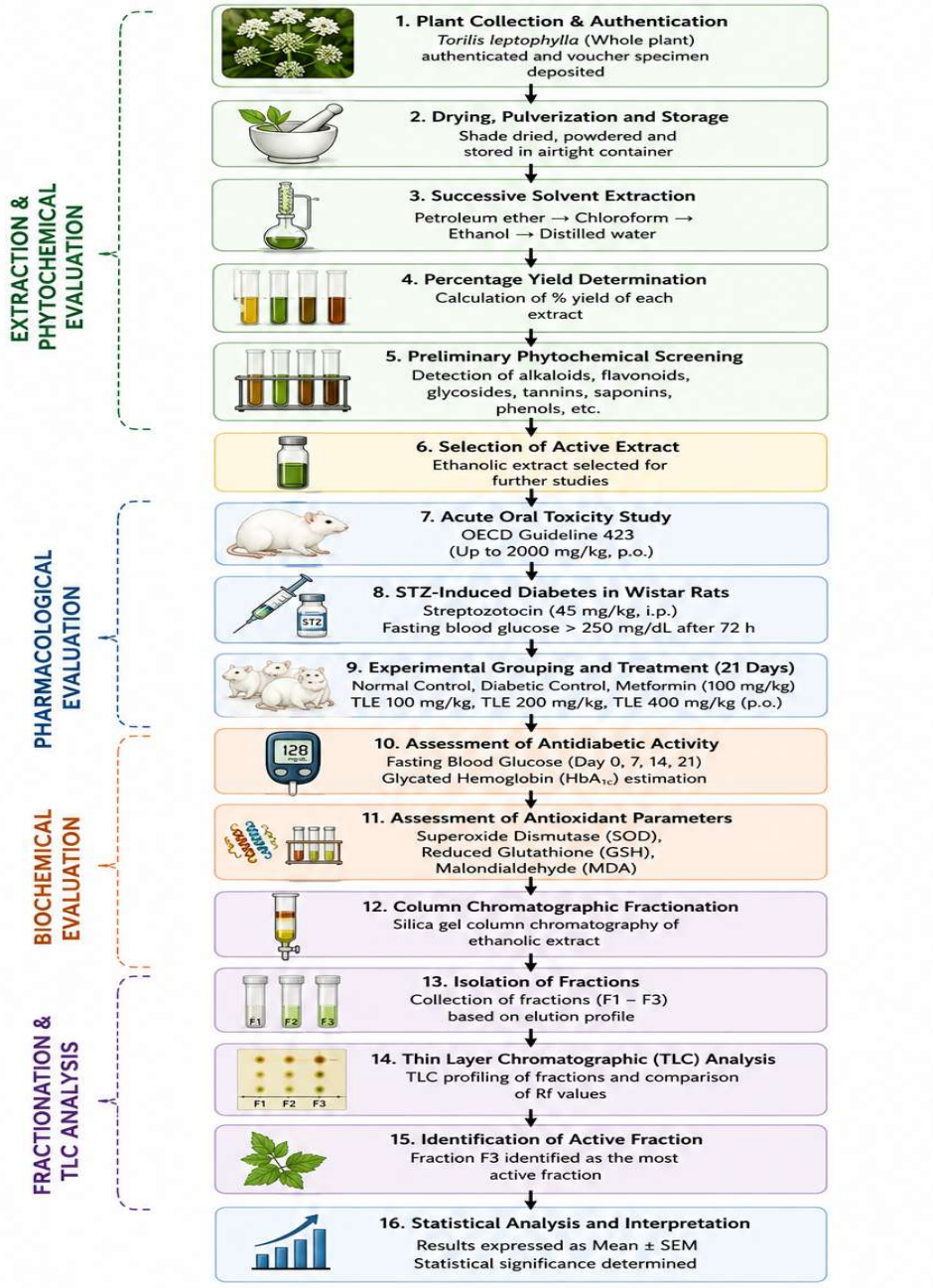
The isolated fractions were subjected to thin layer chromatographic (TLC) analysis to evaluate their phytochemical composition. Samples were applied onto pre-coated silica gel TLC plates and developed using appropriate solvent systems. After development, chromatograms were visualized under ultraviolet light and suitable detecting reagents were employed for spot

identification. Retardation factor (Rf) values were calculated for the observed spots.

2.12 Statistical Analysis

All experimental data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by an appropriate post hoc test. Differences were considered statistically significant at $p < 0.05$.

Figure 1: Experimental Workflow for Evaluation of Antidiabetic Activity of Torilis leptophylla



3. RESULTS

3.1 Percentage Yield of Different Extracts

Successive solvent extraction of *Torilis leptophylla* was carried out using petroleum ether, chloroform, ethanol, and distilled water. The percentage yield varied according to the solvent polarity. Among all extracts, the ethanolic extract exhibited the highest percentage yield, indicating the presence of a greater concentration of polar phytoconstituents. The petroleum ether extract showed the lowest extraction yield, suggesting a lower abundance of non-polar constituents.

The extraction results demonstrated that ethanol was the most efficient solvent for recovering bioactive compounds from *Torilis leptophylla*.

Table 1: Percentage Yield and Phytochemical Constituents of Different Extracts of Torilis leptophylla

Table 1. Percentage Yield and Phytochemical Constituents of Different Extracts of *Torilis leptophylla*

Extract	Yield (%) (w/w)	Alkaloids	Flavonoids	Glycosides
Petroleum Ether Extract	3.70 ± 0.21	+	–	–
Chloroform Extract	4.84 ± 0.18	++	+	+
Ethanolic Extract	12.56 ± 0.34	+++	+++	++
Aqueous Extract	9.72 ± 0.27	++	++	+++

Values are expressed as mean ± SEM (n = 3).

Key: + = Present (Low); ++ = Moderately Present; +++ = Abundantly Present; – = Absent

3.2 Preliminary Phytochemical Screening

Preliminary phytochemical screening revealed the presence of several important secondary metabolites in the different extracts of *Torilis leptophylla*. Alkaloids, flavonoids, and glycosides were detected in varying concentrations depending upon the extraction solvent employed.

The ethanolic extract showed a comparatively richer phytochemical profile and contained higher levels of alkaloids and flavonoids. The aqueous extract demonstrated a substantial presence of glycosides, while the petroleum ether extract contained only a limited number of phytoconstituents. These findings indicate that polar solvents were more effective in extracting biologically active constituents.

3.3 Acute Oral Toxicity Study

The acute oral toxicity study of the ethanolic extract was performed according to OECD guideline 423. No mortality or significant behavioural abnormalities were observed in experimental animals throughout the observation period.

The treated animals showed normal feeding behaviour, locomotor activity, grooming, and physiological responses. The absence of toxic manifestations indicated that the extract was safe at the tested dose levels. Based on these findings, experimental doses were selected for subsequent antidiabetic evaluation.

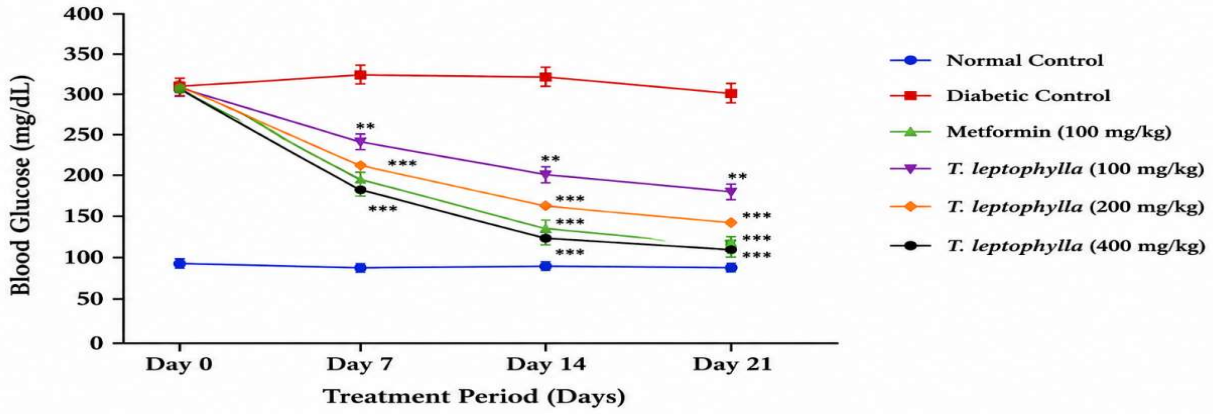
3.4 Effect of Torilis leptophylla on Blood Glucose Levels

Administration of streptozotocin produced a significant elevation in fasting blood glucose levels in diabetic animals compared to the normal control group. Treatment with the ethanolic extract of *Torilis leptophylla* resulted in a dose-dependent reduction in blood glucose levels throughout the study period.

Among the tested doses, the 400 mg/kg treatment group exhibited the greatest reduction in blood glucose concentration and produced results comparable to the standard drug metformin. The antihyperglycemic effect became more pronounced with increasing treatment duration, suggesting sustained glucose-lowering activity.

Graph 1: Effect of Torilis leptophylla on Blood Glucose Levels During the Treatment Period in STZ- Induced Diabetic Rats

Figure 3. Effect of *Torilis leptophylla* on Blood Glucose Levels During the Treatment Period in STZ-Induced Diabetic Rats



Values are expressed as Mean ± SEM ($n = 6$).
$^{**}P < 0.01$, $^{***}P < 0.001$ compared with diabetic control group.

Suggested position: This graph should be placed in the Results section under 3.4 Effect of *T. leptophylla* on Blood Glucose Levels (after the descriptive text of fasting blood glucose) and before the subsection on HbA1c estimation.

3.5 Effect on Glycated Haemoglobin (HbA1c)

Diabetic control animals showed significantly elevated HbA1c levels compared with the normal control group, confirming persistent hyperglycemia during the experimental period.

Treatment with the ethanolic extract significantly reduced HbA1c levels in diabetic rats. The reduction was dose-dependent, with the highest dose producing maximum improvement. The observed decrease in HbA1c indicates improved long-term glycemic control following administration of the extract.

3.6 Effect on Antioxidant Parameters

Table 2: Effect of Torilis leptophylla on Biochemical Parameters in STZ- Induced Diabetic Rats

Table 2. Effect of *Torilis leptophylla* on Biochemical Parameters in STZ-Induced Diabetic Rats

Group	Blood Glucose (mg/dL) Day 21	HbA1c (%)	SOD (U/mg protein)	GSH (µmol/mg protein)	MDA (nmol/mg protein)
Normal Control	93 ± 3	4.8 ± 0.2	9.2 ± 0.4	8.1 ± 0.3	2.3 ± 0.1
Diabetic Control	310 ± 9	9.6 ± 0.4	4.1 ± 0.2	3.4 ± 0.2	7.8 ± 0.3
Metformin (100 mg/kg)	110 ± 3***	5.2 ± 0.2***	8.8 ± 0.3***	7.6 ± 0.2***	2.8 ± 0.1***
Extract 100 mg/kg	180 ± 4**	7.2 ± 0.3**	6.2 ± 0.2**	5.4 ± 0.2**	5.1 ± 0.2**
Extract 200 mg/kg	145 ± 3***	6.3 ± 0.2***	7.4 ± 0.3***	6.6 ± 0.3***	3.9 ± 0.2***
Extract 400 mg/kg	120 ± 4***	5.5 ± 0.2***	8.3 ± 0.3***	7.2 ± 0.2***	3.1 ± 0.1***

Values are expressed as Mean ± SEM ($n = 6$).
Statistical significance: $^{*}P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) compared with diabetic control group.

3.6.1 Superoxide Dismutase (SOD)

Diabetic control animals exhibited a marked reduction in SOD activity compared with normal animals, indicating impairment of endogenous antioxidant defense mechanisms. Treatment with *Torilis leptophylla* significantly restored SOD activity in a dose-dependent manner. The highest dose demonstrated maximum antioxidant protection.

3.6.2 Reduced Glutathione (GSH)

A significant decrease in GSH levels was observed in diabetic control animals. Administration of the ethanolic extract increased GSH concentrations and restored antioxidant balance. The effect was more pronounced in animals receiving higher doses of the extract.

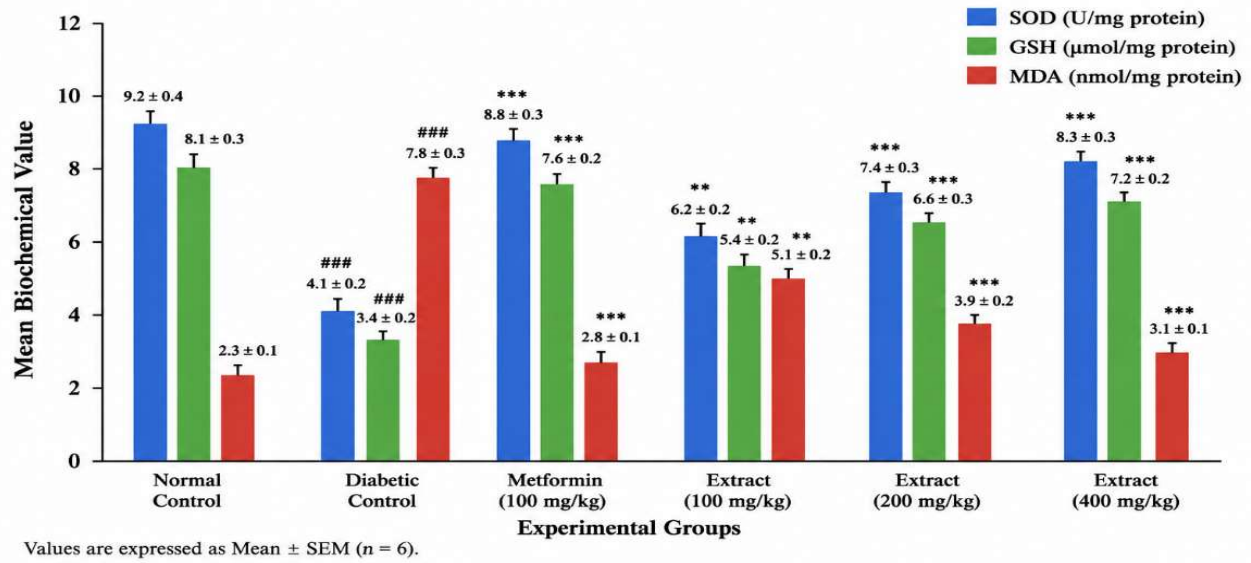
3.6.3 Malondialdehyde (MDA)

MDA levels were significantly elevated in diabetic animals, indicating enhanced lipid peroxidation and oxidative stress. Treatment with *Torilis leptophylla* markedly reduced MDA concentrations compared with diabetic controls. The reduction in MDA was accompanied by improvements in SOD and GSH levels.

Overall, the antioxidant studies demonstrated that the extract effectively attenuated oxidative stress associated with diabetes mellitus.

Graph 2: Effect of Torilis leptophylla on Oxidative Stress Biomarkers in STZ- Induced Diabetic Rats

Figure 4. Effect of *Torilis leptophylla* on Oxidative Stress Biomarkers in STZ-Induced Diabetic Rats



Values are expressed as Mean ± SEM ($n = 6$).
###P < 0.001 compared with Normal Control group.
**P < 0.01, ***P < 0.001 compared with Diabetic Control group.
Abbreviations: SOD = Superoxide Dismutase; GSH = Reduced Glutathione; MDA = Malondialdehyde.

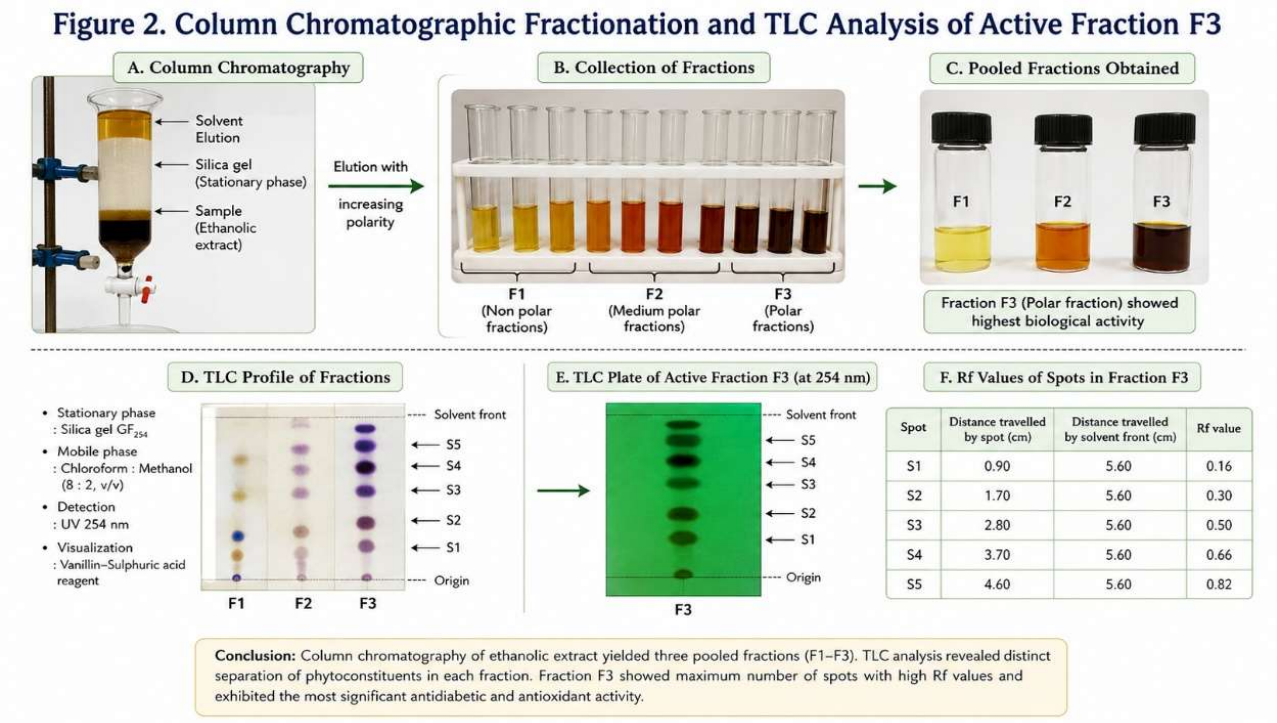
3.7 Fractionation and TLC Analysis

The ethanolic extract exhibiting the highest antidiabetic activity was selected for further fractionation using silica gel column chromatography. Elution with solvent systems of increasing polarity yielded three major fractions designated as F1, F2, and F3.

Thin layer chromatographic analysis revealed distinct chromatographic profiles for each fraction, indicating successful separation of phytoconstituents. Fraction F3 demonstrated a higher number of prominent spots and was considered phytochemically rich compared with other fractions.

Among the isolated fractions, F3 exhibited the most significant biological activity and was therefore identified as the active fraction responsible for the observed antidiabetic effects.

Figure 2: Column Chromatography Fractionation and TLC Analysis of Active Fraction



4. DISCUSSION

4.1 Extraction Yield and Phytochemical Composition

The extraction process yielded different fractions depending upon the polarity of the solvents employed. Among the extracts obtained, the ethanolic extract exhibited the highest percentage yield, indicating the efficient extraction of polar phytoconstituents. Ethanol has been widely reported as an effective solvent for the recovery of flavonoids, phenolics, glycosides, and other biologically active compounds. The higher extraction yield observed in the ethanolic extract suggests the abundance of pharmacologically important constituents within the plant material. Similar observations have been reported by Saeed et al., who demonstrated that methanolic and ethanolic extracts of *Torilis leptophylla* contain higher concentrations of phenolic and flavonoid compounds compared with less polar solvent extracts. [20,21]

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, and glycosides in varying concentrations among different extracts. The ethanolic extract demonstrated the richest phytochemical profile, which may explain its superior biological activity observed during the antidiabetic evaluation. These findings support previous reports indicating that flavonoids and phenolic compounds contribute significantly to the pharmacological potential of medicinal plants. [28,29,47]

4.2 Antidiabetic Activity of Torilis leptophylla

The streptozotocin-induced diabetic rat model employed in the present study successfully produced persistent hyperglycemia and served as a suitable experimental model for evaluating antidiabetic activity. Administration of streptozotocin resulted in a marked increase in fasting blood glucose levels due to pancreatic β-cell damage and insulin deficiency. [44,45]

Treatment with the ethanolic extract of *Torilis leptophylla* produced a significant and dose-dependent reduction in blood glucose levels. The glucose-lowering effect was more pronounced at higher doses and approached the activity of the standard drug metformin. The observed antihyperglycemic activity may be attributed to enhanced insulin secretion, improved peripheral glucose utilization, suppression of hepatic glucose production, and protection of pancreatic β-cells from oxidative damage. Similar antihyperglycemic effects have been reported for several medicinal plants rich in flavonoids and phenolic compounds. [45,46,60]

4.3 Effect on Glycated Haemoglobin (HbA1c)

Glycated haemoglobin (HbA1c) serves as a reliable marker of long-term glycemic control and reflects the average blood glucose concentration over an extended period. Diabetic animals exhibited significantly elevated HbA1c levels compared with normal controls, indicating chronic hyperglycemia. Treatment with *Torilis leptophylla* significantly reduced HbA1c levels,

demonstrating sustained improvement in glucose regulation throughout the treatment period.

The reduction in HbA1c observed in treated groups confirms that the antihyperglycemic activity of the extract was not limited to short-term glucose reduction but was maintained over the entire duration of treatment. Lower HbA1c levels are associated with reduced risk of diabetic complications, emphasizing the therapeutic significance of the extract. [17,18]

4.4 Effect on Oxidative Stress and Antioxidant Defence System

Oxidative stress is a major factor involved in the development and progression of diabetes mellitus and its complications. Persistent hyperglycemia increases the production of reactive oxygen species (ROS), resulting in cellular damage and impairment of antioxidant defence mechanisms. In the present study, diabetic animals exhibited reduced SOD and GSH levels along with elevated MDA concentrations, indicating increased oxidative stress and lipid peroxidation.

Treatment with the ethanolic extract significantly restored antioxidant enzyme activity by increasing SOD and GSH levels while simultaneously reducing MDA concentrations. These findings demonstrate the ability of *Torilis leptophylla* to enhance endogenous antioxidant defenses and protect biological systems against oxidative injury. Similar observations have been reported by Evans et al., Brownlee, and Ceriello, who emphasized the central role of oxidative stress in diabetes and highlighted the therapeutic value of antioxidant interventions. [31–33]

4.5 Relationship Between Phytochemicals and Biological Activity

The significant antidiabetic and antioxidant activities observed in the present study are likely associated with the phytochemical constituents present in the ethanolic extract. Flavonoids, phenolic compounds, alkaloids, and glycosides have been reported to exhibit antihyperglycemic, antioxidant, and cytoprotective properties.

Flavonoids are known to improve insulin sensitivity, enhance glucose uptake, inhibit oxidative stress, and protect pancreatic β-cells. Phenolic compounds act as potent free radical scavengers and contribute to the restoration of antioxidant balance. The combined action of these phytochemicals may be responsible for the observed therapeutic effects of *Torilis leptophylla*. [29,30,47]

4.6 Significance of Fractionation and Identification of Active Fraction

Bioactivity-guided fractionation was performed to identify the fraction responsible for the observed biological activity. Column chromatographic separation of the ethanolic extract yielded three major fractions designated as F1, F2, and F3. Thin-layer chromatographic analysis confirmed successful separation of phytoconstituents and revealed distinct chromatographic profiles among the isolated fractions.

Among the fractions obtained, Fraction F3 demonstrated the highest biological activity and exhibited a richer phytochemical profile. The enhanced activity of F3 suggests that it contains one or more bioactive compounds responsible for the antidiabetic effects of the plant. The identification of an active fraction represents an important step toward isolation and characterization of the compounds responsible for therapeutic activity. [34–36,50]

4.7 Proposed Mechanism of Antidiabetic Action

Based on the experimental findings and available literature, the antidiabetic activity of *Torilis leptophylla* appears to involve multiple complementary mechanisms. The extract reduced fasting blood glucose levels, improved HbA1c values, enhanced antioxidant defenses, and reduced lipid peroxidation. These effects collectively suggest improved glucose metabolism and protection against oxidative damage.

The phytoconstituents present in the active fraction may promote insulin secretion, improve insulin sensitivity, inhibit oxidative stress, and preserve pancreatic β-cell function. Such multi-targeted actions are characteristic of medicinal plants and may provide therapeutic advantages over single-target synthetic drugs.

4.8 Overall Significance of the Study

The present investigation provides scientific evidence supporting the traditional medicinal use of *Torilis leptophylla*. The study demonstrated significant antidiabetic and antioxidant activities in streptozotocin-induced diabetic rats and successfully identified an active phytofraction through chromatographic fractionation. The findings highlight the potential of *Torilis leptophylla* as a promising source of natural antidiabetic agents and establish a foundation for future phytochemical and clinical investigations.

5. CONCLUSION

The present study demonstrated the significant antidiabetic potential of *Torilis leptophylla* through systematic extraction, phytochemical evaluation, biological assessment, and bioactivity-guided fractionation. Among the various solvent extracts, the ethanolic extract exhibited the highest extraction yield and contained a rich diversity of phytoconstituents, including alkaloids, flavonoids, and glycosides, which are known for their pharmacological activities.

In streptozotocin-induced diabetic rats, treatment with the ethanolic extract produced a significant reduction in fasting blood glucose levels and glycated hemoglobin (HbA1c), indicating effective glycemic control.

Furthermore, the extract markedly improved antioxidant status by increasing superoxide dismutase (SOD) and reduced glutathione (GSH) levels while reducing malondialdehyde (MDA) concentrations. These findings suggest that the antidiabetic activity of *Torilis leptophylla* is closely associated with its ability to attenuate oxidative stress and enhance endogenous antioxidant defense mechanisms.

Bioactivity-guided fractionation of the active ethanolic extract through column chromatography yielded multiple fractions, among which Fraction F3 exhibited the most pronounced biological activity. Thin-layer chromatographic analysis confirmed the presence of distinct phytochemical constituents within the active fraction, suggesting that concentrated bioactive compounds may be responsible for the observed therapeutic effects.

Collectively, the findings of this study provide scientific evidence supporting the traditional medicinal use of *Torilis leptophylla* and establish its potential as a promising natural source of antidiabetic agents. The combined antihyperglycemic and antioxidant activities observed in the present investigation indicate that the plant may offer a multi-targeted approach for the management of diabetes mellitus and its associated complications.

Further studies involving isolation, purification, and structural characterization of the active constituents, together with mechanistic investigations and clinical evaluations, are warranted to fully explore the therapeutic potential of *Torilis leptophylla* and facilitate its development into a standardized phytopharmaceutical product.

REFERENCES

1. American Diabetes Association. Standards of medical care in diabetes. Diabetes Care. 2024;47(Suppl 1)–S350.

2. International Diabetes Federation. IDF Diabetes Atlas. 10th ed. Brussels: IDF; 2021.

3. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes estimates. Diabetes Care. 2004;27:1047–1053.

4. Zimmet P, Alberti KG, Shaw J. Global and societal implications of diabetes epidemic. Nature. 2001;414:782–787.

5. DeFronzo RA. Pathogenesis of type 2 diabetes mellitus. Med Clin North Am. 2004;88:787–835.

6. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways in diabetes. Endocr Rev. 2002;23:599–622.

7. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Nature. 2001;414:813–820.

8. Ceriello A. Oxidative stress and glycemic regulation in diabetes mellitus. Diabetes Care. 2003;26:1589–1591.

9. Sharma B, Salunke R, Balomajumder C, Daniel S, Roy P. Oxidative stress and diabetes complications. J Physiol Biochem. 2009;65:267–281.

10. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol. 2002;81:81–100.

11. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TPA. Indian herbs and herbal drugs used for treatment of diabetes. J Clin Biochem Nutr. 2007;40(3):163–173.

12. Patel DK, Kumar R, Laloo D, Hemalatha S. Diabetes mellitus and medicinal plants: a review. Asian Pac J Trop Dis. 2012;2:320–330.

13. Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents. Phytomedicine. 1995;2:137–189.

14. Bailey CJ, Day C. Traditional plant medicines as treatments for diabetes. Diabetes Care. 1989;12:553–564.

15. Newman DJ, Cragg GM. Natural products as sources of new drugs. J Nat Prod. 2007;70:461–477.

16. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts of Torilis leptophylla. BMC Complement Altern Med. 2012;12:221.

17. Saeed N. Phytochemical constituents and antioxidant activity of extract from Torilis leptophylla. Digital Repository; 2015.

18. Noshin N, Semmar N, Farman M, Lacaille-Dubois MA, Ahmed NS. Phytochemical, geographical and pharmacological retrospect of genus Torilis. Curr Top Med Chem. 2023;23(24):2300–2331.

19. Batool S, Qammar SM, Malik T, Ahmad NS, Fatima J. Anti-ulcerogenic evaluation of Torilis leptophylla plant extract on indomethacin induced gastric ulcer. Proc SZMC. 2022;36(4).

20. Fang Y, Lv L, Chai YJ, Yu F. Neuroprotective effect of whole plant extract of Torilis leptophylla in isoflurane-treated rats. Trop J Pharm Res. 2016;15.

21. Fatima J, Shaheen B, Batool S, Malik T, Qammar SM, Naureen S. Evaluation of anti-arthritic effect of Torilis leptophylla and its comparison with indomethacin. J Fatima Jinnah Med Univ. 2020;14:25–29.

22. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman and Hall; 1998.

23. Kokate CK. Practical Pharmacognosy. 4th ed. New Delhi: Vallabh Prakashan; 1994.

24. Trease GE, Evans WC. Pharmacognosy. 15th ed. London: Saunders; 2002.

25. Pari L, Latha M. Antidiabetic effect of medicinal plant extracts in STZ-induced diabetic rats. J Ethnopharmacol. 2002;80:199–204.