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Assessment of the Pharmacognostical, Phytochemicals and Antidiabetic properties of the flowers of *Acacia nilotica* Linn and *Acacia leucophloea* Willd

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Abstract

The present study evaluated the pharmacognostical, phytochemical, pharmaceutical, and pharmacological potential of AN and AL for developing standardized herbal tablets for diabetes mellitus (DM) management. Macroscopic, microscopic, and powder microscopic studies confirmed the identity, purity, and authenticity of the plant materials, while physicochemical parameters indicated acceptable quality. Phytochemical screening revealed flavonoids, phenolics, tannins, saponins, and triterpenoids, with significant total phenolic and flavonoid contents. TLC, HPTLC, and HPLC profiling established characteristic fingerprints and confirmed marker compounds including quercetin and lupeol. Hydroalcoholic extracts were successfully formulated into tablets by direct compression, with satisfactory pre- and post-compression characteristics. Formulation F4 demonstrated optimal performance. In vitro studies showed concentration-dependent α -amylase and α -glucosidase inhibition, while in vivo evaluation demonstrated reduced blood glucose levels and improved lipid profiles. Histopathological findings confirmed protective effects on pancreatic and vital tissues. Overall, the standardized herbal tablets exhibited promising antidiabetic activity and potential as natural therapeutic agents for DM management.

Keywords; *Acacia nilotica* (AN), *Acacia leucophloea* (AL), Herbal tablets, Antidiabetic activity, Phytochemical standardization.

INTRODUCTION: METEOROLOGY AS LITERARY EXPERIENCE

Overview of Diabetes Mellitus

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from impaired insulin production, insulin action, or both. Insulin, secreted by pancreatic β -cells, plays a central role in regulating glucose metabolism by promoting glucose uptake into peripheral tissues and suppressing hepatic glucose production [1,2]. Prolonged hyperglycaemia causes oxidative stress, inflammation, and endothelial dysfunction, leading to progressive damage to organs such as the eyes, kidneys, nerves, heart, and blood vessels [1,3]. The major forms of diabetes are Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM). T1DM results primarily from autoimmune destruction of pancreatic β -cells, causing absolute insulin deficiency and requiring lifelong insulin therapy. T2DM accounts for approximately 90–95% of diabetes cases and is mainly associated with insulin resistance and relative insulin deficiency [4,5]. Obesity, physical inactivity, age, genetic susceptibility, and unhealthy dietary habits are important risk factors. Other forms include gestational diabetes mellitus and secondary diabetes caused by pancreatic disorders, endocrine abnormalities, infections, or certain medications.

Diabetes is diagnosed using laboratory parameters such as fasting plasma glucose, oral glucose tolerance testing, and glycated hemoglobin (HbA1c) [6,7].

Common symptoms include polyuria, polydipsia, polyphagia, unexplained weight loss, fatigue, blurred vision, and delayed wound healing. Untreated diabetes can cause acute complications such as diabetic ketoacidosis and hyperosmolar hyperglycemia, while long-term complications include retinopathy, nephropathy, neuropathy, cardiovascular disease, stroke, and peripheral vascular disease [8,9]. Management involves dietary modification, regular exercise, weight control, glucose monitoring, insulin, and oral antidiabetic drugs. However, limitations such as adverse effects, cost, and poor compliance have increased interest in medicinal plants and herbal preparations as potential complementary therapeutic approaches [10–12].

Role of Medicinal Plants in Diabetes Management

Medicinal plants have been traditionally used for generations to manage diabetes and improve metabolic health through systems such as Ayurveda, Unani, and Traditional Chinese Medicine. Their therapeutic potential, affordability, and relatively fewer side effects have increased scientific interest in their use for diabetes management [13, 14]. Plant-derived bioactive compounds, including alkaloids, flavonoids, glycosides, tannins, terpenoids, and phenolics, may regulate blood glucose by enhancing insulin secretion and sensitivity, promoting peripheral glucose uptake, reducing intestinal glucose absorption, and decreasing hepatic glucose production. Their antioxidant properties may also protect pancreatic β -cells from oxidative damage [15, 16]. Several medicinal plants, including *Gymnema sylvestre*, *Momordica charantia*, *Trigonella foenum-graecum*, *Azadirachta indica*, and *Syzygium cumini*, have demonstrated hypoglycemic and antihyperglycemic effects. Some inhibit carbohydrate-digesting enzymes such as α -amylase and α -glucosidase, thereby reducing postprandial hyperglycemia. The presence of multiple phytochemicals enables herbal medicines to act through several metabolic pathways simultaneously, addressing insulin resistance, oxidative stress, inflammation, and impaired glucose metabolism [17, 18].

Medicinal plants may also help prevent diabetes-related complications through antioxidant, anti-inflammatory, and lipid-lowering effects. Their accessibility and affordability are particularly valuable in developing countries such as India [19]. Advances in HPLC, HPTLC, spectroscopy, and formulation technologies have improved the standardization, quality, stability, bioavailability, and therapeutic effectiveness of herbal medicines. Among these plants, *Acacia* species have attracted attention for their antidiabetic, antioxidant, anti-inflammatory, and

antibacterial properties, particularly due to their flavonoid, tannin, and phenolic contents [20].

OBJECTIVES

The current study's goals are to produce and assess herbal tablet formulations made from AN and AL methodically. The particular goals consist of:

1. **Collection and Authentication of Plant Materials:** To collect plant materials of AN and AL from suitable geographical sources and authenticate them using standard botanical identification procedures [21].
2. **Preparation of Plant Extracts:** To prepare suitable extracts from selected plant parts using appropriate extraction techniques to obtain maximum yield of phytoconstituents [22].
3. **Phytochemical Screening:** To perform qualitative phytochemical screening of plant extracts for the identification of major classes of phytoconstituents such as alkaloids, flavonoids, tannins, glycosides, and phenolic compounds [23].
4. **Pre-formulation Studies:** To conduct pre-formulation studies, including evaluation of flow properties, particle size distribution, moisture content, and compressibility characteristics of herbal powders [24].
5. **Formulation of Herbal Tablets:** To formulate herbal tablets using suitable excipients and pharmaceutical techniques such as wet granulation or direct compression [25].
6. **Evaluation of Tablet Parameters:** To evaluate formulated tablets for physicochemical parameters including hardness, friability, weight variation, disintegration time, thickness, and uniformity of content [25].
7. **Stability Studies:** To conduct stability studies under controlled environmental conditions to assess the stability and shelf life of the formulated herbal tablets [26].
8. **Quality Standardisation:** To establish quality control parameters for the developed herbal tablets in accordance with pharmacopoeial and regulatory guidelines [27].

MATERIALS AND METHODS

Collection and Authentication of Plant Material

To guarantee the highest phytoconstituent output, plant materials of AN and AL were gathered from naturally occurring populations in unpolluted areas at the proper blooming or mature stage. To conserve thermolabile

components, the gathered plant parts were carefully separated, cleaned with distilled water to get rid of surface impurities, and then shade-dried at room temperature. A mechanical grinder was used to ground the dry material, which was then placed in airtight containers for further examination. A certified taxonomist verified the plant material in an accredited herbarium, where a voucher specimen was stored for later use. Reproducibility and scientific validation of pharmacognostic and pharmacological research were guaranteed by proper identification [28-30].

Physicochemical Evaluation

The quality and purity of the crude medication were assessed physicochemically using established metrics such as ash values, extractive values, moisture content, and drying loss. These factors are crucial for evaluating plant material stability, solvent-soluble components, and inorganic contaminants. The investigations were carried out using approved pharmacopoeial techniques that have been documented in previous studies on herbal standardization. These assessments guarantee that the raw material satisfies the quality requirements necessary for the creation of pharmaceutical formulations [35-37].

Ash Values: Accurately weighed plant material was burned at 450–600°C in a silica crucible to quantify total ash, acid-insoluble ash, and water-soluble ash. Total ash represents the total inorganic content, while acid-insoluble ash indicates silica and earthy matter. The quantity of inorganic salts soluble in water may be measured with the use of water-soluble ash. When assessing the overall purity, adulteration, and contamination of crude pharmaceuticals, these factors are essential. The results are contrasted with conventional limitations for quality assurance that have been documented in pharmacognosy literature [36, 37].

Extractive Values

Solvents with increasing polarity, such as water, ethanol, and petroleum ether, were used to calculate extractive values. Each solvent was used to macerate a known amount of powdered medication for 24 to 48 hours while being shaken periodically. After the extracts were filtered and dried by evaporation, the yield percentage was computed. Extractive values reveal the chemical makeup of the plant material and show the existence of active phytoconstituents soluble in various solvents. This technique is often used to assess extraction efficiency in herbal standardization research [38, 39].

$$\text{Extractive Value [\%]} = \frac{\text{Weight of sample}}{\text{Weight of extract}} \times 100$$

Loss on Drying

A carefully weighed sample was heated to 105°C until a consistent weight was achieved in order to calculate the loss on drying. The sample's moisture and volatile components are reflected in the weight loss. One important factor affecting the stability, enzymatic breakdown, and microbiological development of herbal medications is moisture content. The plant material's stability and shelf life are enhanced by maintaining a low moisture level. Pharmacopoeial guidelines for the evaluation of herbal quality generally support this approach [40].

Moisture Content

Depending on the appropriateness of the sample, either Karl Fischer titration or gravimetric procedures were used to determine the moisture content. The amount of free and bound water in the sample was measured. Microbial contamination and phytoconstituent breakdown might result from excessive moisture in herbal medications. For pharmaceutical formulation to be stable and appropriate, moisture management is crucial. Moisture determination is a crucial quality criterion in the standardization of herbal drugs, according to recent research [41].

Phytochemical Standardization

To guarantee uniformity in the bioactive components found in the plant material, phytochemical standardization was carried out. Major secondary metabolites, including alkaloids, flavonoids, tannins, and phenolic substances, were analyzed both qualitatively and quantitatively. The antidiabetic action is mostly caused by these phytoconstituents. A crucial stage in the creation of herbal drugs is standardization, which guarantees the repeatability of pharmacological effects. Phytochemical profiling is the basis for quality control in herbal formulations, according to recent research [42-44].

Preparation of Extract

Soxhlet extraction was performed on the powdered plant material using a hydroalcoholic solvent [ethanol:water 70:30 v/v]. A thimble was filled with between 100 and 500 g of powdered medication, which was constantly withdrawn until it was completely exhausted. To conserve thermolabile components, the extract was concentrated using a rotary evaporator at low temperature and low pressure. For future research, the concentrated extract was dried and kept in airtight containers at 4°C. Herbal pharmacological research has extensively documented this technique, which guarantees the effective extraction of both polar and semi-polar phytoconstituents [45-47].

Preliminary Phytochemical Screening

Standard qualitative chemical assays were used for preliminary phytochemical screening in order to identify the presence of main phytochemical groups. Alkaloids were tested using Mayer's and Dragendorff's methods, flavonoids using the Shinoda test, tannins using the ferric chloride test, saponins using the foam test, and glycosides using the Keller-Killiani test. The findings verified the existence of bioactive secondary metabolites that have antidiabetic properties. In herbal pharmacological research, these screening techniques are often used as first instruments [48-49].

Quantitative Estimation

Spectrophotometric techniques were used to quantify the TPC [TPC], TFC [TFC], and total tannin content. TPC was estimated using the Folin-Ciocalteu reagent, TFC was estimated using the aluminum chloride technique, and tannins were estimated using the Folin-Denis method. Gallic acid and quercetin equivalents were used to express the findings. The antioxidant and antidiabetic activity of plant extracts is shown by these quantitative estimates. Antihyperglycemic action is closely associated with high phenolic and flavonoid content [50-52]. The TPC of the extract was determined using the Folin-Ciocalteu method, which is based on the reduction of the Folin-Ciocalteu reagent by phenolic compounds under alkaline conditions, resulting in the formation of a blue-colored complex. In the procedure, a known volume of the plant extract was mixed with diluted Folin-Ciocalteu reagent, followed by the addition of sodium carbonate solution. The mixture was incubated at room temperature for a specified time, and the absorbance was measured at 765 nm using a UV-Visible spectrophotometer. The TPC was calculated using a calibration curve prepared with gallic acid and expressed as mg gallic acid equivalents (GAE) per gram of extract.

Similarly, the TFC was estimated using the aluminium chloride colorimetric method, which is based on the formation of a yellow-colored complex between flavonoids and aluminium chloride. In this method, the plant extract was treated with aluminium chloride and potassium acetate, followed by incubation at room temperature. The intensity of the developed color was measured at 415 nm using a UV-Visible spectrophotometer. The flavonoid content was determined using a calibration curve of quercetin and expressed as mg quercetin equivalents (QE) per gram of extract. These methods provide a reliable estimation of phenolic and flavonoid contents, which are important indicators of the antioxidant potential of plant extracts.

Chromatographic Analysis

Phytoconstituent measurement and fingerprint profiling were done using TLC, HPTLC, and HPLC methods. HPLC was used for accurate analysis of active ingredients, HPTLC for densitometric quantification, and TLC for initial compound identification. The batch uniformity and chemical standardization of plant extracts are guaranteed by these chromatographic methods. Chromatographic fingerprinting is a crucial quality control method in the creation of herbal drugs, according to recent study [53-57].

Formulation of Herbal Tablets

The formulation of herbal tablets using standardized plant extracts has become an effective phytopharmaceutical approach for improving dose accuracy, product stability, and patient compliance. Among the available manufacturing methods, direct compression is widely preferred because it involves minimal processing and reduces the exposure of phytoconstituents to heat and moisture, thereby preserving the stability of thermolabile bioactive compounds. This technique also offers simplicity, cost-effectiveness, and good reproducibility in herbal tablet production.

The successful development of herbal tablets depends largely on the selection of suitable excipients that enhance powder flow and compressibility. Microcrystalline cellulose is commonly employed as a diluent because of its excellent compressibility and compatibility with plant-derived materials. Superdisintegrants such as sodium starch glycolate and croscarmellose sodium improve tablet disintegration and dissolution, ensuring efficient release of active phytoconstituents. Polyvinylpyrrolidone (PVP) functions as a binder by increasing particle cohesion and mechanical strength, while magnesium stearate and talc are used as lubricant and glidant, respectively, to reduce interparticle friction and improve die filling and weight uniformity.

Pre-compression evaluation of powder blends, including bulk density, tapped density, Carr's compressibility index, and Hausner's ratio, is essential for predicting flow behavior and ensuring smooth compression. Similarly, post-compression quality parameters such as hardness, friability, disintegration time, and weight variation are critical for assessing tablet performance. Overall, direct compression provides a reliable and reproducible method for producing standardized herbal tablets with consistent quality, stability, and therapeutic effectiveness. [50, 52, 54].

Selection of Excipients

The selection of proper excipients is a very important step during the development of herbal tablet formulations, because the excipients play an important role in powder flow properties, compressibility, stability and drug release behavior. Recent pharmaceutical studies have shown that herbal extracts usually have poor flowability and compressibility because of their fibrous nature and hygroscopic properties, thus the appropriate excipients should be used to improve formulation performance [58]. Microcrystalline cellulose is a commonly used diluent in herbal formulations because of its good compressibility, inertness and ability to enhance tablet hardness without any adverse effect on drug release. Commonly included are superdisintegrants like sodium starch glycolate and croscarmellose sodium which help the tablet to absorb water and swell rapidly leading to faster disintegration of tablets and improved dissolution efficiency [59].

Binders are crucial in imparting mechanical strength to tablets. Polyvinylpyrrolidone [PVP K-30] has been widely reported as a suitable binder because of its compatibility with phytoconstituents and its ability to improve particle cohesion during compression. Lubricants, such as magnesium stearate, are added in small amounts to ensure that the surfaces of the tablets and the walls of the dies between which they are compressed do not stick or pick during compression. In addition, glidants, such as talc, improve powder flow and provide uniform die filling, leading to consistent tablet weight and uniform drug distribution [60]. The compatibility of herbal extracts with excipients is usually checked by Fourier Transform Infrared [FTIR] spectroscopy or differential scanning calorimetry [DSC] in order to make sure that no chemical interaction occurs that could affect stability or therapeutic efficacy [61]. Selection of excipients should be based on functional requirements and compatibility studies to produce herbal tablets with desirable mechanical strength, rapid disintegration and consistent therapeutic performance [62].

Method of Tablet Preparation

The direct compression method is well known as a simple and efficient method for the preparation of solid dosage forms with minimum of processing steps. The herbal tablets were prepared by using the direct compression method with standardized extracts of AN/AL. Recent studies have revealed the direct compression technique particularly advantageous for the herbal formulations by minimizing the exposure of the phytoconstituents to moisture and heat, and therefore, preserving the stability and bioactivity of the

thermolabile compounds [63]. In this method, accurate weighed quantity of dried herbal extract and selected excipients were individually passed through sieve No. 40 to have uniform particle size distribution and to remove agglomerates. The sieved materials were geometrically diluted and mixed thoroughly to ensure uniform distribution of the active constituents in the powder blend [64].

Lubricants like magnesium stearate and glidants like talc were added in the last stage of blending to minimize friction between powder particles and die walls during compression. The prepared powder blend was then filled in the hopper of rotary tablet compression machine using suitable punches of predetermined diameter. Compression parameters such as compression force and turret speed were optimized to get the tablets with uniform weight, adequate hardness and minimum friability. It has been reported that mechanical integrity and physical quality of tablets prepared by direct compression technique are significantly affected by careful control of compression pressure and blending time [65, 66]. The tablets prepared were visually inspected after compression for any defects such as capping, lamination or sticking and were stored in air tight containers under controlled environmental conditions to protect from moisture and environmental degradation. The direct compression method is still one of the most reliable methods for preparing herbal tablets due to its reproducibility, scalability and consistent tablet quality characteristics [67].

Optimization of Formulation

The herbal tablet formulation was optimized to obtain suitable mechanical strength, rapid disintegration, and consistent drug-release characteristics. In the present study, six formulation batches (F1–F6) were prepared by varying the concentrations of key excipients, particularly the binder and disintegrant, while maintaining a constant amount of standardized AN/AL extract. Optimization of excipient concentrations is essential for controlling the physicochemical properties and therapeutic performance of herbal tablets. A trial-based approach incorporating factorial design principles was used to assess the effects of independent variables, namely PVP K-30 and sodium starch glycolate concentrations, on dependent parameters such as hardness, friability, disintegration time, and dissolution rate [68], [69].

Before compression, each formulation was evaluated for powder-flow properties, while post-compression studies assessed mechanical strength and tablet integrity. Increasing binder concentration generally improved tablet hardness and reduced friability; however, excessive binder could prolong

disintegration and delay drug release. Conversely, higher concentrations of sodium starch glycolate promoted faster tablet disintegration through increased water uptake and swelling within the tablet matrix. Therefore, appropriate balancing of formulation variables was necessary to achieve pharmacopoeial quality requirements [70].

Statistical optimization approaches, including response surface methodology (RSM) and factorial design, can further identify suitable formulation compositions with fewer experimental trials by predicting interactions among formulation variables and their combined effects on tablet performance [71]. Comparative evaluation of F1–F6 enabled selection of the optimized batch based on acceptable hardness, friability below 1%, uniform weight variation, rapid disintegration, and satisfactory dissolution. The optimized formulation was considered suitable for subsequent in-vitro and in-vivo antidiabetic studies, supporting reproducible and consistent therapeutic performance [72].

Evaluation of Herbal Tablets

Prepared herbal tablets were subjected to evaluation for their physical integrity, mechanical strength, uniformity and drug release characteristics. These evaluations are important in the assessment of the quality of the formulated tablets to ensure the tablets meet pharmacopoeial specifications and exhibit a uniform quality profile for therapeutic use. The pre-compression and post compression investigations of the optimized formulation batches were performed according to standard procedures mentioned in recent pharmaceutical research. The parameters of such evaluation provide useful insight into flow behavior of powder blends and performance characteristics of compressed tablets and therefore ensuring reproducibility and reliability of the developed herbal dosage form [73, 74].

Pre-compression Parameters

Precompression parameters were employed to determine the flow properties and compressibility behavior of the powder blends before compression into tablets. Good flow properties are required to ensure even die filling during compression and to minimize weight variation between tablets. Standard pharmacopoeial procedures were followed for the evaluation of angle of repose, bulk density, tapped density, Carr's compressibility index and Hausner's ratio of the powder blends of different formulation batches. These parameters are generally accepted as reliable parameters of the flowability and compressibility properties of the powder during the tablet manufacturing process [75].

- i. **Angle of Repose:** The flowability of the powder blend was determined by measuring the angle of repose by the fixed funnel method. The powder was poured from a funnel held at a fixed height on to a flat surface and formed a conical heap. The measurements of height and radius of the heap were used to obtain the angle of repose by the standard mathematical formula. When the angle of repose value is minimum, then the flow properties of powder blend are better. Good flowability is important for uniform die filling during compression which affects the weight uniformity and dosage accuracy of the tablets.
- ii. **Density Bulk:** Bulk density was determined by carefully pouring a known amount of powder into a graduated cylinder without tapping. The volume occupied by the powder was measured and the bulk density was calculated by dividing the mass of the powder by the bulk volume. This parameter gives information about the packing behavior and the porosity of the powder blend. The values of bulk density can be used to determine the appropriate tablet size and compression force needed during the manufacturing process.
- iii. **Density [bulk]:** The tapped density was determined by mechanically tapping the graduated cylinder containing the powder blend with a tapped density apparatus until a constant volume was obtained. The last tapped volume was recorded and tapped density was calculated on the basis of it. This parameter is a measure of the degree of packing obtained by mechanical tapping. It is used in the calculation of compressibility indices. The higher the tapped density value, the greater the packing efficiency and lesser inter-particulate void spaces within the powder blend.
- iv. **Carr's compressibility index:** The compressibility properties of the powder blend were determined by calculating Carr's compressibility index from the bulk density and tapped density values. This parameter gives information on the tendency of the particles in the powder to consolidate and their cohesion during compression. Lower compressibility index values of powders are considered to be suitable for direct compression methods due to their better flow and compressibility behavior.
- v. **The Hausner Ratio:** The Hausner's ratio was calculated as the ratio of the bulk density to the tapped density. This parameter is widely used to

predict the flow properties of powders and is considered to be a reliable indicator of inter-particle friction. The lower the value of Hausner's ratio, the better is the flowability, which is essential for consistent tablet weight and quality during industrial large-scale manufacturing processes.

Parameters After Compression.

The physical and mechanical properties of the compressed tablets were evaluated after compression. The tests ensure the tablets are hard enough not to break when handled, packaged and transported. Prepared tablets were evaluated for thickness, hardness, friability, weight variation, disintegration time, dissolution profile and uniformity of drug content by standard pharmacopeial methods. These parameters are important indicators for tablet quality and performance properties [76].

- i. **Tablet Thickness:** Thickness of the tablet was measured by using a calibrated vernier caliper to maintain uniformity in tablet dimension. Variation in thickness might indicate that the die filling or compression force was not consistent during tablet manufacturing. Tablet thickness uniformity is important for tablet weight uniformity and compatibility with packaging.
- ii. **Hardness of Tablet:** The mechanical strength of tablets was evaluated by using a Monsanto hardness tester for the determination of tablet hardness. The hardness should be sufficient to avoid breakage in handling and transport. Tablets with suitable hardness values are more likely to retain their structural integrity during storage and distribution.
- iii. **Tablet Friability:** In order to test for friability, the tablets were placed in a Roche friabilator to determine their resistance to abrasion and mechanical shock. A known number of tablets was rotated at a fixed speed for a fixed time and the percentage loss in weight was determined. Tablets with friability values of <1% are acceptable by pharmacopeial standards.
- iv. **Weight Fluctuations:** The uniformity of tablet weight was assessed by weighing individual tablets using analytical balance and by conducting the weight variation test. Uniformity of weight of tablets implies uniformity of active ingredients and accuracy of dosage. The tablets were analyzed for compliance to pharmacopeial limits for standard quality specifications.

- v. **Collapse Moment:** Distilled water was kept at a constant temperature and used for disintegration testing with a USP disintegration test apparatus. Noted time taken for tablets to break into smaller pieces. The rapid disintegration is required to increase the dissolution and to improve the bioavailability of the active ingredients.
- vi. **Dissolution Study:** Dissolution testing was performed to evaluate the release behavior of active constituents from the tablets using USP dissolution apparatus [Type II paddle method]. Samples were taken at pre-determined times under controlled conditions of dissolution medium, temperature and rotation speed. The release kinetic profiles were prepared and the release kinetics were determined. Spectrophotometric determination of drug release was performed.
- vii. **Content Uniformity of Drug:** The uniformity of drug content was determined by crushing individual tablets and assaying the amount of active constituent by UV-visible spectrophotometer or chromatographic methods. The tablets with the uniform distribution of drug will provide consistent therapeutic performance and will comply with the pharmacopeial standards.

Antidiabetic Evaluation

The antidiabetic activity of the optimized herbal tablet formulation containing standardized extract of AN / AL was evaluated by in-vitro and in-vivo experimental models. Several experimental approaches should be evaluated to establish the mechanism of antihyperglycemic activity and to validate the therapeutic potential of plant-based formulations. Recent pharmacology research has shown that the combination of enzyme inhibition assays and animal diabetic models can reliably demonstrate the glucose lowering activity and pancreatic protective effects [286]. The present work involved in-vitro α amylase and α -glucosidase inhibitory assays followed by in-vivo studies on chemically induced diabetic animal models. These methods provide a systematic evaluation of the herbal formulation for its ability to regulate carbohydrate metabolism, improve glucose tolerance and restore pancreatic function [77].

In-vitro Antidiabetic Studies

α -Amylase Inhibition Assay: The inhibition of starch hydrolysis by the herbal extract was determined by a modified colorimetric method for the measurement of α -amylase inhibition [24]. The tablet powder was extracted and dissolved in phosphate buffer and incubated with α -

amylase enzyme at controlled temperature conditions. Soluble starch solution was added as substrate after incubation and allowed to react for a certain period of time. The reaction was terminated by the addition of dinitrosalicylic acid [DNS] reagent and heating in a boiling water bath for color development. The absorbance of the obtained solution was measured at the suitable wavelength using UV-Visible spectrophotometer. Enzyme activity was expressed as percentage inhibition relative to control samples. Recently, it has been shown that inhibition of the enzyme α -amylase reduces postprandial hyperglycemia and plays an important role in the management of diabetes.

α -Glucosidase Inhibition Assay: The anti-glucosidase activity of the herbal extract was measured as an inhibition of formation of glucose from carbohydrate substrates by α -glucosidase inhibition assay. In this method the α -glucosidase enzyme was incubated with different concentrations of herbal extract at controlled temperature conditions. A synthetic substrate, p-nitrophenyl- α -D glucopyranoside [pNPG], was added to start the reaction. After incubation, the reaction was terminated by the addition of an alkaline solution and the amount of p-nitrophenol released was measured spectrophotometrically. The inhibitory activity of the extracts was estimated by comparing the absorbance values with control samples. α -Glucosidase inhibition has been shown to delay glucose absorption from the intestine and is a major contributor to the reduction of postprandial blood glucose levels [78, 79].

In-vivo Antidiabetic Studies

The in-vivo antidiabetic study was performed to evaluate the therapeutic potential of the optimized herbal tablet formulation in experimental animal models. For herbal drugs, animal studies offer detailed data on pharmacodynamic activity, metabolic regulation and effects on tissue levels. The experimental animals were Wistar albino rats, because of the physiological similarity to the metabolic responses in humans. The animals were housed in the laboratory under controlled conditions of temperature, humidity and light:dark cycles. Ethics approval and consent to participate section the animal experiments were approved by the Institutional Animal Ethics Committee [IAEC] according to the national guidelines for animal research [80, 81].

- i. **Experimental Animals:** Experimental studies were conducted on healthy adult Wistar albino rats with a body weight of 150–200 g. Animals were housed in polypropylene cages and given standard laboratory diet and water ad libitum. Animals were

acclimated to laboratory conditions for at least seven days prior to experimentation. Throughout the study, animal handling and environmental control were performed to reduce stress and variability in experimental results.

- ii. **Induction of Diabetes [STZ] model:** Experimental animals were made diabetic by intraperitoneal administration of streptozotocin [STZ]. Animals were fasted overnight before induction of diabetes. Streptozotocin was dissolved in freshly prepared citrate buffer and administered at an optimized dose based on body weight. Animals were observed for signs of hyperglycemia after administration and blood glucose level was measured 48-72 hours later. Those animals with fasting blood glucose levels above the pre-determined diabetic levels were considered as diabetic and selected for further experimental studies. Chemical induction of diabetes with STZ or alloxan has been reported as a reliable method to study the antidiabetic activity of herbal formulations.
- iii. **Experimental Group:** Experimental animals were divided into different groups like normal control, diabetic control, standard drug treated group and test formulation treated groups. The herbal tablet formulation was administered orally at a fixed dose according to the body weight. Metformin was used as the standard antidiabetic drug as reference control. Blood glucose levels were checked at different intervals to measure therapeutic response for a fixed duration of the experiment.
- iv. **Estimation of Blood Sugar:** Blood glucose measurements were taken from the tail vein at predetermined intervals. Glucose was determined by digital glucometer or biochemical assay kits. The antihyperglycemic effect of herbal tablet formulation was evaluated by periodic monitoring of blood glucose level. The test formulation exhibited significant antidiabetic activity by reducing the blood glucose levels in comparison to diabetic control group.
- v. **Oral Glucose Tolerance Test [OGTT]:** The oral glucose tolerance test was used to assess the efficiency of glucose utilization in treated animals. The animals were fasted overnight and then dosed with a glucose solution by gavage. Blood glucose levels were taken at timed intervals after glucose administration. The groups treated with the compounds showed improvement in glucose

tolerance as compared to the diabetic control animals indicating improved glucose metabolism and insulin sensitivity.

- vi. **Biochemical Properties Estimation:** Serum biochemical parameters such as total cholesterol, triglycerides, high density lipoprotein [HDL], low density lipoprotein [LDL], glycated hemoglobin [HbA1c] and insulin levels were estimated by standard biochemical assay kits. These parameters are involved in lipid metabolism, glycemia control. The improved biochemical profile indicates the protective effects of herbal formulation against the metabolic complications associated with diabetes.
- vii. **Histopathology Studies:** Histopathological analysis of pancreatic tissues was carried out to examine the structural alterations associated with diabetes and treatment effects. At the end of the experimental study, animals were humanely sacrificed and pancreatic tissues were collected and fixed in 10% formalin solution. The tissue samples were processed, embedded in paraffin, sectioned by microtome and stained by haematoxylin and eosin stain technique. Microscopic study of the morphology of cells and the integrity of pancreatic β -cells. The restoration of normal pancreatic architecture indicates the protective and regenerative potential of the herbal formulation.

Statistical Analysis

Statistical analysis of the experimental data was performed in order to evaluate the significance and reliability of the observed findings from pre-compression, post compression, in-vitro and in-vivo antidiabetic studies. All experiments were performed in triplicate unless otherwise stated and the results are presented as mean $\pm$ standard deviation [SD]. Statistical analysis was done to find out the level of variation between different batches of formulation and experimental groups. Suitable statistical tools are used to confirm the reproducibility and scientific validity of pharmaceutical and pharmacological studies.

The analysis of variance [ANOVA] was performed to analyze the differences between multiple experimental groups such as control, standard drug treated and test formulation treated groups. The one-way ANOVA was the main statistical method used to determine statistically significant differences of the mean values of different experimental groups. If there were significant differences, post hoc multiple comparison tests such as Tukey's test or Dunnett's test were employed to detect the significant

differences among groups. These statistical methods are widely recommended in the pharmaceutical and biomedical research for the assessment of efficacy of herbal formulations and determination of treatment response in diabetic models.

All statistical calculations were performed using validated statistical software such as GraphPad Prism [version 8.0 or greater] or SPSS statistical software. These software tools allow accurate graphical representation of the experimental data, and also help in interpretation of the results by plotting bar graphs, line graphs and dose-response curves. Statistical significance was defined as $p < 0.05$, the accepted criterion for statistically significant differences between experimental groups. The smaller the p-values, the greater the statistical significance and the better the therapeutic response of the tested herbal formulation.

Hypotheses were tested and relationships between formulation variables and performance parameters like dissolution rate, disintegration time and levels of glucose reduction were established using correlation analysis, regression modeling, as and when required. These statistical approaches offer a more holistic perspective on the interaction of formulation components with biological responses. The results obtained were reliable, reproducible and suitable for scientific interpretation and further validation of the developed herbal tablet formulation by systematic application of statistical methods.

RESULTS

Physicochemical Evaluation Results

1. **Determination of Ash Values:** To assess the total inorganic content in the powdered medication samples, ash values were calculated. These criteria aid in the detection of contamination and adulteration by exogenous elements such silica, sand, and dirt. It was discovered that AN had a slightly greater ash content than AL, perhaps as a result of variations in tissue density and mineral composition.

Table 1: Ash Value of Plant Materials

Parameter	AN [% w/w]	AL [% w/w]
Total Ash	7.82 $\pm$ 0.18	6.94 $\pm$ 0.14
Acid Insoluble Ash	1.42 $\pm$ 0.06	1.18 $\pm$ 0.05

Water Soluble Ash	1.76 ± 0.11	1.31 ± 0.09
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2. **Extractive Values:** The amount of active ingredients soluble in various solvents was estimated using extractive values. AN was found to have a greater hydroalcoholic extractive value than AL, indicating a comparatively larger concentration of polar phytoconstituents such as flavonoids and tannins.

Table 2: Extractive Values of Plant Materials

Parameter	AN [% w/w]	AL [% w/w]
Water Soluble Extractive	18.45 ± 0.32	16.28 ± 0.27
Alcohol Soluble Extractive	14.72 ± 0.25	12.96 ± 0.22
Pet Ether Extractive	19.13 ± 0.09	17.06 ± 0.14
Hydroalcoholic Extractive	21.63 ± 0.41	19.87 ± 0.36

3. **Loss on Drying:** The quantity of moisture in powdered medication samples was measured using loss on drying. In plant materials, moisture content is crucial for preserving stability and inhibiting microbial development. The findings showed that the moisture content of both plant samples was within permissible pharmacognostical bounds, guaranteeing stability during storage.
4. **Content of Moisture:** Moisture content was determined to evaluate the hygroscopic nature of powdered drug samples. Lower moisture content is desirable to prevent degradation and microbial contamination. Both samples were adequately dried before being stored and processed, according to the measured moisture content values.

Table 3: LOD & MOC

Parameter	AN [% w/w]	AL [% w/w]
Loss on Drying	5.36±0.12	4.89±0.10
Moisture content	4.92±0.09	4.37±0.08

Phytochemical Analysis Results

1. **Preliminary Phytochemical Screening:** Standard qualitative chemical assays were used for preliminary phytochemical screening in order to identify the presence of main phytoconstituent groups. Both plant species' hydroalcoholic extracts had favorable responses for flavonoids, phenolic compounds, and tannins, demonstrating their abundance of polyphenolic components. Alkaloids

and saponins were found in moderate amounts, and both extracts included glycosides and carbohydrates. There were trace amounts of proteins and amino acids. The discovered phytochemical profile supports the use of both plant species in the creation of herbal preparations and validates their therapeutic potential.

Table 4: Preliminary Phytochemical Screening

Phytoconstituent	AN	AL
Alkaloids	++	+
Flavonoids	+++	++
Tannins	+++	++
Phenolic Compounds	+++	++
Saponins	++	+
Glycosides	++	++
Carbohydrates	++	++
Proteins	+	+
Steroids	+	+

+++ = Strong presence, ++ = Moderate presence, + = Mild presence, – = Absent

2. **Quantitative Estimation of Phytochemicals:** The total phenolic, flavonoid, and tannin content in the hydroalcoholic extracts of both plant species was determined by quantitatively estimating the key phytoconstituents. These metrics provide important insights into the potential antidiabetic and antioxidant properties of plant materials. The findings showed that AN has much more phenolics and tannins than AL. Environmental factors influencing plant development and species-specific variations in phytochemical production might be the cause of this variance.

Table 5: Quantitative Phytochemical Estimation

Parameter	AN	AL
TPC [mg GAE/g extract]	186.42 ± 3.21	162.58 ± 2.94
TFC [mg QE/g extract]	124.36 ± 2.18	109.74 ± 1.96
Total Tannin Content [mg TAE/g extract]	148.27 ± 2.76	132.65 ± 2.41

3. **Chromatographic Analysis:** TLC analysis revealed distinct bands under ultraviolet light in both plant extracts, confirming the presence of multiple phytoconstituents. AN exhibited a greater number and intensity of bands than AL, indicating a richer phytochemical composition. TLC, HPTLC, and HPLC were further employed to establish

characteristic phytochemical fingerprints and assess phenolic and flavonoid contents in hydroalcoholic extracts. Comparative chromatographic profiles showed different peak patterns between AN and AL, confirming differences in their phytochemical composition and supporting authentication and standardization.

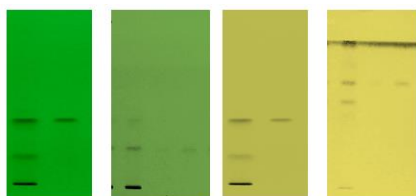


Figure 1: TLC Profile of Extracts

Table 6: TLC Profile of Extracts

Parameter	AN	AL
Number of Bands	6	5
Major Rf Values	0.21, 0.36, 0.48, 0.62, 0.74, 0.85	0.24, 0.39, 0.53, 0.67, 0.79
Band Intensity	Strong	Moderate
Observation under UV	Bright yellow-green bands	Pale yellow bands

Table 7: HPTLC Fingerprinting with Phytochemical

Peak No.	Rf Value	Peak Area [%] - AN	Peak Area [%] - AL	Phytochemical
1	0.05–0.07	6.42	5.38	Phenolic acid derivative

2	0.12–0.14	8.75	7.92	Gallic acid-like compound
3	0.21–0.26	10.84	11.63	Tannin component
4	0.33–0.38	14.67	14.18	Flavonoid glycoside
5	0.45–0.51	17.92	16.42	Catechin derivative
6	0.57–0.63	19.38	13.55	Polyphenolic compound
7	0.69–0.76	13.74	9.46	Quercetin-like flavonoid
8	0.81–0.88	6.95	6.02	Flavonoid derivative
9	0.92	1.33	—	Non-polar constituent

[—] indicates absence or negligible peak detection.

Table 8: HPLC Fingerprinting with Phytochemical

Peak No.	Retention Time [min]	Peak Area [%] - AN	Peak Area [%] - AL	Tentative Phytochemical Identification
1	2.18–2.36	11.28	9.74	Gallic acid [Phenolic acid]
2	3.72–4.02	18.45	15.83	Catechin derivative
3	5.28–5.89	24.67	21.36	Tannin compound
4	6.84–7.21	21.94	19.48	Quercetin-like flavonoid
5	8.42–8.94	14.83	13.22	Kaempferol derivative
6	9.76	8.83	6.41	Polyphenolic compound

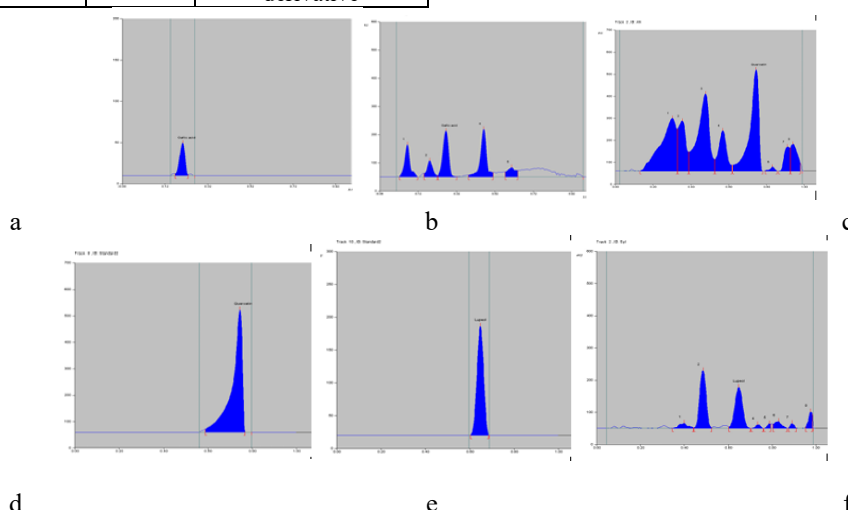


Figure 2: HPTLC fingerprinting of (a) standard Gallic acid; (b) AN containing Gallic acid; (c) standard Quercetin; (d) AN containing Quercetin acid; (e) standard Lupeol; (f) AN containing Lupeol

4. **Analysis of Comparative HPLC:** The main phytochemical components found in the

hydroalcoholic extracts of AN and AL were measured using High Performance Liquid

Chromatography [HPLC] analysis. Several distinct peaks that corresponded to phenolic acids, flavonoids, and tannin derivatives were obtained from the chromatographic separation. In comparison to AL, the chromatographic profiles showed higher peak intensities in AN, indicating a larger number of polyphenolic compounds. To create distinctive chemical fingerprints that are helpful for standardization and authentication, the retention time values and peak area percentages were noted and compared between the two species.

Table 9: Analysis of HPLC

Peak No.	Retention Time [min]	Peak Area [%] - AN	Peak Area [%] - AL	Tentative Phytochemical Identification
1	2.18–2.36	11.28	9.74	Gallic acid [Phenolic acid]
2	3.72–4.02	18.45	15.83	Catechin derivative
3	5.28–5.89	24.67	21.36	Tannin compound
4	6.84–7.21	21.94	19.48	Quercetin-like flavonoid
5	8.42–8.94	14.83	13.22	Kaempferol derivative
6	09.11 - 9.76	8.83	6.41	Polyphenolic compound

Pre-compression Assessment Outcomes [F1–F6]

Before tablet compression, the flow characteristics and compressibility of powder blends made for various formulation batches [F1–F6] were assessed. These factors are essential for maintaining constant tablet weight during compression and uniform die filling. Using established methods, the powder blends were assessed for angle of repose, bulk density, tapped density, Carr's compressibility index, and Hausner's ratio. All formulation batches showed appropriate flow behavior in the findings, suggesting that the blends were suitable for the direct compression technique.

Table 10: Pre-compression Parameters of Powder Blends

Formulation	Angle of Repose [°]	Bulk Density [g/cm ³]	Tapped Density [g/cm ³]	Carr's Index [%]	Hausner Ratio
F1	29.84 ± 0.42	0.46 ± 0.02	0.54 ± 0.03	14.81	1.17
F2	28.62 ± 0.36	0.48 ± 0.01	0.55 ± 0.02	12.72	1.14

F3	27.95 ± 0.41	0.49 ± 0.02	0.56 ± 0.02	12.5	1.14
F4	26.74 ± 0.38	0.51 ± 0.01	0.57 ± 0.02	10.52	1.12
F5	27.18 ± 0.44	0.50 ± 0.02	0.57 ± 0.03	12.28	1.14
F6	28.05 ± 0.39	0.48 ± 0.01	0.55 ± 0.02	12.72	1.14

Table 11: Flow Property Classification

Parameter	Acceptable Range	Observation
Angle of Repose	< 30°	Good flow
Carr's Index	5–15%	Good compressibility
Hausner Ratio	1.00–1.25	Acceptable flow

Tablet Formulation Result

The direct compression technique was successfully used to prepare six herbal tablet formulations (F1–F6) from hydroalcoholic extracts of AN and AL by varying the sodium starch glycolate concentration while keeping other excipients constant. Microcrystalline cellulose served as diluent, PVP K-30 as binder, and magnesium stearate and talc as lubricant and glidant. All formulations produced uniform, round, flat-faced, light-brown tablets with adequate mechanical strength and acceptable powder flow. No capping, sticking, lamination, or cracking was observed, while superdisintegrant concentration influenced hardness, friability, and disintegration.

Table 12: Composition of Herbal Tablet Formulations

Ingredients [mg/tablet]	F1	F2	F3	F4	F5	F6
AN Extract	150	150	150	150	150	150
AL Extract	150	150	150	150	150	150
Microcrystalline Cellulose	120	115	110	105	100	95
Sodium Starch Glycolate	10	15	20	25	30	35
PVP K-30	25	25	25	25	25	25
Talc	10	10	10	10	10	10
Magnesium Stearate	5	5	5	5	5	5
Total Weight [mg]	470	470	470	470	470	470



Figure 3: Prepared tablet

Table 13: Physical Appearance of Tablets

Parameter	Observation
Shape	Round
Color	Light brown
Surface	Smooth
Odor	Characteristic
Taste	Slightly astringent
Defects	None observed

Post Formulation Evaluation of Tablet

The physical quality, mechanical strength, and medicinal efficacy of herbal tablets made from hydroalcoholic extracts of AN and AL were evaluated after compression. The thickness, hardness, friability, weight fluctuation, disintegration duration, homogeneity of drug content, and in-vitro dissolving parameters of the compressed tablets from each of the six formulations [F1–F6] were assessed. For tablet dosage forms to be consistent, stable, and effective, these factors are crucial. The produced tablets had consistent dimensions, smooth surfaces, and a light brown hue, indicating that the herbal powders had been properly blended and compressed. During compression, no obvious flaws such as capping, lamination, or cracking were seen, suggesting that the chosen excipients and plant extracts were compatible.

Table 14: Post-compression Parameters of Herbal Tablets

Parameter	F1	F2	F3	F4	F5	F6
Thickness [mm]	4.21 ± 0.04	4.18 ± 0.03	4.15 ± 0.05	4.12 ± 0.02	4.16 ± 0.04	4.20 ± 0.03
Hardness [kg/cm ²]	4.62 ± 0.12	4.78 ± 0.10	5.12 ± 0.14	5.46 ± 0.11	5.28 ± 0.13	5.04 ± 0.15
Friability [%]	0.78	0.71	0.64	0.52	0.59	0.63

Weight Variation [mg]	471 ± 3.6	469 ± 3.2	470 ± 2.9	468 ± 2.4	472 ± 3.1	469 ± 2.8
Disintegration Time [min]	12.8 ± 0.6	11.6 ± 0.5	10.2 ± 0.4	8.4 ± 0.3	9.1 ± 0.4	9.8 ± 0.5
Drug Content [%]	96.4 2 ± 0.82	97.1 8 ± 0.74	98.3 6 ± 0.69	99.2 4 ± 0.58	98.7 5 ± 0.63	98.1 2 ± 0.71

Table 15: Tablet Quality Compliance Summary

Parameter	Pharmacopeial Limit	Observation
Hardness	4–6 kg/cm ²	Within limit
Friability	< 1%	Within limit
Disintegration	< 15 min	Within limit
Weight Variation	±5%	Within limit
Drug Content	95–105%	Within limit

In-vitro Dissolution Study Results: In-vitro dissolution studies were performed to evaluate the release characteristics of active phytoconstituents from herbal tablets prepared using hydroalcoholic extracts of AN and AL. The dissolution study was conducted using USP dissolution apparatus under standardized conditions. Samples were withdrawn at predetermined time intervals and analyzed spectrophotometrically to determine the percentage drug release. The obtained results demonstrated gradual release of phytoconstituents from all formulations, with noticeable variation in release profiles depending on the concentration of superdisintegrant used in each formulation batch. The dissolution profile indicated that increasing the concentration of sodium starch glycolate significantly enhanced drug release due to improved tablet disintegration and increased surface area exposure. Among all batches, formulation F4 exhibited the most desirable release pattern with rapid and consistent release of active constituents within the specified time period.

Table 16: In-vitro Dissolution Profile of Herbal Tablets

Time [min]	F1 [%]	F2 [%]	F3 [%]	F4 [%]	F5 [%]	F6 [%]
0	0	0	0	0	0	0
5	18.6	21.4	24.8	28.9	26.3	25.7
10	32.8	36.7	41.5	46.9	44.2	43.6
15	44.9	49.6	55.8	61.7	59.3	57.8

20	55.2	60.8	66.9	72.8	70.6	69.4
30	67.4	72.5	78.3	85.6	82.9	80.7
45	78.6	83.7	88.9	94.8	92.3	90.6
60	86.2	90.4	94.6	98.7	96.5	95.2

Table 17: Dissolution Efficiency Comparison

Formulation	% Drug Release at 60 min	Release Behavior
F1	86.20%	Moderate
F2	90.40%	Good
F3	94.60%	Very Good
F4	98.70%	Excellent
F5	96.50%	Very Good
F6	95.20%	Very Good

Antidiabetic Study Results

- In-vitro Antidiabetic Activity Results:** α -amylase and α -glucosidase inhibition tests were used to assess the in vitro antidiabetic efficacy of hydroalcoholic extracts and an optimized herbal tablet formulation [F4] made from AN and AL. The potential of plant extracts to block carbohydrate-hydrolyzing enzymes, hence lowering glucose absorption and regulating postprandial hyperglycemia, is often assessed using these enzymatic tests. At various doses, the % inhibition of enzymes was computed and compared with acarbose, a common antidiabetic medication. The findings showed that both enzymes were inhibited in a concentration-dependent manner, suggesting that plant extracts and tablet formulations had considerable antidiabetic potential.

Assay for α -Amylase Inhibition: Plant extracts and tailored formulations were tested for their ability to inhibit α -amylase at doses between 20 and 100 $\mu\text{g/mL}$. The findings suggested dose dependent action as the inhibition percentage gradually increased with increasing concentration.

Table 18: α -Amylase Inhibition Activity

Concentration [$\mu\text{g/mL}$]	AN Extract [%]	AL Extract [%]	Optimized Tablet F4 [%]	Standard [Acarbose] [%]
20	24.6	20.8	26.3	35.4
40	38.2	33.7	40.5	49.8

60	51.9	46.4	54.6	63.2
80	64.7	58.1	67.3	76.5
100	76.5	69.8	78.9	88.4

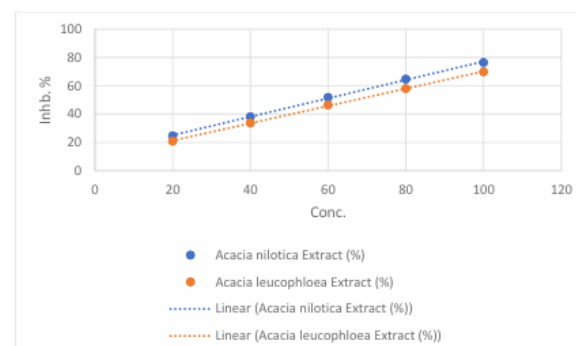


Figure 4: α -Amylase Inhibition Activity

Assay for α -Glucosidase Inhibition: Similar concentration ranges were used to assess the α -glucosidase inhibitory activity. When compared to the α -amylase experiment, the findings demonstrated more inhibitory activity, suggesting that the digestion of carbohydrates was effectively inhibited.

Table 19: α -Glucosidase Inhibition Activity

Concentration [$\mu\text{g/mL}$]	AN Extract [%]	AL Extract [%]	Optimized Tablet F4 [%]	Standard [Acarbose] [%]
20	28.4	24.2	30.7	41.6
40	42.7	37.5	45.3	56.8
60	57.9	51.8	60.2	69.4
80	71.6	65.4	73.9	82.7
100	84.3	78.2	86.5	92.6

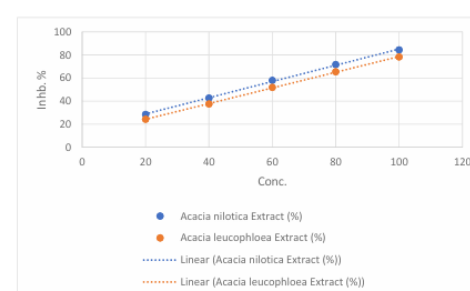


Figure 5: α -Glucosidase Inhibition Activity

Table 20: IC₅₀ Values of Enzyme Inhibition

Sample	α -Amylase IC ₅₀ [$\mu\text{g/mL}$]	α -Glucosidase IC ₅₀ [$\mu\text{g/mL}$]
AN Extract	58.4	46.2
AL Extract	64.9	52.8
Optimized Tablet [F4]	55.7	43.6
Standard [Acarbose]	38.2	32.5

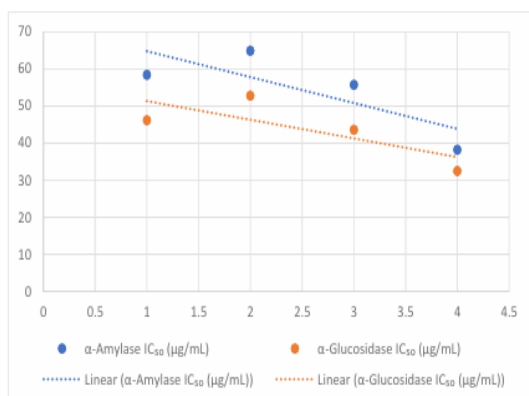


Figure 6: IC₅₀ Values of Enzyme Inhibition

Results of In-vivo Antidiabetic Activity: Using chemically induced diabetes animal models, the in-vivo antidiabetic efficacy of hydroalcoholic extracts of AN, AL, and optimized herbal tablet formulation [F4] was assessed. Streptozotocin [STZ] was used to produce diabetes, and therapy lasted for 21 days. Antihyperglycemic activity was assessed by frequently checking blood glucose levels. When compared to diabetic control animals, the treated groups' fasting blood glucose levels were much lower, according to the data. Similar to the conventional antidiabetic medication, the improved tablet formulation [F4] had a significant glucose-lowering impact.

Table 21: Experimental Group Design

Group	Treatment	Description
Group I	Normal Control	Normal saline
Group II	Diabetic Control	STZ-induced diabetes
Group III	Standard	Standard drug [Metformin 100 mg/kg]
Group IV	AN Extract	200 mg/kg
Group V	AL Extract	200 mg/kg
Group VI	Optimized Tablet [F4]	Equivalent dose

Table 22: Effect on Fasting Blood Glucose Levels [mg/dL]

Group	Day 0	Day 7	Day 14	Day 21
Normal Control	92 ± 4.2	94 ± 3.8	95 ± 3.6	93 ± 4.1
Diabetic Control	268 ± 7.4	279 ± 8.2	286 ± 7.8	294 ± 8.6
Standard [Metformin]	271 ± 6.9	198 ± 5.7	142 ± 4.9	108 ± 3.6
AN	269 ± 7.2	214 ± 6.4	165 ± 5.2	124 ± 4.3
AL	270 ± 7.5	226 ± 6.9	178 ± 5.8	139 ± 4.8
Tablet F4	272 ± 7.1	205 ± 6.2	150 ± 5.1	116 ± 3.9

Table 23: OGTT Results [Blood Glucose mg/dL]

Time [min]	Normal	Diabetic	Standard	<i>A. nilotica</i>	<i>A. leucophloea</i>	Tablet F4
0	94	276	268	270	272	269
30	128	342	295	304	316	298
60	112	356	268	278	291	271
90	102	349	235	248	259	239
120	96	332	198	212	224	206

Table 24: Biochemical Parameters

Parameter	Normal	Diabetic	Standard	<i>A. nilotica</i>	<i>A. leucophloea</i>	Tablet F4
Total Cholesterol [mg/dL]	98 ± 3.2	186 ± 6.4	118 ± 4.1	126 ± 4.5	134 ± 4.9	121 ± 4.2
Triglycerides [mg/dL]	84 ± 2.8	168 ± 5.6	104 ± 3.6	112 ± 3.8	118 ± 4.2	108 ± 3.9
HDL [mg/dL]	48 ± 1.9	32 ± 1.4	44 ± 1.7	41 ± 1.6	39 ± 1.5	43 ± 1.7
LDL [mg/dL]	62 ± 2.1	142 ± 5.1	82 ± 3.0	90 ± 3.2	97 ± 3.6	85 ± 3.1

Histopathological Findings: To assess structural alterations after therapy, pancreatic tissue was examined histopathologically. The pancreatic islets in the normal control group had normal cellular architecture and were undamaged. Reduced islet size and significant pancreatic β-cell degeneration were seen in the diabetic control group.

Pancreatic islet cell regeneration and cellular integrity restoration were seen after treatment with AN, AL, and improved tablet formulation. Comparable to the regular drug-treated group, the optimized tablet formulation [F4] showed a strong protective effect with almost normal pancreatic architecture across the treatment groups.

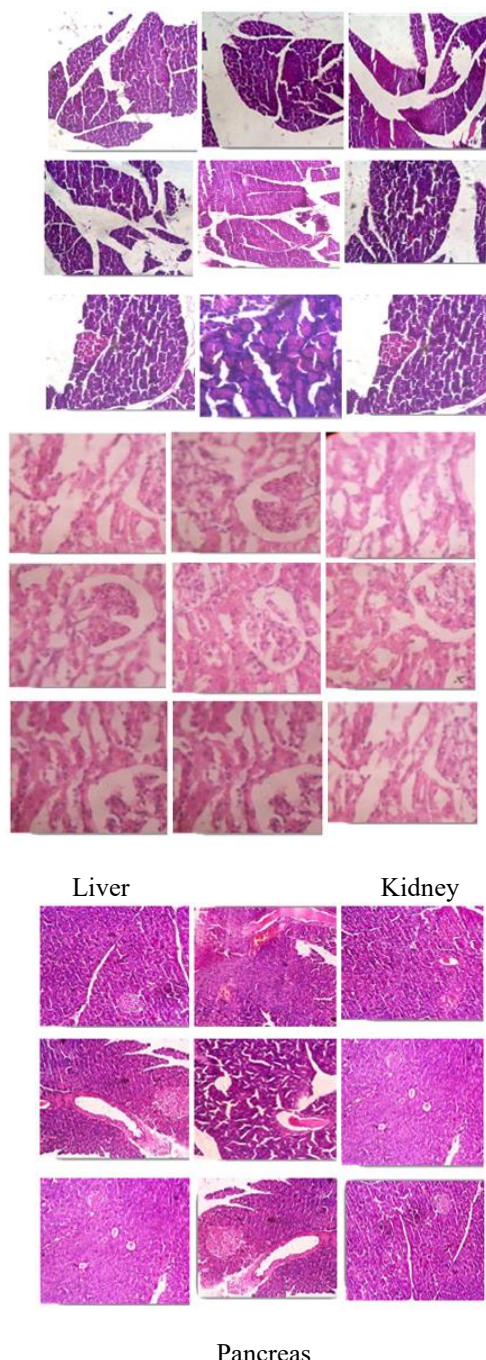


Figure 7: Histology of different organs

Findings from Statistical Analysis

To ascertain the significance of the differences between the treatment and control groups, statistical analysis of the experimental data was carried out. The mean $\pm$ standard error of mean [SEM] was used to represent all findings. One way analysis of variance [ANOVA] and a post-hoc comparison test were used in the statistical assessment to ascertain the degree of significance between various groups. Statistical significance was defined as a probability value of

$p < 0.05$, while high significance was defined as $p < 0.01$ and $p < 0.001$.

When compared to diabetic control mice, the statistical analysis verified that treatment with AN, AL, and optimized tablet formulation [F4] dramatically lowered blood glucose levels and improved biochemical parameters. Comparable to the group treated with the regular medicine, the improved formulation [F4] showed statistically significant improvement.

Table 25: One-way ANOVA Results for Blood Glucose Levels [Day 21]

Source of Variation	Sum of Squares [SS]	Degrees of Freedom [df]	Mean Square [MS]	F-value	p-value
Between Groups	52486.3	5	10497.3	128.74	$p < 0.001$
Within Groups	2446.18	30	81.54	—	—
Total	54932.5	35	—	—	—

Table 26: ANOVA Results for Lipid Profile Parameters

Parameter	F-value	p-value	Significance Level
Total Cholesterol	96.52	$p < 0.001$	Highly Significant
Triglycerides	88.34	$p < 0.001$	Highly Significant
HDL	42.18	$p < 0.01$	Significant
LDL	73.95	$p < 0.001$	Highly Significant

Table 27: Post-hoc Significance Comparison

Group	Blood Glucose Reduction	Significance
Standard vs Diabetic Control	Highly Reduced	*** $p < 0.001$
AN vs Diabetic Control	Significantly Reduced	** $p < 0.01$
AL vs Diabetic Control	Significantly Reduced	** $p < 0.01$
Tablet F4 vs Diabetic Control	Highly Reduced	*** $p < 0.001$

* $p < 0.05$ [Significant] ** $p < 0.01$ [Highly Significant] *** $p < 0.001$ [Very Highly Significant]

DISCUSSION

The goal of the current study was to create and assess standardized herbal pills made from certain medicinal plants that may have antidiabetic properties. Systematic pharmacognostical evaluation, phytochemical

standardization, formulation creation, in-vitro enzyme inhibition investigations, in-vivo antidiabetic evaluation, and histopathological analysis were all part of the research. The work's main goal was to determine the therapeutic potential of extracts from AN and AL in the treatment of DM and to use the direct compression method to manufacture them into stable and powerful herbal tablets.

1. **Physicochemical and Pharmacognostical Assessment:** Pharmacognostical evaluation confirmed the authenticity and quality of AN and AL flowers through macroscopic, microscopic, and powder microscopic studies. Distinctive morphological and anatomical features, including inflorescence characteristics, stamens, pollen grains, vascular elements, fibers, and tannin-containing cells, supported their identification. Physicochemical parameters, including ash values, extractive values, and moisture content, indicated good purity, controlled moisture, and the presence of water- and alcohol-soluble phytoconstituents, confirming their suitability for further investigation.
2. **Chromatographic Analysis and Phytochemical Standardization:** Phytochemical screening identified flavonoids, tannins, phenolics, saponins, and triterpenoids, while quantitative analysis demonstrated significant tannin, flavonoid, and phenolic contents. TLC, HPTLC, and HPLC established characteristic fingerprint profiles and confirmed marker compounds such as quercetin and lupeol. AN showed comparatively higher marker concentrations, supporting its medicinal potential and the importance of chromatographic standardization.
3. **Herbal Tablet Formulation Development:** Herbal tablets were successfully prepared by direct compression using suitable excipients. Microcrystalline cellulose improved compressibility, sodium starch glycolate enhanced disintegration, while polyvinylpyrrolidone, magnesium stearate, and talc improved strength, lubrication, and flow. Pre-compression parameters showed acceptable powder flow, and all formulations met pharmacopeial requirements. F4 demonstrated superior mechanical strength, rapid disintegration, and consistent dissolution, identifying it as the optimized formulation.
4. **Antidiabetic Activity In Vitro:** Both extracts demonstrated concentration-dependent α -amylase

and α -glucosidase inhibition. The optimized formulation showed greater inhibitory activity than individual extracts, potentially due to improved active-ingredient availability. Flavonoids and phenolics may contribute to these effects by limiting carbohydrate breakdown and postprandial glucose elevation.

5. **Antidiabetic Activity In Vivo:** Plant extracts and the optimized formulation significantly reduced fasting blood glucose and improved glucose tolerance. The formulation showed greater antihyperglycemic activity, suggesting synergistic effects of phytoconstituents. Improvements in LDL, triglycerides, total cholesterol, and HDL further indicated beneficial effects on lipid metabolism and diabetes-related cardiovascular risk.
6. **Results of Histopathology:** Histopathological findings demonstrated reduced pancreatic β -cell degeneration and restoration of pancreatic islet architecture in treated animals. Reduced hepatic and renal damage, necrosis, tubular injury, and fatty degeneration further indicated protective effects against diabetes-induced organ damage.

Overall, the integrated findings demonstrate substantial antidiabetic potential of AN and AL. The optimized herbal tablet exhibited better activity than individual extracts, potentially through enzyme inhibition, antioxidant effects, and tissue protection, supporting their potential as natural therapeutic agents for diabetes management.

CONCLUSION

The study demonstrated the pharmacognostical, phytochemical, pharmaceutical, and pharmacological potential of AN and AL for developing standardized herbal tablets for diabetes management. Pharmacognostical evaluations, including macroscopic, microscopic, and powder microscopic studies, confirmed the authenticity, purity, and diagnostic characteristics of the plant materials. Physicochemical parameters such as ash values, extractive values, and moisture content were within acceptable limits, supporting their suitability for pharmaceutical processing. Preliminary phytochemical screening identified flavonoids, phenolics, tannins, saponins, and triterpenoids, while quantitative analysis confirmed substantial phenolic and flavonoid contents. TLC, HPTLC, and HPLC fingerprinting established characteristic profiles and confirmed marker compounds such as quercetin and lupeol. Herbal tablets were successfully formulated by direct compression using

suitable excipients, with all formulations satisfying pharmacopeial requirements. Among them, formulation F4 demonstrated superior mechanical strength, rapid disintegration, and consistent dissolution. In vitro studies showed concentration-dependent inhibition of α -amylase and α -glucosidase, indicating potential for controlling carbohydrate digestion and postprandial glucose. In vivo studies demonstrated significant reductions in fasting blood glucose, improved glucose tolerance, and favorable lipid-profile changes. Histopathological findings further indicated protective effects on pancreatic, hepatic, and renal tissues. The integration of authentication, standardization, chromatographic profiling, formulation, and pharmacological evaluation represents the study's novelty. However, limitations include the use of crude extracts, animal models, and absence of long-term stability and clinical validation. Future studies should include pharmacokinetics, toxicity, advanced analytical techniques, clinical trials, and innovative drug-delivery systems. Overall, the formulation shows promising antidiabetic potential.

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