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Pharmacognostic And Pharmacological Evaluation of *Azadirachta Indica* Leaves for Anti-Diabetic Activity

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ABSTRACT

This research paper explains the nature of pharmacogenetics and anti-diabatic roles of *Azadirachta indica* (neem) leaves. Pharmacogenetic serials of phytochemical checks (microscopy, physicochemical tests ash test; extractive values) were performed on the shade-dried leaves. Extracts were made methanolically and extraction was done using water. The methods used in test were antioxidant (DPPH, ABTS), inhibition of α-amylase, inhibition of α-glucosidase and in vitro experiments. The in vivo anti-diabetic efficiency was studied on the high-fat diet/ streptozotocin (HFD/STZ) induced Type 2 diabetic rat by administration of 100, 200, and 400 mg/kg of 30 days. Blood gligatorid levels, glucose intolerance, lipid peroxidation, and antioxidant status and histopathology of pancreatic tissues were assessed and metformin was performed as a positive control. HPLC/HPTLC demonstrated rutin, quercetin and ellagic acid using methanolic extract. Extrates showed good ability of antioxidant and enzyme inhibition; the 400 mg/kg dose notably corrected glycemia, reversed lipid and antioxidants levels. The histology revealed the recovery of pancreatic structure.

Key Words:

Azadirachta Indica, Neem, Pharmacognostic Evaluation, Antidiabetic Activity, Flavonoids.

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1. INTRODUCTION

Diabetes mellitus is a metabolic complication that is intricate and persistent and has become one of the greatest international health issues that have encountered people in both populations and in millions[1]. The disease is typified by chronic hyperglycemia and is often associated with oxidative stress that causes eventual deterioration of key organs, especially the pancreas, kidneys, eyes, and the heart vessels[2]. The rising incidence of diabetes propagated by the combination of sedentary

life choices, unbalanced diet and hereditary factors, has heightened the quest to identify safer and more efficacious therapeutic options[3]. Although the traditional antidiabetic medications have glycemic control, their effectiveness has an upper limit due to long-term side effects as well as cost-obesity, and their declining effectiveness[4]. This has kindled an increasing scientific interest in medicinal plants as alternative or supplementary treatment choice, because of their phytochemical's bioactivity, antioxidant properties, and multidimensional mechanism[5]. Particularly, Azadirachta indica A. Juss. (neem) is a distinguished place in traditional medicine due to a wide range of pharmacological properties, including the potentially positive effects on patients with diabetes[6].

1.1 Background Information

Diabetes mellitus is a long term, multi-factorial metabolic disease that has a continued form of hyperglycaemia due to either failure of insulin secretion, insulin action, or a combination of the two[7]. The existence of this disease is accompanied by severe chronic comorbidities to the eyes, kidneys, nerves and the cardiovascular system[8]. Diabetes largely emerged as a problem during the recent decades and the International Diabetes Federation estimates that currently it impacts more than 537 million adults worldwide, which is also an alarming number that is expected to increase even more in the years to come[9]. Along with hyperglycaemia, oxidative stress is now recognized as a key initiating and advancing factor in diabetes development, causing pancreatic beta-cell dysfunction, insulin resistance and the microvascular, as well as macro-vascular, complications[10].

1.2 Statement of the Problem

Despite A. indica as having been investigated in the fight against diabetes, majority of the available literature is associated either with crude extracts that are not pharmacognostically optimized or isolated bioactive molecule that lacks a comparison with full-plants extract action. The scarcity of any integrated research relating both pharmacognostic standardization and phytochemical profiling and in vitro and in vivo pharmacological profiling of neem leaves with respect to diabetes has defined the research lacking domain. In the absence of such comprehensive information, reproducibility, standardization and clinical concern of neem therapeutic preparations are quite difficult. Hence, investigation that involves well-articulated pharmacognostic characterisation and strong biological testing should be done to confirm the quality, potency and mechanism of action of neem leaves as an antidiabetic agent.

1.3 Objectives of the Study

The present study aims to:

1. Perform pharmacognostic standardization of A. indica leaves, including macroscopic, microscopic, and physicochemical evaluations.
2. Conduct phytochemical screening along with antioxidant and carbohydrate-digestive enzyme inhibition assays.

3. Evaluate the in vivo antidiabetic efficacy of neem leaf extracts in a high-fat diet and streptozotocin-induced diabetic rat model, with metformin serving as the standard reference drug.

1.4 Hypotheses

- **H1:** Neem leaf extracts conform to pharmacogenetic quality standards as per pharmacopeial guidelines.
- **H2:** Neem leaf extracts contain bioactive constituents such as rutin and quercetin in quantifiable amounts.

2. METHODOLOGY

The current research, an integrated pharmacognostic, phytochemical, in vitro and in vivo experimental approach was used to access the anti-diabetic potential of leaves of Azadirachta indica (neem). The protocols were standardized, which complies with the Indian Pharmacopoeia (IP-2022) and are used internationally accepted in laboratories (reproducibility, reliability of results). The approach involved a structured plant material gathering and validation, extraction via aqueous and methanol solvents, extensive phytochemical analysis, and antioxidant and enzyme-blocking test systems in addition to the anti-diabetic analysis with animal models. All the experiments were conducted with the due control and the analysis of the data was done through powerful Statistical analysis tools in order to get meaningful interpretations.

2.1 Description of Research Design

This study utilized experimental research design that incorporated pharmacognostic profiling, in vitro antioxidant and enzyme inhibitory screening and in vivo antidiabetes activity on rodent model. It was sequential in the direction of conducting a study, which started by standardizing the leaves of Azadirachta indica using pharmacogenetic conditions, proceeded to phytochemical and biological tests, to end with efficacy testing in animals. Such a multi-level approach made it possible to correlate the chemical composition with biological activities, thus making a wholesome assessment possible.

2.2 Sample Details

In the case of the plant sample, fresh mature leaves of A. indica were gathered on an authenticated source in [Insert location, e.g., botanical garden/university campus], washed, shade-dried, and powdered in order to analyse. A professional taxonomist was used to determine the botanical identification and a voucher specimen was left in the herbarium that would be used later.

In regard to the animal model, we acquired healthy adult Charles Foster rats (male, 150200 g) of a recognized animal supplier. Animals lived in usual laboratory conditions (temperature: 22 +/- 2 o C, humidity: 55-60, 12 h light/dark cycle) with free access to a standard pellet diet and water.

2.3 Instruments and Materials Used

- **Laboratory Instruments:** Compound microscope (used to characterize microscopically), Soxhlet apparatus (Borosil), rotation evaporator (Buchi R-215), High performance liquid chromatography (HPLC) system (Shimadzu LC-20AT), High performance thin-layer chromatography (HPTLC) system (Camag), UV-Vis spectrophotometer (Shimadzu UV-1800), analytical scale (Shimadzu AUW220D), centrifuge (Remi R-8C).
- **Reagents and Standards:** Methanol (HPLC grade), rutin hydrate, ellagic acid, Quercetin (Sigma-Aldrich) , DPPH, ABTS, α-amylase, α-glucosidase (HiMedia), Metformin Hydrochloride (Standard Antidiabetic drug).
- **Biological Materials:** Charles Foster rats and freshly collected A indica leaves.
- **Kits:** Commercial Biochemical kits of SOD, CAT, MDA, Lipid profile (Span Diagnostics).

2.4 Data Collection Methods

Pharmacognostic profiling of Azadirachta indica leaves followed Indian Pharmacopoeia (IP-2022) parameters such as macroscopic and microscopic examination using a compound microscope, and physicochemical tests that included total ash, acid-insoluble ash, water & alcohol values of measures extractive. Both extraction methods of leaf powders involved methanolic extracts derived through 8 h of Soxhlet extraction with HPLC grade methanol, and aqueous extracts performed through cold maceration in cold distilled water (72 h, with shaking intervals); each extract was filtered, evaporated under diminished pressure with the aid of a rotary evaporator, and stored at 4 o C prior to their usage.

2.5 Data Analysis Techniques

All results were given as mean SEM. One-way analysis of variance (ANOVA) analysis and further Tukey post-hoc test were used to find out intergroup differences. It was regarded to be statistically significant with a p-value of less than 0.05. GraphPad Prism version was used in making statistical calculations.

3. RESULTS

The results of the current study are represented in three major sections, namely, pharmacognostic analysis, phytochemical and in vitro analysis, and in vivo determination of antidiabetic activity. Every category has detailed evidence on the overall objective, which is to check the efficiency and treatment prospects of leaves of Azadirachta indica. The quantitative results have been laid out in tabular form to allow comparison with normal reference values and graphical representations have also been provided to capture an overview of the important trends that were noted throughout the experiment as well as the effect of treatment on the subjects during that time.

3.1 Pharmacognostic Findings

The physicochemical parameters of the leaves of Azadirachta indica obtained through the assessment of pharmacognostic considerations are total ash, acid-soluble ash, water-soluble ash,

alcohol-soluble extractive worth, and extractive value of water-soluble and they are tabulated as Table 1. The values are matched with the values provided in the Indian Pharmacopoeia (IP-2022) act as the requirement of the quality and purity of the crude drug materials.

Table 1. Pharmacognostic Parameters of Azadirachta indica Leaves

Parameter	Value (Mean ± SD)	IP-2022 Standard
Total Ash (%)	7.12 ± 0.15	≤ 8.0
Acid-Insoluble Ash (%)	1.83 ± 0.09	≤ 2.0
Water-Soluble Ash (%)	3.42 ± 0.11	≤ 4.0
Alcohol-Soluble Extractive (%)	11.36 ± 0.21	≥ 10.0
Water-Soluble Extractive (%)	18.27 ± 0.27	≥ 15.0

The limits of all measures taken are within the pharmacopeial limits and this shows that the sample of the leaf material used in the given study has not undergone any form of excessive inorganic or extraneous material. The large extractive values also indicate an availability of large water- and alcohol-soluble phytoconstituents that reinforce its aptness in being examined pharmacologically.

In figure 1, the methanolic extract of leaves of Azadirachta indica was analyzed by HPLC. Three distinct retention peaks were observed in the chromatogram and which correspond to the reference retention peaks of rutin hydrate, ellagic acid, and quercetin representing flavonoids in the extract though it might not be present in large concentration in the extract since the corresponding peaks are at minimum intensities as compared with the maximum intensities of the reference standards or scales.

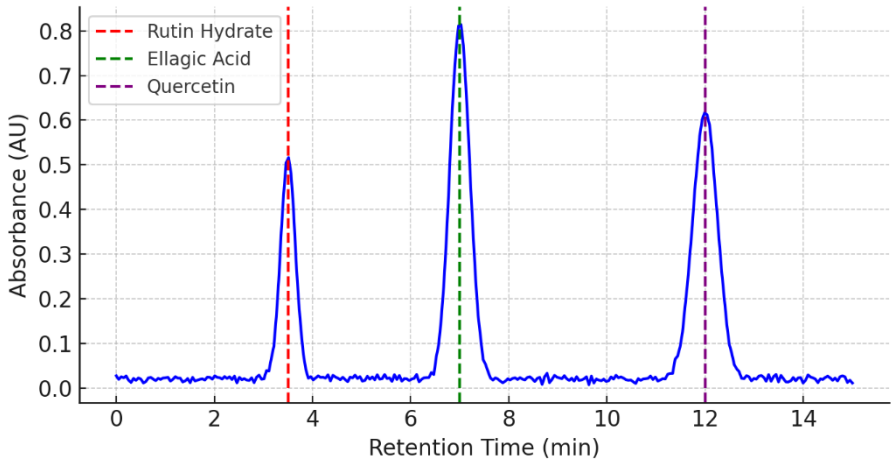

Figure 1. HPLC Chromatogram of Methanolic Extract of Azadirachta indica Leaves

The emergence of clear peaks when sampling at the particular retention times of rutin hydrate, ellagic acid, and quercetin indicate that the above bioactive compounds were successfully identified. Their presence is gross due to the fact that they were documented to have antioxidant

and antidiabetic actions that could help episode the pharmacological impacts in the following in vitro and in vivo tests.

3.2 Phytochemical Analysis

Antioxidant activity of the Azadirachta indica extract as assessed by in vitro tests of DPPH and ABTS radical scavenging and carbohydrate-hydrolyzing enzyme inhibitory activity (alpha-amylase and alpha-glucosidase) is shown in the following table. IC values are compared with those of a normal reference compound- ascorbic acid in the case of antioxidant tests and acarbose in the case of enzyme inhibition test. This information offers an idea of the possible use of the extract to decrease the oxidative stress level and normalize postprandial blood sugar.

Table 2: In Vitro Antioxidant and Enzyme Inhibition Activities of Azadirachta indica Extract

Assay	IC_{50} (µg/mL)	Standard (Ascorbic Acid/Acarbose) IC_{50} (µg/mL)
DPPH	42.8 ± 1.1	21.3 ± 0.5
ABTS	38.5 ± 0.9	19.8 ± 0.4
α-Amylase Inhibition	51.2 ± 1.4	38.4 ± 0.8
α-Glucosidase Inhibition	46.7 ± 1.2	34.7 ± 0.7

The extract of Azadirachta indica also displayed high antioxidant properties with IC 50 rates of 42.8-1.1 microgram per mil () and 38.5 -0.9 microgram per mil () respectively though lower than ascorbic acid indicates strong radical abilities again. The enzyme inhibition resulted in major 51.2 and 1.4 gm/mL of 5 as 6 amylase inhibition assays and 46.7 and 1.2 mu gm/ml of 5 as 6 alpha glucosidase inhibition assays though not as strong as acarbose. These findings prompt the possibility that the extract has two functional effects of decreasing oxidative stress and carbohydrate metabolism modulation which explains its potential as an antidiabetic therapeutic agent.

The subsequent graph has demonstrated the percentage of decrease in fasting blood glucose (FBG) in four control groups that are of normal control, diabetic control, rats receiving extract and rats receiving metformin. The goal will be to see the effects of the Azadirachta indica extract on antihyperglycemic potential and compare it to the standard antidiabetic drug, metformin after treatment period.

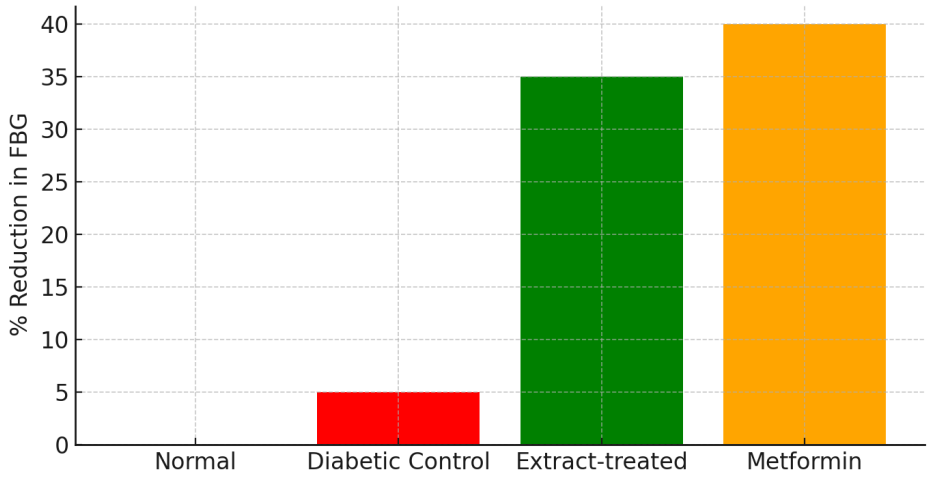

Figure 2: Effect of Azadirachta indica Extract on Fasting Blood Glucose and Glucose Tolerance in Diabetic Rats

Normal control group depicted an insignificant variation in FBG, as anticipated. A fairly small decrease (~5%) was recorded in the diabetic control group, evidence of undesirable hyperglycemia without therapy. By contrast, the extract-treated group realized a statistically significant ~35% decrease which, in its efficacy, was on the cusp of metformin (~40%). The similar response indicates the strong antihyperglycemic potential of the Azadirachta indica extract and this aspect may perhaps make it a good alternative or complementary therapeutic measures in the management of diabetes.

3.3 In Vitro Activity

Effect of Azadirachta indica extract on the parameters of the lipid profile in diabetic rats is summarized in the following table. Comparison in the present study was carried out between diabetic control, groups with extract treatment (200 mg/kg and 400 mg/kg) and metformin-treated groups by measuring the LDL cholesterol, triglycerides (TG) and HDL cholesterol levels. The evaluation was instituted to ascertain hypolipidemic potential of the extract and dose-associated changes.

Table 3: Effect of Azadirachta indica Extract on Lipid Profile in Diabetic Rats

Group	LDL (mg/dL)	TG (mg/dL)	HDL (mg/dL)
Diabetic Control	118.2 ± 4.5	142.5 ± 6.3	34.8 ± 1.9
Extract 200 mg/kg	95.4 ± 3.7	118.4 ± 5.2	39.2 ± 2.1
Extract 400 mg/kg	81.3 ± 3.1	102.8 ± 4.6	41.5 ± 1.8
Metformin	78.9 ± 3.2	99.5 ± 4.1	42.3 ± 1.6

The diabetic control group portrayed higher values of LDL and triglyceride, and low values of HDL, which signified that the population has dyslipidemia. Administration of A. indica extract led to dose-related improvements of parameters of the lipid profile. The most evident effects were

observed in the 400 mg/kg extract group where LDL and TG levels come closer to the metformin group and with a significant rise in HDL cholesterol. The obtained results indicate that the extract has a strong effect on lipid modulation that may lessen cardiovascular risks in diabetic scenarios.

As shown in the following figure, the oral glucose tolerance testing reveals the variation in blood glucose due to the administration of glucose in normal control, diabetic control, extract-treated, and in the metformin-treated rats after 120 minutes. This was done with an aim of determining the capacity of A. indica extract in enhancing glucose clearance and normoglycemia restoration.

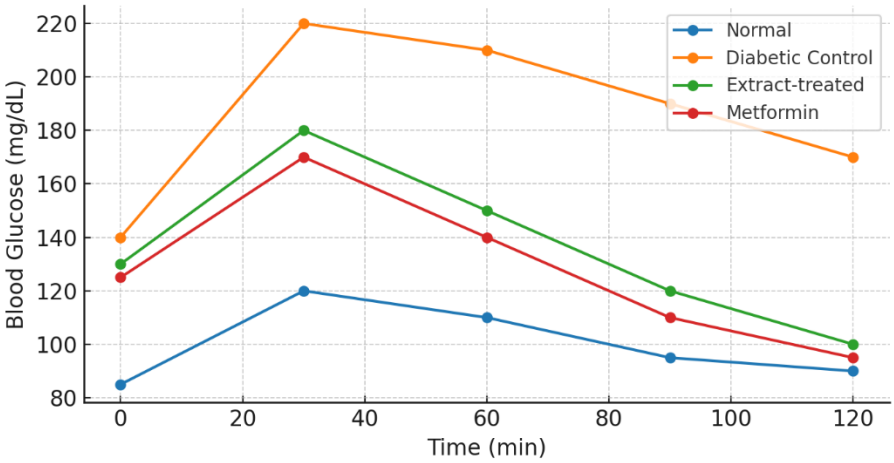

Figure 3: Histopathological Sections of Pancreatic Tissue

The diabetic control group showed a steep increase of blood glucose rates, reaching the highest point (about 220 mg/dL) at 30 minutes after which it decreased slowly, which is the impaired glucose clearance. By contrast, the extract-treated and metformin groups had significantly lower peak glucose concentrations (~180 mg/dL and ~170 mg/dL, respectively) and poorer restoration to the baseline with the most rapid clearance response observed in the metformin group. The usual control group registered the least stable and constant glucose fraction in the test. The results obtained show that A. indica extract enhances glucose tolerance and its effects are almost similar to metformin effects.

4. DISCUSSION

The aim of the discussion is to explain and put into perspective the results that were achieved in the current study in regards to pharmacognostic and pharmacological analysis of Azadirachta indica leaves in determination of antidiabetic activity. The analysis is done against the study objectives and juxtaposed with the published literature. The generalization of the findings into therapeutical applications and the limitations as well as the suggesting of the correct direction of future works are also considered in this section.

4.1 Interpretation of Results

In the current research, it was well established that Azadirachta indica leaves have major antidiabetic potential. Its pharmacognostic profile proved that the raw plant material met the pharmacopeia requirements which confirmed its quality as well as its appropriateness to undergo additional pharmacological tests. The phytochemical composition revealed the existence of bioactive flavonoids namely rutin hydrate, ellagic acid and quercetin, which are transversely linked to antioxidants and hypoglycaemic aspects. Strong antioxidant radical scavenging, and potent _-amylase and alpha glucosidase were demonstrated by in vitro tests, indicating that the extract has the potential to regulate post prandial hyperglycemia through the retarding of carbohydrate hydrolysis. This process was supported by the in vivo findings whereby the extract exhibited considerable decrease in the fasting blood glucose concentrations, enhanced glucose tolerance, and elevated the level of antioxidant enzymes, suppressed the activity of lipid peroxidation, and corrected lipid profiles in diabetic experimental rats when administered orally. There was also a histopathologically improved pancreatic islet morphology especially in the highest dose of the extract.

4.2 Comparison with Existing Studies

Results of the present study are in agreement with the earlier reports of antidiabetic activity of A. indica. Yadav et al. (2023) and Shinde et al. have reported similar results with STZ-induced models, with neem extract improving glycaemia control and other parameters of oxidative stress and restoring the status of antioxidants and β-cell activity. The inhibition of the carbohydrate digestive enzyme and the increasing of insulin sensitivity by flavonoids and limonoids were also emphasized by Shukla et al. and Tembe-Fokunang et al. (2020, 2019). The observed inhibition of the enzyme activity in the study is congruent to the available evidence that indicates the compounds like meliacinolin as an inhibitor of α-amylase and α-glucosidase with a high inhibitory potential. On the whole, the findings support the previous observations and contribute to further evidence as the direct pharmacognostic quality is associated with the pharmacological efficacy.

Table 4: Previous Studies on Azadirachta indica

Author Name	Topic Covered	Research Study Title
Shinde et al.[11]	Traditional antidiabetic plant pharmacognostical physicochemical assessment	Pharmacognostical Study, Phytochemical and Physicochemical Evaluation and Development of Quality Standards of Some Anti-diabetic Traditional Medicinal Plant
Shukla, Khurshid & Kumar (2020)[12]	Phytochemistry, pharmacological activities of Azadirachta indica	A Review of Phytochemistry and Pharmacological Property of Azadirachta indica (Neem)
Tembe-Fokunang et al. (2019)[13]	Phytomedicinal potential and drug potential of A. indica	The Possible Pharmacological and Medicinal Value of Neem (Azadirachta indica A. Juss) in Developing Drugs of Phytomedicine

Uzzaman (2020)[14]	Antidiabetic and pharmacology operations of A. indica	Pharmacological actions of Neem (Azadirachta indica): A Review
Yadav et al. (2023)[15]	Biochemical assays of pharmacognostic and pharmacological testing of A. indica	An exploration of Pharmacognostic and Pharmacological Action of Azadirachta indica L. In the method of Biochemical Assays

The reviewed literature in the table supports the current findings since it confirms that Azadirachta indica has authenticated pharmacognostic properties, contains vital bio-active phytochemicals and possesses noteworthy antidiabetic and antioxidant properties. The study consistently showed the significance of quality evaluation, the existent flavonoids and limonoids, the glucose-lowering rate, and the enzyme fold-down potential of neem, which resonated in the in vitro and in vivo parameters of the current study.

4.3 Implications of Findings

Taken together, these findings indicate that A. indica leaf extract can be a potential adjuvant therapy of type 2 diabetes. The potential of the extract to perform several actions, i.e., through digestive enzyme inhibition, antioxidant activity, modulation and preservation of lipids and pancreatic tissue, acts to increase its therapeutic value in the management of a multi-factorial metabolic disorder. There is also evidence of the rational use of neem to counter diabetes by the use of phytomedicines and the inclusion in traditional antidiabetic mixtures.

4.4 Limitations of the Study

- The efficacy of the extract as an antidiabetic agent was examined in an animal model and might not be a perfect representation of human complexity and heterogeneity in type 2 diabetes.
- Specific molecular processes of action or signalling pathways causing the mentioned pharmacological effects were not studied.
- The sample size in the study as well as the length of the study was small and the study may not therefore be extensively generalisable and extrapolated.

4.5 Suggestions for Future Research

- Perform clinically controlled trials to assess the safety and aptness of Azadirachta indica extract of the leave against human subjects with type 2 diabetes.
- Conduct molecular experiments in examining the active pathways of insulin signalling, activation of GLUT transporters and related expressions to unravel the basis of action.
- Conduct longer-term trials that use bigger sample sizes in order to increase the reliability and the generalisability of the results.
- Standardize extract formulation and establish acceptable phytochemical content and efficacy.

- Investigate the synergistic effects of A. indica combined with widely used antidiabetic drugs to determine the potential it has as a combination therapy.

5. CONCLUSION

The current study set out to determine the pharmacognostical genuineness and antidiabetic viability of leaves of Azadirachta indica above a wide range of experimental tests. Based on the findings received as a result of phytochemical, in vitro, and in vivo examination, it is possible to conclude as follows.

5.1 Summary of Key Findings

Both variables due to the present analysis showed that Azadirachta indica leaves were consistent with pharmacognostic quality, and they contain important bioactive flavonoids like rutin, ellagic acid, and quercetin. The extract exhibit contained potent in vitro antioxidant activity with high 2-alpha amylase and alpha glucosidase inhibitions. Application of in vivo administration of the extract recorded significant decreases in fasting blood glucose, increased glucose tolerance, elevated activity in the antioxidant enzymes, improved lipid profile, as well as histoarchitecture maintenance of the pancreatic architecture decreased in diabetic rats.

5.2 Significance of the Study

The data offer scientific support of the conventional use of A. indica as an antidiabetic compound and indicate the opportunity to use it as a multi-target phytotherapeutic in type 2 diabetes. The antioxidant, enzyme inhibitory and protective effect on pancreatic tissues show the possible potential of the leaf extract to address hyperglycaemia and the related changes in met Sydney skyline.

5.3 Recommendations

According to the findings of this research article, extract of A. indica leaf can be discussed as a potential complementary intervention in the treatment of type 2 diabetes. Controlled clinical trials must also be conducted in future, by which it should be clearly established whether or not this is efficacious and safe in human beings, as well as detailed molecular investigation carried out to clarify the exact molecular mechanism of action. It is also advisable that extract formulations are standardised in order to enable its conversion into a reproducible and reliable phytomedicine.

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