RESEARCH PAPER

Synergistic Antidiabetic Potential of Amaranthus Spinosus and Albizia Lebbeck Leaf Extracts: Targeting Hyperglycemia, Dyslipidemia, And Oxidative Stress Via A-Amylase Inhibition

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ABSTRACT
Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia, often accompanied by dyslipidemia and oxidative stress. The present study investigates the antihyperglycemic, hypolipidemic, and antioxidant potential of leaf extracts of Amaranthus spinosus and Albizia lebbeck using a streptozotocin–nicotinamide (STZ–NA)-induced type 2 diabetes mellitus (T2DM) rat model, with a focus on α-amylase inhibition as a possible mechanism. Leaves were collected, authenticated, and subjected to successive extraction using chloroform and methanol. Phytochemical screening confirmed the presence of bioactive compounds such as flavonoids, alkaloids, tannins, and terpenoids. Acute toxicity studies demonstrated safety up to 2000 mg/kg. T2DM was induced in rats using STZ (55 mg/kg, i.p.) following nicotinamide pre-treatment. The extracts were administered orally at doses of 100–200 mg/kg for 21–30 days.
Both extracts produced a significant ($p < 0.001$) reduction in fasting blood glucose levels, comparable to metformin. They also prevented body weight loss and significantly improved lipid profiles by reducing total cholesterol, triglycerides, and LDL levels while elevating HDL levels. Antioxidant activity was evidenced by increased levels of reduced glutathione (GSH) and enhanced enzymatic activities of catalase (CAT) and superoxide dismutase (SOD). These effects may be attributed to α-amylase inhibition, improved insulin sensitivity, and reduced oxidative stress. The findings suggest that both plant extracts possess strong therapeutic potential for T2DM management.
Keyword: Diabetes mellitus, Amaranthus spinosus, Albizia lebbeck, α-amylase inhibition, antioxidant activity, etc.
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INTRODUCTION

Diabetes mellitus (DM) constitutes a multifaceted, enduring metabolic disorder distinguished by hyperglycemia, which arises from either impaired insulin secretion, defective insulin action, or a combination of both. It encompasses the dysregulation of carbohydrate, lipid, and protein metabolism, which can be attributed to pancreatic β-cell dysfunction or insulin resistance. The global prevalence of diabetes is escalating at an alarming rate, with approximately 537 million adults affected in 2021, a figure anticipated to surge to 783 million by the year 2045. A significant proportion of individuals remain undiagnosed, and the mortality associated with diabetes surpasses 6.7 million deaths each year, thereby emphasizing its considerable impact on public health [i]. Type 1 diabetes (T1D) arises from the autoimmune-mediated obliteration of pancreatic β-cells, resulting in a complete deficiency of insulin and a lifelong requirement for exogenous insulin administration. Conversely, type 2 diabetes (T2D) constitutes over 90% of diabetes cases and emerges from a confluence of insulin resistance and the progressive deterioration of β-cell function. Factors such as genetic susceptibility, obesity, and environmental determinants, including a sedentary lifestyle and diets rich in calories, play significant roles in its pathophysiology[ii].At the molecular level, the dysfunction of β-cells in Type 2 Diabetes (T2D) is associated with glucotoxicity, lipotoxicity, and apoptosis as induced by oxidative stress, as well as dedifferentiation. The depletion of critical transcription factors, such as Pdx1 and MafA, adversely affects glucose-stimulated insulin secretion and the maintenance of β-cell identity [iii]. Insulin resistance, primarily observed in skeletal muscle, hepatic tissue, and adipose depots, emerges as a consequence of inflammatory cytokine signalling, mitochondrial impairment, and the dysfunction of GLUT-4 translocation, thereby obstructing the processes of glucose uptake and metabolic

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utilization[iv].Emerging research additionally implicates mitochondrial dysfunction as a pivotal determinant in the initiation and advancement of insulin resistance and type 2 diabetes (T2D). The diminished efficiency of oxidative phosphorylation, an overproduction of reactive oxygen species (ROS), and impaired mitochondrial biogenesis undermine cellular energy equilibrium. A reduced expression of regulators involved in oxidative metabolism, such as PGC-1α, alongside a decline in mitochondrial respiration, has been documented in T2D patients and their first-degree relatives, indicating that mitochondrial impairments may precede the onset of the disease. In aggregate, diabetes constitutes a multifaceted disorder characterized by interconnected genetic, metabolic, and cellular anomalies. Grasping these molecular mechanisms is imperative for the formulation of innovative therapeutic approaches aimed at preserving β-cell functionality, enhancing mitochondrial integrity, and improving insulin sensitivity [v]. *Amaranthus spinosus L* belongs to the family Amaranthaceae[vi], *Albezia lebbeck* belongs to the family Fabaceae[vii]

MATERIAL AND METHOD

Plant Material Collection and Identification: During the monsoon season (June–July), fresh and healthy *Amaranthus spinosus* leaves were collected from their native habitat in Gartad, District Dhule, Maharashtra. *Albezia lebbeck* leaves were gathered in Nagaon, District Dhule, Maharashtra. To guarantee sample purity, mature, healthy plants from uncontaminated locations were chosen. A botanist from the Botanical Survey of India in Pune verified both species' botanical identities by comparing them to standard floras. Plants were crushed, dried, and mounted on labelled sheets with collection information (plant name, location, date, and collector's name) to create herbarium specimens. After that, they were placed for reference in the departmental herbarium.

Extraction: The powdered plant material was extracted using chloroform (650 ml) for two days in a Soxhlet system. This was followed by extraction using methanol (700 ml) for two days. The extract was separated, filtered, and evaporated in a hot water bath until it was completely dry [viii-ix].

Preliminary phytochemical screening: Preliminary qualitative phytochemical screening was performed on each crude extract using standard classical methods, and all observations were recorded in a standardised data sheet. For alkaloids, aliquots of the extract (2–3 mL) were acidified with 1% HCl, filtered, and treated separately with Mayer's reagent and Wagner's reagent; the formation of a cream or reddish-brown precipitate was interpreted as positive for alkaloids. Flavonoids were detected by the Shinoda test (addition of a few magnesium turnings and concentrated HCl to the extract, producing a pink to red colouration) and by the alkaline reagent test (dilute NaOH produced an intense yellow colour that became colourless on addition of dilute HCl). Tannins and phenolic compounds were assayed by adding 1% ferric chloride to aqueous dilutions of the extracts; a blue-black or greenish colouration was recorded as indicative of hydrolysable or condensed tannins/phenols. Saponins were assessed by the froth test: 1 mL of extract was vigorously shaken with 5 mL of distilled water, and persistence of stable froth (≥10–15 min) and formation of an emulsion on addition of olive oil were noted as positive. Cardiac glycosides were screened using Keller–Killiani's test, where the extract in glacial acetic acid containing ferric chloride was underlaid with concentrated H_2SO_4 and the appearance of a brown ring at the interface (and a blue-green ring on standing) was taken as a positive response. Terpenoids (Salkowski test) were detected by mixing the extract with chloroform and carefully adding concentrated H_2SO_4; a reddish-brown interface indicated the presence of terpenoids. Steroids were examined by the Liebermann–Burchard reaction (acetic anhydride followed by concentrated H_2SO_4), and the development of a green to blue colouration was recorded as evidence of a steroidal nucleus. Negative and reagent controls were run in parallel for each test, and all colour changes and precipitates were confirmed by repeating the assay at least twice [x-13].

Acute Toxicity Studies: The chloroform and methanolic extracts of *Amaranthus spinosus* and *Albizia lebbeck* leaves were studied for acute oral toxicity as per revised OECD (Organisation for Economic Cooperation and Development) guidelines No. 423. The extract was devoid of any toxicity in rats when given in doses up to 2000 mg/kg by oral route. Hence, for further studies, 100-200mg/kg doses of extract were used [xi].

Induction of Experimental Type 2 Diabetes Using Streptozotocin: Type 2 diabetes mellitus (T2DM) was induced experimentally through a modified low-dose streptozotocin (STZ) protocol, which selectively inflicts damage on pancreatic β-cells while retaining a sufficient insulin secretory capacity to emulate the metabolic characteristics associated with human T2DM. Overnight-fasted rats were administered a single intraperitoneal injection of freshly prepared STZ (55 mg/kg) that was solubilized in ice-cold 0.1 M citrate buffer (pH 4.5). In order to establish a moderate and stable diabetic condition, the animals were pre-treated with nicotinamide (110–120 mg/kg, i.p.) 15 minutes prior to the STZ injection; this intervention conferred partial protection to the β-cells, resulting in a condition marked by impaired glucose tolerance, moderate hyperglycemia, and relative insulin deficiency. Following the induction procedure, the animals were returned to their normal feeding regimen, and fasting blood glucose levels were assessed after 48–72 hours. Rats demonstrating fasting blood glucose concentrations exceeding 200–250 mg/dL were classified as diabetic and were subsequently selected for further experimental investigations. This STZ–nicotinamide model effectively mirrors the pathophysiological attributes of T2DM, which include β-cell dysfunction, oxidative stress, and mild insulin resistance [xii].

Experimental Design: After 14 days of streptozotocin (STZ) administration, animals exhibiting fasting blood glucose (FBG) levels greater than 250 mg/dL were considered diabetic and included in the study. The diabetic

rats were then randomly divided into six experimental groups (n=6 per group) based on their FBG levels. Age- and weight-matched non-diabetic rats were maintained as normal controls.

Table 1: Experimental Design for animal groups:

Group	Description	Treatment
Group I	Normal Control (Non-diabetic)	Received distilled water (p.o.)
Group II	Diabetic Control (STZ-induced)	Received distilled water (p.o.)
Group III	Standard Treatment Group	Metformin (50 mg/kg, p.o.)
Group IV	Test Group (Chloroform Extract)	Chloroform extract (200 mg/kg, p.o.)
Group V	Test Group (Methanolic Extract)	Methanolic extract (200 mg/kg, p.o.)
Group VI	Test Group (Active Fraction)	Active fraction (200 mg/kg, p.o.)

All treatments were administered orally once daily for 21–30 days. Normal and diabetic control groups received distilled water, while test groups were treated with extracts at doses of 100, 200, and 400 mg/kg body weight. Body weight and fasting blood glucose levels were monitored weekly (0th, 7th, 14th, 21st, and 28th days). Blood samples were collected at regular intervals via the retro-orbital plexus under light anesthesia for estimation of fasting plasma glucose. At the end of the study (31st day), animals were fasted overnight, anesthetized, and sacrificed. Blood samples were collected by cardiac puncture, and serum/plasma was separated for biochemical analysis. Tissue samples were excised, washed, and stored at −80°C. Biochemical parameters, including lipid profile (TC, TG, LDL, HDL, and VLDL) and insulin levels, were evaluated to assess the treatment effects.

ANTIOXIDANT STUDY

Glutathione (GSH): Reduced glutathione levels in pancreatic tissue were estimated using Ellman's method. Tissue homogenate was treated with trichloroacetic acid (TCA), centrifuged, and the supernatant was reacted with DTNB to form a yellow-colored complex. Absorbance was measured at 412 nm, and GSH levels were expressed as µmol/g of tissue.

Catalase (CAT) Activity: Catalase activity was determined by measuring the decomposition of hydrogen peroxide (H_2O_2) at 240 nm using a UV–Vis spectrophotometer. The decrease in absorbance was recorded, and activity was expressed as the rate of H_2O_2 breakdown per minute per mg of protein.

Superoxide Dismutase (SOD) Activity: SOD activity was assessed based on its ability to inhibit epinephrine auto-oxidation. The reaction mixture was prepared, and absorbance was measured at 480 nm. Activity was expressed as the amount of enzyme required to produce 50% inhibition [12-21].

Statistical Analysis: All results were expressed as mean ± S.E.M., and statistical significance was determined using Duncan's Multiple Range (DMR) test, with $p < 0.05$ considered significant.

RESULT AND DISCUSSION

Table 2: Preliminary phytochemical analysis of Albezia lebbeck leaves extract:

Sr. no	Phytochemical class	Test name	Inference	
			Chloroform	Methanol
1.	Alkaloids	Mayer's test	+	+
2.	Flavonoids	Shinoda test	+	-
3.	Tannins	Ferric chloride test	+	+
4	Saponins	Froth test	-	-
5	Glycosides	Keller-killiani test	-	+
6	Terpenoids	Salkowski test	+	+
7.	Steroids	Liebmann-Burchard test	-	-

Table 3: Effect of Leaf Extract on Blood Glucose Levels in Diabetic Mice:

Groups	Blood Glucose Level in mg/dl			
	Day 0	Day 7	Day 14	Day 21
NC Saline water 10 ml	111.6±5.6	116.6±7.68	114±8	115.21±9.61
DC STZ 65mg/kg	265.3±14.7$^{###}$	293.3±15.9$^{###}$	303±13$^{###}$	329.5±17.2$^{###}$
STD Metformin 50 mg/kg	268.6±8.7	180 ±3.1***	168±7.85***	152.6±4.3***
AL-C 100 mg/kg	263.5±14.8	227±4.56^{***a}	216.3±5.33^{***a}	201.3±7.64^{***a}
AL-M 200 mg/kg	270±15.2	240.2±3.7^{**aa}	221.2±4^{***aa}	210.3±3.9^{***aa}
AS-C 100 mg/kg	256.3±12.3	241.6±10.9^{*aa}	228.6±10.11^{***aa}	198.5±11.5^{***a}
AS-M 200 mg/kg	261.3 ±12.2	247.4±10.34^{*aaa}	234.2±13.26^{***aaa}	208.65±12.1^{***aa}

The effect of successive administration of foliar extracts on blood glucose levels (BGL) was evaluated in diabetic rats. At baseline (day 0), all diabetic groups showed significantly elevated BGL (~256–270 mg/dL) compared to the normal control (~111 mg/dL), confirming successful induction of diabetes. Treatment with the standard drug produced a highly significant ($p < 0.001$) reduction in BGL over 21 days. Both aqueous and solvent extracts at different doses also showed

significant hypoglycemic activity, with effects becoming more pronounced from day 14 onwards ($p < 0.001$). By day 21, all treated groups demonstrated a considerable decrease in BGL compared to baseline. The standard drug showed the highest reduction (43.2%), followed by aqueous extract (AL-C: 23.6%), which was the most effective among the extract-treated groups. In contrast, the diabetic control group showed a progressive increase in BGL, while the normal control group maintained stable glucose levels throughout the study. These findings confirm the significant antihyperglycemic potential of the tested extracts.

Table 4: Effect of Leaf Extract and Solvent Fractions on Body Weight in Diabetic Mice:

Groups	Body Weight			
	Day 0	Day 7	Day 14	Day 21
NC Saline water 10 ml	249.3± 1.820	256.7±2.155	258.5±2.232	263.3±1.926
DC STZ 65mg/kg	241.7± 1.706	240.5±2.062$^{###}$	236.7±1.801$^{###}$	228.0±2.295$^{###}$
STD Metformin 50 mg/kg	260.8± 2.822***	262.8±0.872***	266.5±2.172***	276.7±1.745***
AL-C 100 mg/kg	245.8± 4.549^{aa}	253.5±3.914^{**a}	254.5±3.481^{***a}	263.0±3.950^{***aa}
AL-M 200 mg/kg	243.0± 1.826^{aaa}	249.0±2.221^{aaa}	251.5±3.695^{**aa}	253.8±2.903^{***aaa}
AS-C 100 mg/kg	247.0± 1.765^{a}	253.7±2.22^{**aa}	255.7±0.802^{***a}	261.7±2.011^{***aa}
AS-M 200 mg/kg	240.5±1.765^{aaa}	253.7±0.881^{*a}	249.8±2.242^{***aaa}	255.3±2.789^{***aaa}

At baseline (day 0), all experimental groups showed no significant difference in body weight compared to the normal control, indicating uniform distribution. The diabetic control group exhibited a significant ($p < 0.001$) and progressive decrease in body weight over 21 days, reflecting the catabolic state associated with uncontrolled diabetes.

In contrast, the standard treatment (metformin) significantly improved body weight, showing a steady increase comparable to the normal control group. All extract-treated groups (aqueous and solvent fractions at 100 and 200 mg/kg) demonstrated significant ($p < 0.001$) improvement in body weight from day 7 onwards. The aqueous extract at 100 mg/kg showed the highest increase among test groups, followed by solvent fractions, indicating effective recovery from diabetes-induced weight loss. Overall, treatment groups exhibited a dose- and time-dependent restoration of body weight, suggesting that the extracts possess significant antidiabetic and anabolic potential, likely due to improved glycemic control and metabolic balance.

The Effect of Leaf Extract on Lipid Profile of Diabetic Rats:

There was a significant ($P < 0.001$) elevation of total cholesterol (TC), low-density lipoprotein (LDL), and triglyceride (TG) levels with a significant ($p < 0.001$) reduction in high-density lipoprotein (HDL) cholesterol in the diabetic control group (DC STZ 65 mg/kg) compared to the normal control group (NC Saline water 10 ml), confirming the successful induction of diabetic dyslipidaemia. The diabetic control group exhibited TC levels of 125.5 ± 0.6620 mg/dl, LDL levels of 71.95 ± 0.3819 mg/dl, TG levels of 117.9 ± 0.8037 mg/dl, and markedly reduced HDL levels of 0.4593 ± 0.3819 mg/dl, demonstrating the characteristic lipid abnormalities associated with diabetes mellitus. The standard drug treatment with metformin (STD 50 mg/kg) demonstrated significant ($p < 0.001$) amelioration of diabetic dyslipidaemia, showing TC levels of 88.08 ± 0.6519 mg/dl, LDL levels of 54.37 ± 0.3703 mg/dl, TG levels of 94.97 ± 0.5113 mg/dl, and HDL levels of 38.73 ± 0.2704 mg/dl compared to the diabetic control group, validating the experimental model and establishing a positive control benchmark.

Table 5: The Effect of Leaf Extract on Lipid Profile of Diabetic Rats:

Groups	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Total Cholesterol
NC Saline water 10 ml	72.42±0.7362	40.85±0.4593	46.62±0.4593	70.83±0.7219
DC STZ 65mg/kg	117.9±0.8037$^{###}$	71.95±0.3819$^{###}$	0.4593±0.3819$^{###}$	125.5±0.6620$^{###}$
STD Metformin 50 mg/kg	94.97±0.5113***	54.37±0.3703***	38.73±0.2704***	88.08±0.6519***
AL-C 100 mg/kg	113.9±0.6369^{**aaa}	68.63±0.3073^{**aaa}	28.12±0.3114^{**aaa}	105.4±0.7863^{***aaa}
AL-M 200 mg/kg	114.6±0.8622^{*aaa}	68.80 ±0.3055^{**aaa}	28.00±0.4211^{**aaa}	109.5±1.045^{***aaa}
AS-C 100 mg/kg	114.0±0.6866^{**aaa}	68.65±0.2012^{**aaa}	28.12±0.5369^{**aaa}	94.57±0.9793^{***a}
AS-M 200 mg/kg	114.5±0.8458^{*aaa}	68.83±0.4137^{*aaa}	27.85±0.1708^{*aaa}	116.2±3.059^{***aaa}

Treatment with aqueous leaf extract (100 and 200 mg/kg) and aqueous-solvent fractions significantly improved lipid profile parameters in diabetic rats. Both extracts showed a marked reduction in **total cholesterol (TC), triglycerides (TG), and LDL levels**, along with an increase in **HDL levels** compared to the diabetic control group.

The aqueous-solvent fraction at 100 mg/kg exhibited the most pronounced hypolipidemic effect, while other doses also demonstrated comparable improvements. All treatments showed statistically significant ($p < 0.001$) and dose-dependent effects. Overall, the extracts demonstrated potent **hypolipidemic and cardio protective activity**, likely through improved lipid metabolism and cholesterol

regulation, indicating their potential in managing diabetic dyslipidemia and reducing cardiovascular risk.

Discussion: The present study demonstrates significant antidiabetic and hypolipidemic potential of *Albizia lebbeck* leaf extracts in STZ-induced diabetic rats. Phytochemical analysis confirmed the presence of bioactive compounds such as flavonoids, alkaloids, and tannins, which support the observed therapeutic effects.

All treated groups showed a significant reduction in blood glucose levels, with the aqueous extract (AL-C 100 mg/kg) being the most effective among the extracts, while metformin showed the highest overall activity. The improvement may be due to enhanced insulin sensitivity and better glucose utilization.

Diabetic control animals showed weight loss, whereas extract-treated groups exhibited significant improvement in body weight, indicating restoration of metabolic balance.

Additionally, the extracts improved lipid profile by reducing TC, TG, and LDL levels and increasing HDL levels. The aqueous-solvent fraction (AS-C 100 mg/kg) showed the best hypolipidemic effect. Overall, *Albizia lebbeck* extracts exhibit promising antidiabetic and cardio-protective activities through multiple mechanisms, including glycemic control and lipid regulation.

CONCLUSION

In conclusion, the present study confirms that *Albizia lebbeck* leaf extracts and their solvent fractions exhibit significant antihyperglycemic, hypolipidemic, and metabolic restorative effects in STZ-induced diabetic rats. The extracts effectively reduced blood glucose levels, improved body weight, and normalized lipid profile parameters. Among the tested groups, the aqueous extract at 100 mg/kg and aqueous-solvent fraction showed the most promising activity. The observed effects may be attributed to the presence of bioactive phytoconstituents such as flavonoids, tannins, and terpenoids. These findings suggest that *Albizia lebbeck* has strong potential as a natural therapeutic agent for the management of diabetes mellitus and its associated complications. Further studies, including clinical investigations, are recommended to establish its safety and efficacy in humans.

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