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Pharmacognosstical And Pharmacological Screening of Bioactive Compound Obtained from Plant *Aphanamixis Polystachya* and Its In Vivo Potential for the Management of Type-Ii Diabetes

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ABSTRACT:

Aphanamixis polystachya (Meliaceae) is a traditionally valued medicinal tree rich in limonoids and polyphenolic constituents. The present study aimed to establish pharmacognostical standards of the leaf and evaluate the antidiabetic potential of a polyphenol-enriched fraction (PEF) in a high-fat diet/low-dose streptozotocin (HFD-STZ) induced type-II diabetic rat model. Pharmacognostical investigation included macroscopic and microscopic features, physicochemical parameters, and preliminary phytochemical screening. The PEF was prepared by hydroalcoholic extraction followed by enrichment and standardized using total phenolic content and HPTLC fingerprint. In vivo evaluation was performed for 28 days with endpoints including fasting blood glucose, oral glucose tolerance, serum insulin, HOMA-IR, lipid profile, hepatic glycogen, oxidative stress markers, and pancreatic histology. Treatment with PEF (200 and 400 mg/kg) significantly reduced fasting glucose, improved glucose tolerance and insulin sensitivity, normalized dyslipidemia, increased hepatic glycogen, restored antioxidant defenses, and preserved pancreatic architecture compared with diabetic control. The study supports A. polystachya leaf PEF as a promising antidiabetic candidate, warranting further isolation and mechanistic studies.

INTRODUCTION

Type-II diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from insulin resistance, progressive β-cell dysfunction, and impaired regulation of hepatic glucose production [1-2]. The disease is strongly associated with obesity, sedentary lifestyle, and dietary factors and is frequently accompanied by dyslipidemia, oxidative stress, low-grade inflammation, and endothelial dysfunction. Uncontrolled T2DM contributes to long-term microvascular complications (retinopathy, nephropathy, neuropathy) and macrovascular complications (cardiovascular and cerebrovascular diseases), making it a major global public health challenge [3].

Despite the availability of several antidiabetic drug classes (e.g., biguanides, sulfonylureas, DPP-4 inhibitors, SGLT-2 inhibitors, GLP-1 receptor agonists, and insulin), limitations such as adverse effects (hypoglycemia, gastrointestinal intolerance, weight changes), high long-term cost, variable patient response, and poor adherence often compromise therapy [4]. Furthermore, T2DM is multifactorial; hence multi-target interventions that improve insulin sensitivity, reduce oxidative stress, modulate carbohydrate digestion, and protect β-cell integrity are of increasing interest. In this context, medicinal plants remain a valuable source of bioactive molecules, particularly polyphenols, flavonoids, terpenoids, alkaloids, and limonoids, many of which demonstrate antihyperglycemic, antihyperlipidemic, antioxidant, and anti-inflammatory effects [5].

The development of plant-based antidiabetic candidates requires two complementary pillars: pharmacognostical standardization and pharmacological validation. Pharmacognostical evaluation ensures correct botanical identification and establishes quality control parameters including macroscopic and microscopic characteristics, powder microscopy, physicochemical constants (ash values, extractive values, loss on drying), fluorescence behavior, and chromatographic fingerprints (HPTLC/HPLC) [6]. Such standardization is essential to prevent adulteration, assure batch-to-batch consistency,

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and provide reproducible biological outcomes. Pharmacological screening, particularly using in vivo models that mimic insulin resistance and β-cell impairment, is critical for substantiating traditional claims and identifying effective lead fractions or isolated compounds [7].

Aphanamixis polystachya (Wall.) R. Parker (family: Meliaceae), also known in several regions for its medicinal importance, has been reported to contain structurally diverse secondary metabolites such as limonoids, triterpenoids, and other phenolic constituents. Plants of the Meliaceae family are widely recognized for bioactive compounds exhibiting antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and metabolic regulatory activities [8]. However, comprehensive work integrating pharmacognostical profiling with bioactivity-guided evaluation of *A. polystachya*—particularly focusing on type-II diabetes—remains limited. A systematic approach that begins with standardization of the crude drug, followed by extraction, isolation/characterization of bioactive constituent(s), and in vivo evaluation of antidiabetic potential can provide scientifically robust evidence and facilitate development of a phytopharmaceutical lead [9].

In vivo models such as high-fat diet (HFD) combined with low-dose streptozotocin (STZ) are especially relevant because they simulate key features of human T2DM, including insulin resistance, impaired insulin secretion, hyperglycemia, and dyslipidemia [10]. Using such models allows evaluation of multiple endpoints: fasting blood glucose, oral glucose tolerance, insulin and insulin resistance indices (e.g., HOMA-IR), lipid profile, hepatic glycogen, oxidative stress biomarkers (MDA, SOD, catalase, GSH), inflammatory mediators, and histopathological changes in pancreatic tissue [11]. These endpoints collectively help to understand whether the test extract/compound acts through improved insulin sensitivity, protection of β-cells, reduction of oxidative stress, inhibition of carbohydrate-digesting enzymes, or a combination of mechanisms [12].

Therefore, the present study is designed to perform pharmacognostical and pharmacological screening of bioactive compound(s) obtained from *Aphanamixis polystachya* and to evaluate their in vivo potential for the management of type-II diabetes [13]. Establishing standardized identification and quality parameters for the plant material, coupled with systematic in vivo validation, may support *A. polystachya* as a promising natural source for antidiabetic lead molecules and may provide a foundation for future mechanistic, toxicity, and formulation studies [14].

Objectives of the study

1. To authenticate and standardize *Aphanamixis polystachya* using pharmacognostical parameters (macro-microscopy, physicochemical constants, fluorescence analysis, and chromatographic fingerprinting).
2. To prepare extract/fractions and obtain bioactive compound(s) through suitable isolation strategy.
3. To evaluate the antidiabetic potential of the extract/fraction/isolated compound(s) in a T2DM animal model (HFD-STZ).
4. To assess associated biochemical (lipid profile, insulin, glycogen), oxidative stress markers, and pancreatic histopathology to support mechanistic interpretation.

MATERIALS AND METHODS

Plant Material

Collection of Plant Material

Fresh and healthy plant material of *Aphanamixis polystachya* (Wall.) R. Parker (Family: Meliaceae) was collected during the month of July, 2024 from Bhopal, Madhya Pradesh, India). The plant was selected based on ethnomedicinal relevance and availability. The collected material leaves were free from visible infection, insect damage, or contamination [15].

Authentication and Identification

The plant specimen was authenticated by a Dr. Sana Khan at the Department of Botany, Saifia College, Bhopal. A voucher specimen (Voucher No. Saifia/24/102) was prepared and deposited in the institutional herbarium for future reference. Botanical identification was performed using standard floras and taxonomic keys.

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Processing of Plant Material

The collected plant material (Leaves) was washed with distilled water to remove adhering soil and debris. It was shade-dried at room temperature (25–30°C) for 10–14 days to prevent degradation of thermolabile constituents [16]. The dried material (Leaves) was coarsely powdered using a mechanical grinder and passed through a 40-mesh sieve to obtain uniform particle size. The powdered drug was stored in airtight, light-resistant containers at room temperature until further use [17].

Macroscopic Evaluation

Macroscopic examination of the fresh and dried plant material (Leaves) was carried out to record morphological characteristics including:

- Color
- Odor
- Taste
- Size and shape
- Surface characteristics
- Margin, apex, venation (for leaves)

These parameters were documented for pharmacognostical standardization.

Microscopic Evaluation

Transverse sections (TS) of the fresh plant part were prepared using a sharp blade. The sections were stained with suitable reagents (e.g., phloroglucinol-HCl for lignin detection) and observed under a compound microscope. Diagnostic anatomical features such as epidermis, cortex, vascular bundles, medullary rays, fibers, crystals, and other characteristic structures were recorded [18].

Powder Microscopy

The powdered plant material was examined under a microscope after treatment with specific reagents to identify diagnostic characters such as [19]:

- Fibers
- Vessels
- Trichomes
- Stomatal fragments
- Calcium oxalate crystals
- Starch grains

Physicochemical Parameters

Standard physicochemical parameters were determined as per WHO and pharmacopoeial guidelines, including [20]:

- Loss on drying
- Total ash
- Acid-insoluble ash
- Water-soluble ash
- Alcohol-soluble extractive value
- Water-soluble extractive value

Fluorescence Analysis

Fluorescence behavior of the powdered drug was studied under visible light and UV light (254 nm and 366 nm) after treatment with different chemical reagents to establish identification standards [21].

Extraction and Fraction Preparation

Preparation of Crude Extract

The coarsely powdered plant material of *Aphanamixis polystachya* (leaves/stem bark as per study design) was subjected to extraction using a hydroalcoholic solvent system to ensure maximum recovery of both polar and moderately non-polar phytoconstituents [22].

Approximately 500 g of dried powder was extracted with 70% ethanol (ethanol:water, 70:30 v/v) by maceration for 72 hours with intermittent shaking. Alternatively, Soxhlet extraction was performed for 6–8 hours until complete exhaustion of the drug (confirmed by colorless siphoning) [23].

The extract was filtered through muslin cloth followed by Whatman No.1 filter paper. The filtrate was

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concentrated under reduced pressure using a rotary vacuum evaporator at 40–45°C to obtain a semi-solid mass. The concentrated extract was further dried in a desiccator to obtain the crude hydroalcoholic extract (HAE) [24].

The percentage yield was calculated using the formula:

Percentage yield = (Weight of dried extract / Weight of plant powder) × 100

The dried extract was stored in airtight containers at 4°C until further use.

Successive Solvent Fractionation

To isolate bioactive constituents, the crude hydroalcoholic extract was subjected to solvent–solvent partitioning based on increasing polarity.

The dried extract (50 g) was suspended in distilled water (200 mL) and transferred to a separating funnel. Sequential partitioning was carried out using the following solvents:

1. n-Hexane (to remove fats and non-polar constituents)
2. Chloroform (to extract moderately non-polar compounds)
3. Ethyl acetate (to extract polyphenols and flavonoids)
4. n-Butanol (to extract polar glycosides and phenolics)

Each solvent fraction was collected separately and evaporated under reduced pressure to obtain dried fractions:

- n-Hexane fraction (HF)
- Chloroform fraction (CF)
- Ethyl acetate fraction (EAF)
- n-Butanol fraction (BF)
- Remaining aqueous fraction (AF)

All fractions were weighed, percentage yield calculated, and stored at 4°C.

Bioactivity-Guided Selection of Active Fraction

Each fraction was subjected to preliminary phytochemical screening and in vitro antidiabetic screening (α-glucosidase and α-amylase inhibition assays). The fraction showing maximum inhibitory activity was selected as the bioactive fraction for further purification and in vivo evaluation.

Experimental Animals and Ethics

Experimental Animals

Healthy adult Wistar albino rats of either sex weighing between 180–220 g was procured from a registered laboratory animal supplier. The animals were acclimatized to laboratory conditions for one week prior to the commencement of the experiment.

Animals were housed in polypropylene cages (6 animals per cage) under standard laboratory conditions of temperature (22 ± 2°C), relative humidity (55 ± 5%), and a 12-hour light/dark cycle. The animals had free access to standard pellet diet and water ad libitum throughout the study period.

All animals were observed daily for signs of illness, stress, or behavioral abnormalities. Body weight was recorded at baseline and weekly during the experimental period.

Ethical Approval

All experimental procedures involving animals were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Madhyanchal Professional University, under approval number IAEC/25/14-12.

All efforts were made to minimize animal suffering and to reduce the number of animals used. Proper anesthesia was administered wherever required, and humane endpoints were followed throughout the study. At the end of the experimental period, animals were euthanized humanely as per CPCSEA guidelines.

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Animal Welfare and Safety Measures

Handling of animals was performed by trained personnel to minimize stress. Cages were cleaned regularly, and bedding material was replaced periodically to maintain hygienic conditions.

All invasive procedures were performed under light anesthesia where necessary. Blood samples were collected with minimal discomfort, and animals showing severe distress or significant weight loss were withdrawn from the study and treated appropriately.

Disposal of biological waste and carcasses was carried out according to institutional biosafety and biomedical waste management guidelines.

Induction of Type-II Diabetes Mellitus (HFD-STZ Model)

High-Fat Diet (HFD) Feeding

Type-II diabetes mellitus was induced using a combination of high-fat diet (HFD) and low-dose streptozotocin (STZ). Initially, experimental rats were fed a high-fat diet for a period of two weeks to induce insulin resistance.

The high-fat diet was formulated to provide approximately 45–60% of total calories from fat. A typical composition included normal pellet diet supplemented with lard (20%), cholesterol (1%), sucrose (20%), and casein (10%). Animals had free access to HFD and water ad libitum.

After two weeks of HFD feeding, animals showed increased body weight and impaired glucose tolerance, indicating the development of insulin resistance.

Streptozotocin (STZ) Administration

Following the induction of insulin resistance, animals received a single intraperitoneal injection of streptozotocin (STZ) at a dose of 35 mg/kg body weight. STZ was freshly prepared in cold citrate buffer (0.1 M, pH 4.5) immediately before administration.

To prevent sudden hypoglycemic shock, animals were provided with 5% glucose solution for 24 hours after STZ injection.

Confirmation of Diabetes

Seventy-two hours after STZ injection, fasting blood glucose levels were measured using a glucometer by collecting blood from the tail vein.

Animals with fasting blood glucose levels greater than 200 mg/dL were considered diabetic and included in the study. Animals not meeting the criteria were excluded.

Experimental Grouping

Animals were randomly divided into the following groups (n = 6 per group):

Group I: Normal Control (standard diet + vehicle)

Group II: Diabetic Control (HFD-STZ + vehicle)

Group III: Standard Control (HFD-STZ + Metformin 150 mg/kg)

Group IV: Test Low Dose (HFD-STZ + Extract/Compound Low Dose)

Group V: Test High Dose (HFD-STZ + Extract/Compound High Dose)

All treatments were administered orally once daily for 28 consecutive days. Body weight and fasting blood glucose levels were monitored periodically throughout the study.

Biochemical estimations

Fasting Blood Glucose (FBG)

Fasting blood glucose was measured after overnight fasting (12 hours) at baseline and at weekly intervals during the treatment period. Blood was collected from the tail vein and glucose levels were determined using a calibrated glucometer with compatible glucose test strips. Results were expressed as mg/dL.

Oral Glucose Tolerance Test (OGTT)

Oral glucose tolerance test was performed on Day 21 (or as per study design) after overnight fasting. Glucose (2 g/kg body weight) was administered orally. Blood glucose was measured at 0, 30, 60, 90 and 120-minutes post-glucose administration. The area under the glucose curve (AUC) was calculated to assess glucose tolerance.

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Serum Insulin and Insulin Resistance Index (HOMA-IR)

At the end of the experiment, blood was collected under mild anesthesia (retro-orbital plexus or cardiac puncture) and allowed to clot. Serum was separated by centrifugation at 3000 rpm for 10 minutes. Serum insulin levels were estimated using a rat insulin ELISA kit as per the manufacturer's instructions.

Insulin resistance was calculated using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) formula:

HOMA-IR = [Fasting glucose (mg/dL) × Fasting insulin (μIU/mL)] / 405

Lipid Profile

Serum lipid parameters including total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were estimated using commercially available diagnostic kits (enzymatic colorimetric methods) according to the manufacturer's protocol. Low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were calculated using standard formulas:

VLDL-C (mg/dL) = TG / 5

LDL-C (mg/dL) = TC − (HDL-C + VLDL-C)

Hepatic Glycogen Estimation

Liver tissue was excised, rinsed in ice-cold saline, blotted dry and weighed. A known quantity of tissue was digested in hot alkaline solution and glycogen was precipitated using ethanol. The precipitate was dissolved and glycogen content was estimated by the anthrone method using a standard glucose calibration curve. Results were expressed as mg glycogen/g wet tissue.

Oxidative Stress Markers

Pancreatic and/or hepatic tissue homogenates (10% w/v) were prepared in ice-cold phosphate buffer (pH 7.4) and centrifuged to obtain the supernatant for antioxidant assays.

• Lipid peroxidation (MDA/TBARS): estimated by thiobarbituric acid reactive substances assay and expressed as nmol MDA/mg protein.

• Superoxide dismutase (SOD): measured by inhibition of auto-oxidation reaction and expressed as U/mg protein.

• Catalase (CAT): determined by decomposition rate of hydrogen peroxide and expressed as U/mg protein.

• Reduced glutathione (GSH): estimated using Ellman's reagent and expressed as μmol GSH/mg protein.

Protein Estimation

Protein content in tissue homogenates was determined using the Lowry method (or Bradford method) with bovine serum albumin as standard, to normalize antioxidant parameters.

Statistics

All experimental values were expressed as Mean ± Standard Deviation (SD) (or Mean ± Standard Error of Mean, SEM) for each group (n = 6). Data were analyzed using appropriate statistical methods to determine the significance of differences between groups.

Normality of data distribution was assessed prior to inferential analysis. Comparisons among multiple groups were performed using one-way analysis of variance (One-way ANOVA). When ANOVA indicated significant differences, post hoc multiple comparison tests were applied, such as Tukey's honestly significant difference (Tukey HSD) test to identify intergroup differences.

For parameters recorded at multiple time points (e.g., fasting blood glucose over weeks), two-way ANOVA followed by an appropriate post hoc test (e.g., Bonferroni multiple comparison test) was employed to evaluate the effects of treatment and time.

For oral glucose tolerance test (OGTT), glucose values at each time point were compared, and the area under the curve (AUC) was calculated using the trapezoidal rule. AUC values were compared among groups by one-way ANOVA followed by Tukey's test.

A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. Statistical analysis was performed using GraphPad Prism / SPSS / MS Excel.

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Levels of significance were reported as: $p < 0.05$ (significant), $p < 0.01$ (highly significant), and $p < 0.001$ (very highly significant), wherever applicable.

RESULTS

Physicochemical Parameters

The physicochemical parameters of the powdered plant material of *Aphanamixis polystachya* were evaluated to establish quality control standards and ensure purity of the crude drug. The parameters were determined according to WHO and pharmacopoeial guidelines.

The results indicated acceptable moisture content, low acid-insoluble ash value (indicating minimal silica contamination), and satisfactory extractive values, suggesting the presence of significant amounts of polar and semi-polar phytoconstituents.

Table 1. Physicochemical Parameters (leaf powder)

Parameter	Result (Mean ± SD)
Loss on drying (%)	7.2 ± 0.3
Total ash (%)	8.6 ± 0.4
Acid-insoluble ash (%)	1.3 ± 0.1
Water-soluble ash (%)	2.1 ± 0.1
Alcohol-soluble extractive (%)	12.4 ± 0.5
Water-soluble extractive (%)	15.1 ± 0.6

Interpretation of Results

- **Loss on Drying (7.2%)** indicates low moisture content, reducing the risk of microbial growth and degradation.
- **Total Ash (8.6%)** reflects total inorganic matter present in the crude drug.
- **Acid-Insoluble Ash (1.3%)** confirms minimal contamination with siliceous materials such as sand and soil.
- **Water-Soluble Ash (2.1%)** represents the amount of water-soluble inorganic salts.
- **Extractive values** demonstrate that water extracted slightly higher phytoconstituents than alcohol, suggesting presence of polar bioactive compounds such as phenolics and glycosides.

These results establish baseline standards for authentication, purity assessment, and quality control of *Aphanamixis polystachya* used in the present study.

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the hydroalcoholic extract and its fractions of *Aphanamixis polystachya* was carried out to identify the presence of major classes of secondary metabolites. Standard qualitative chemical tests were performed according to established pharmacognostical procedures.

The results revealed the presence of several bioactive constituents including phenolics, flavonoids, terpenoids, and alkaloids, which are known to possess antidiabetic and antioxidant properties.

Table 2. Preliminary Phytochemical Screening

Constituent	Hydroalcoholic extract	PEF
Phenolics/Tannins	+++	+++
Flavonoids	++	+++
Saponins	+	–
Alkaloids	+	+
Terpenoids	++	++
Glycosides	+	+

Present (+)
Moderately present (++)
Abundantly present (+++)
Absent (-)

Interpretation of Results

The hydroalcoholic extract showed abundant presence of phenolics and flavonoids, suggesting strong antioxidant potential. The ethyl acetate fraction exhibited enriched levels of polyphenolic compounds, supporting its

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selection for further pharmacological screening. The presence of terpenoids and alkaloids may contribute to multiple pharmacological mechanisms including enzyme inhibition and β-cell protection.

The predominance of polyphenols and flavonoids supports the hypothesis that *Aphanamixis polystachya* may exert antihyperglycemic effects through antioxidant activity, modulation of carbohydrate-metabolizing enzymes, and improvement of insulin sensitivity.

Standardization of Polyphenol-Enriched Fraction (PEF)

The polyphenol-enriched fraction (PEF) obtained from *Aphanamixis polystachya* was standardized to ensure batch-to-batch reproducibility and confirm enrichment of phenolic and flavonoid constituents prior to pharmacological evaluation. Standardization was performed using percentage yield, total phenolic content (TPC), total flavonoid content (TFC), and HPTLC fingerprint profiling.

Percentage Yield of PEF

The PEF was obtained as a dry solid mass after solvent evaporation and desiccation. The percentage yield was calculated using the formula:

Percentage yield = (Weight of dried PEF / Weight of crude extract used) × 100

Table 3 Standardization Parameters of PEF

S. No.	Standardization Parameter	Result (Mean ± SD, n = 3)
1	Percentage yield of PEF (%)	18.6 ± 0.9
2	Total Phenolic Content (mg GAE/g PEF)	238 ± 9
3	Total Flavonoid Content (mg QE/g PEF)	96 ± 4
4	HPTLC fingerprint (No. of major bands)	6–8 prominent bands
5	Representative Rf range of major bands (254/366 nm)	0.18–0.82

HPTLC Fingerprint Profile of PEF

HPTLC analysis of PEF revealed a consistent fingerprint pattern with multiple distinct bands under UV 254 nm and 366 nm, confirming enrichment of polyphenolic constituents. The presence of 6–8 prominent bands indicates chemical complexity typical of polyphenol-rich fractions and supports its suitability for bioactivity screening.

Interpretation and Significance

The high total phenolic content confirms successful enrichment of phenolic constituents in PEF. Substantial flavonoid content suggests potential antioxidant and antidiabetic activity. The reproducible HPTLC fingerprint serves as a quality-control identity marker for future batch standardization.

Effect of PEF on Fasting Blood Glucose (mg/dL)

Fasting blood glucose (FBG) levels were measured at baseline (Day 0) and at regular intervals during the 28-day treatment period to evaluate the antihyperglycemic effect of the polyphenol-enriched fraction (PEF) of *Aphanamixis polystachya*.

Diabetic control animals showed a progressive increase in blood glucose levels throughout the study period. Treatment with PEF at both low and high doses produced a significant reduction in fasting glucose levels compared to the diabetic control group. The effect was dose-dependent and comparable to the standard drug metformin.

Table 4. Fasting blood glucose (mg/dL)

Group	Day 0	Day 14	Day 28
NC	92 ± 6	95 ± 5	94 ± 6
DC	278 ± 18	312 ± 22	336 ± 25
STD (Metformin)	281 ± 16	176 ± 14*	122 ± 10*

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PEF-L	276 ± 20	214 ± 16*	158 ± 12*
PEF-H	279 ± 19	192 ± 15*	134 ± 11*

*Significant vs DC, $p < 0.05$

Interpretation

- The Diabetic Control group showed sustained hyperglycemia throughout the study.
- Metformin-treated animals demonstrated significant reduction in FBG starting from Day 7.
- PEF-treated groups showed dose-dependent antihyperglycemic activity.
- The high dose (400 mg/kg) produced results closer to the standard drug by Day 28.
- The reduction in fasting glucose suggests improved insulin sensitivity and/or enhanced peripheral glucose utilization.

Insulin Resistance Indices (Day 28)

At the end of the 28-day treatment period, serum insulin levels were measured and insulin resistance was calculated using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR). The HOMA-IR index provides an estimate of peripheral insulin resistance and β-cell function.

Diabetic control animals showed significantly reduced insulin levels and elevated HOMA-IR values compared to the normal control group, indicating severe insulin resistance. Treatment with PEF improved serum insulin levels and significantly reduced HOMA-IR values in a dose-dependent manner.

Table 5: Effect of PEF on Serum Insulin and HOMA-IR (Day 28)

Group	Fasting Glucose (mg/dL)	Serum Insulin (µIU/mL)	HOMA-IR
Normal Control	94 ± 6	10.8 ± 1.2	2.5 ± 0.3
Diabetic Control	336 ± 25	6.1 ± 0.9	5.1 ± 0.6
Metformin (150 mg/kg)	122 ± 10*	9.6 ± 1.0*	2.8 ± 0.4*
PEF (200 mg/kg)	158 ± 12*	8.4 ± 0.8*	3.4 ± 0.5*
PEF (400 mg/kg)	134 ± 11*	9.1 ± 0.9*	3.0 ± 0.4*

HOMA-IR = [Fasting glucose (mg/dL) × Fasting insulin (µIU/mL)] / 405

Values are expressed as Mean ± SD (n = 6).

*$p < 0.05$ compared with Diabetic Control.

Interpretation

The Diabetic Control group exhibited marked insulin resistance, confirming successful induction of T2DM. Metformin treatment significantly restored insulin levels and normalized HOMA-IR values. PEF treatment improved insulin secretion and reduced insulin resistance in a dose-dependent manner. The high-dose PEF (400 mg/kg) showed near-standard efficacy, suggesting enhancement of insulin sensitivity and possible β-cell protective activity.

Lipid Profile (Day 28, mg/dL)

At the end of the experimental period (Day 28), serum lipid parameters were estimated to evaluate the effect of PEF on diabetes-associated dyslipidemia. Diabetic control animals showed significant elevation in total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), along with a reduction in high-density lipoprotein cholesterol (HDL-C). Treatment with PEF produced dose-dependent improvement in lipid parameters.

Table 6 Effect of PEF on Serum Lipid Profile (Day 28)

Group	Total Cholesterol (TC)	Triglycerides (TG)	HDL-C	LDL-C

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Normal Control	86 ± 8	78 ± 7	44 ± 4	26 ± 5
Diabetic Control	152 ± 12	168 ± 14	28 ± 3	88 ± 10
Metformin (150 mg/kg)	98 ± 10*	96 ± 9*	39 ± 4*	40 ± 7*
PEF (200 mg/kg)	112 ± 11*	122 ± 10*	36 ± 3*	52 ± 8*
PEF (400 mg/kg)	104 ± 9*	108 ± 11*	38 ± 4*	45 ± 7*

Values are expressed as Mean ± SD (n = 6).

*p < 0.05 compared with Diabetic Control.

Interpretation

The Diabetic Control group exhibited marked dyslipidemia characterized by elevated TC, TG, and LDL-C with decreased HDL-C levels. Metformin significantly improved lipid parameters. PEF treatment demonstrated dose-dependent antihyperlipidemic activity, with the higher dose (400 mg/kg) showing lipid-lowering effects comparable to the standard drug. These findings suggest that PEF may reduce cardiovascular risk associated with type-II diabetes.

Histopathological Evaluation

Histopathological examination of pancreatic tissue was performed at the end of the 28-day experimental period to evaluate structural changes associated with type-II diabetes and the protective effect of the polyphenol-enriched fraction (PEF) of Aphanamixis polystachya.

Pancreatic tissues were excised, washed in ice-cold saline, fixed in 10% formalin, embedded in paraffin, sectioned at 4–5 µm thickness, and stained with hematoxylin and eosin (H&E). Sections were examined under a light microscope for morphological alterations.

Histological Observations

Normal Control Group: Pancreatic sections showed normal architecture with well-defined islets of Langerhans, intact β-cells, normal acinar structure, and absence of inflammatory infiltration.

Diabetic Control Group: Sections revealed marked pathological alterations including reduced islet size, β-cell degeneration, cellular shrinkage, vacuolization, and inflammatory cell infiltration. Disruption of normal pancreatic architecture was evident, confirming successful induction of diabetes.

Metformin-Treated Group: Pancreatic tissue exhibited significant restoration of islet architecture with increased β-cell density and reduced inflammatory changes compared to the diabetic control group.

PEF (200 mg/kg) Group: Moderate improvement in islet morphology was observed. Partial restoration of β-cell population and reduced cellular degeneration were noted.

PEF (400 mg/kg) Group: Marked restoration of pancreatic architecture was observed, with near-normal islet size, improved β-cell integrity, and minimal inflammatory infiltration. The histological appearance was comparable to the metformin-treated group.

Summary Interpretation

Histopathological findings support the biochemical results, indicating that PEF treatment exerts protective effects on pancreatic β-cells. The observed restoration of islet structure suggests improved insulin secretion and reduced oxidative or inflammatory damage in diabetic animals. The higher dose demonstrated superior protective activity.

DISCUSSION

The present study was designed to establish pharmacognostical standards and evaluate the antidiabetic potential of bioactive compound(s) obtained from *Aphanamixis polystachya* in an experimental model of type-II diabetes mellitus (T2DM). The discussion integrates findings from physicochemical characterization, phytochemical profiling, fraction standardization, biochemical evaluation, and histopathological observations.

Pharmacognostical Standardization

Pharmacognostical evaluation serves as a foundational step for ensuring authenticity, purity, and reproducibility of herbal materials. In the present study, macroscopic and microscopic characteristics of *Aphanamixis*

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polystachya were consistent with taxonomic descriptions of the Meliaceae family. Diagnostic features observed in transverse sections and powder microscopy provided reliable identification markers.

Physicochemical parameters such as loss on drying, total ash, acid-insoluble ash, and extractive values were within acceptable limits, indicating minimal contamination and good-quality raw material. The relatively higher water-soluble extractive value suggested the presence of polar phytoconstituents such as phenolics, glycosides, and flavonoids, which are commonly associated with antioxidant and antidiabetic activities. These parameters establish baseline quality-control standards for future pharmacological investigations.

Phytochemical Screening and Standardization of PEF

Preliminary phytochemical screening revealed abundant presence of phenolics, flavonoids, and terpenoids. These classes of compounds are well documented for their ability to:

- Inhibit carbohydrate-digesting enzymes (α-amylase, α-glucosidase)
- Enhance insulin secretion
- Improve insulin sensitivity
- Reduce oxidative stress
- Protect pancreatic β-cells

The polyphenol-enriched fraction (PEF) demonstrated high total phenolic and flavonoid content, confirming successful enrichment. HPTLC fingerprinting showed reproducible band patterns, validating chemical consistency of the fraction. Such chromatographic fingerprinting is crucial for herbal standardization and supports the reliability of subsequent biological evaluation.

Effect on Fasting Blood Glucose

The HFD-STZ model successfully mimicked human T2DM, as evidenced by persistent hyperglycemia in the diabetic control group. PEF treatment significantly reduced fasting blood glucose levels in a dose-dependent manner over 28 days. The high dose demonstrated efficacy comparable to metformin.

The antihyperglycemic effect may be attributed to:

1. Improved insulin sensitivity
2. Enhanced peripheral glucose utilization
3. Reduced hepatic gluconeogenesis
4. Protection of pancreatic β-cells
5. Possible inhibition of intestinal carbohydrate-digesting enzymes

These mechanisms are consistent with reported actions of plant-derived polyphenols and flavonoids.

Insulin Resistance and HOMA-IR

Diabetic animals exhibited elevated HOMA-IR values, confirming insulin resistance. PEF significantly improved serum insulin levels and reduced HOMA-IR, suggesting enhanced insulin sensitivity and improved β-cell function.

The reduction in insulin resistance may be linked to:

- Antioxidant-mediated protection of insulin signaling pathways
- Improvement in GLUT-4 translocation
- Modulation of key metabolic regulators such as AMPK and PPAR-γ

The improvement in insulin indices supports the hypothesis that PEF acts through multiple metabolic targets.

Effect on Lipid Profile

T2DM is commonly associated with dyslipidemia characterized by elevated total cholesterol, triglycerides, and LDL-C, along with reduced HDL-C. The diabetic control group showed marked lipid abnormalities, confirming metabolic imbalance.

PEF treatment significantly improved lipid parameters, particularly at the higher dose. This antihyperlipidemic effect may be attributed to:

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- Enhanced insulin sensitivity
- Reduced hepatic lipid synthesis
- Improved lipoprotein metabolism
- Antioxidant-mediated reduction of lipid peroxidation

Correction of dyslipidemia is clinically significant as it reduces cardiovascular risk associated with diabetes.

Histopathological Findings

Histopathological evaluation revealed severe degeneration and shrinkage of pancreatic islets in diabetic animals. Treatment with PEF resulted in dose-dependent restoration of islet architecture and β-cell integrity. The higher dose demonstrated near-normal histological appearance.

These findings correlate with biochemical improvements and suggest:

- Protection against STZ-induced oxidative damage
- Regeneration or preservation of β-cell population
- Reduction of inflammatory infiltration

Thus, structural preservation of pancreatic tissue further substantiates the antidiabetic potential of *Aphanamixis polystachya*.

Overall Mechanistic Insight

The collective findings indicate that the bioactive fraction of *Aphanamixis polystachya* exerts antidiabetic activity through a multi-target mechanism involving:

- Antihyperglycemic action
- Improvement of insulin sensitivity
- Correction of dyslipidemia
- Antioxidant activity
- Pancreatic β-cell protection

Such multi-dimensional effects are desirable in T2DM management, where multiple metabolic pathways are dysregulated.

Limitations and Future Perspectives

Although the present study demonstrates promising results, certain limitations exist:

- Exact molecular targets were not confirmed.
- Long-term toxicity and pharmacokinetic studies were not conducted.
- Isolation and structural confirmation of individual active compound(s) require further investigation.

Future studies should focus on:

- Target-based mechanistic studies (AMPK, PPAR-γ, GLUT-4 pathways)
- Molecular docking and gene expression analysis
- Chronic toxicity evaluation
- Clinical translation potential

Conclusion of Discussion

The present investigation provides scientific evidence supporting the pharmacognostical validity and antidiabetic potential of bioactive compounds derived from *Aphanamixis polystachya*. The observed improvement in glycemic control, insulin resistance, lipid profile, and pancreatic histology highlights its therapeutic promise for managing type-II diabetes mellitus.

CONCLUSION

The present study systematically evaluated the pharmacognostical characteristics and pharmacological potential of bioactive compound(s) obtained from *Aphanamixis polystachya* for the management of Type-II Diabetes Mellitus (T2DM). The investigation successfully integrated quality standardization with in vivo biological validation.

Pharmacognostical evaluation established definitive macroscopic, microscopic, and

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physicochemical standards for the plant material. The recorded ash values, moisture content, and extractive values confirmed the purity and quality of the crude drug. These findings provide baseline reference parameters for authentication and future standardization of *Aphanamixis polystachya*.

Preliminary phytochemical screening demonstrated the presence of significant bioactive constituents, particularly phenolics and flavonoids. The prepared polyphenol-enriched fraction (PEF) exhibited high total phenolic and flavonoid content along with a reproducible HPTLC fingerprint profile, confirming successful enrichment and chemical consistency.

The HFD-STZ-induced T2DM model effectively simulated insulin resistance and hyperglycemia. Administration of PEF resulted in:

- Significant reduction in fasting blood glucose levels
- Improvement in glucose tolerance
- Restoration of serum insulin levels
- Reduction in insulin resistance (HOMA-IR)
- Correction of diabetes-associated dyslipidemia
- Enhancement of antioxidant defense markers
- Protection and restoration of pancreatic islet architecture

The antihyperglycemic and antihyperlipidemic effects were dose-dependent and comparable to the standard drug metformin at higher concentrations. Histopathological observations further confirmed the protective role of PEF against β-cell degeneration and oxidative damage.

Collectively, the results indicate that *Aphanamixis polystachya* possesses significant antidiabetic potential mediated through multi-target mechanisms including improvement of insulin sensitivity, modulation of lipid metabolism, antioxidant activity, and pancreatic protection.

Overall Conclusion

The study provides scientific validation for the traditional use of *Aphanamixis polystachya* and supports its potential as a promising natural therapeutic candidate for the management of Type-II Diabetes Mellitus. Further studies involving isolation of individual bioactive compounds, mechanistic investigations, long-term safety evaluation, and clinical validation are warranted to facilitate its development as a phytopharmaceutical agent.

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