Research Article

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Antihyperglycemic, antiglycation, anti-hypercholesteremic, and toxicity evaluation with gas chromatography mass spectrometry profiling for *Aloe armatissima* leaves

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Abstract: *Aloe* species are known for the treatment of various conditions including diabetes mellitus, hypocholesteremia, and glycation end products. Nevertheless, the biological activity of *Aloe armatissima* is yet to be reported. It is a first-time report to evaluate the *Aloe armatissima* leaves (AAL) extract for its antioxidant, anti-glycation, anti-hyperglycemic, and anti-hyperlipidemic potential. *In vitro* tests of 1,1-diphenyl-2-picrylhydrazyl for the antioxidant and HSA for the antiglycation activity whereas *in vivo* models were used to assess the toxicity, antihyperglycemic, and anti-hypercholesteremic effects. The volatile profile was determined via gas chromatography-mass spectrometry. The IC_{50} values of 116 ± 0.66 (μg/mL) for antioxidant activity and 0.21 ± 0.009 (mg/mL) for antiglycation activity were observed for the AAL extract. The acute toxicity in the animal model revealed a lack of toxicity for the extract. The *in vivo* models exhibited a dose-dependent hypoglycemic and anti-hyperglycemic effects with significant ($P < 0.01$) blood glucose levels reduction. Moreover, a profound decrease in serum cholesterol, triglyceride, and LDL along with a significant ($P < 0.05$) increase in HDL and serum insulin levels was recorded. The statistical analysis demonstrated the values of $F_{(24,125)} = 23.95$, $P = 0.001$, effect size = 1.95 (normoglycemic mice), $F_{(24,125)} = 143.21$, $P = 0.001$, effect size = 4.79 (glucose loaded mice), and $F_{(24,125)} = 82.69$, $P = 0.001$, effect size = 3.6 (diabetic model). GCMS showed the presence of eleven compounds with tetratetracontane (100%), β-sitosterol (27.76), and vitamin E (18.68) in major amounts. The results underscore the extract's capacity to effectively combat various ailments; however, the active phytochemicals need to be isolated and the pharmacological activities may be established at the molecular level.

Keywords: *Aloe armatissima*, anti-glycation, anti-diabetic, anti-hyperglycemic, GC-MS

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Abbreviations

AAL	*Aloe armatissima* leaves Extract
ANOVA	analysis of variance
BGL	blood glucose level
DPPH	1,1-diphenyl-2-picrylhydrazyl
DM	diabetes mellitus
GLIB	glibenclamide
HAS	human serum albumin
IDF	International Diabetes Federation
OECD	Organization for Economic Cooperation and Development
OGTT	oral glucose tolerance test
STZ	streptozotocin
WHO	World Health Organization

1 Introduction

Diabetes mellitus (DM) is a chronic condition with a substantial impact on an individual, family, and community

health. According to The International Diabetes Federation's 2017 report, 352 M are at risk of developing type II diabetes. The health experts foresee a staggering figure of 439M adults being afflicted by diabetes by the year 2030 [1]. DM disrupts the body's metabolic processes, impacting carbohydrate and lipid metabolism, consequently giving rise to an array of complications including the macrovascular (neuropathy, retinopathy, and renal issues) and microvascular complications (closely related to cardiovascular diseases) [2]. The adverse health outcomes with diabetes stem from processes driven by oxidative stress, intricately linked to the onset of diabetes and its ensuing complications. Prior investigations have demonstrated that oxidative stress precipitates diminished insulin secretion and compromised glucose metabolism in peripheral tissues during hyperglycemic states. It indicates that blocking or neutralizing the formation of reactive oxygen species (ROS) in the hyperglycemic context holds the potential to mitigate diabetes-related complications [3]. The emergence of ROS in DM arises from disrupted insulin synthesis due to the apoptotic cell death in the pancreas. To bolster the body's antioxidant defense mechanism, supplements and plant-based compounds are integrated into regimens to mitigate oxidative stress and thwart the disease's inception at its nascent stages [4]. Moreover, the formation of glycation end products in the body plays a vital role in the pathogenesis of complications in diabetic individuals. Glycation products are the covalent adducts of glucose and plasma proteins produced through a non-enzymatic process in the body where glucose reacts with the free amino group of the amines producing amadori products. These amadori products turn into advanced glycation end products (AGEs) in the later stage where their interaction with the AGEs receptor (RAGE) causes the release of free radicals, pro-inflammatory molecules, altered intracellular-signaling and gene expression. This ultimately turns into diabetic complications of neuropathy, retinopathy, cardiomyopathy, aging, and osteoporosis [5]. The metabolic risk factor of hypercholesteremia is another contributing factor to the development of cataracts and retinopathy in type II DM patients [6]. Though a complete exploratory mechanism is awaited to date, certain lipoproteins in the body have been reported to have a close association with diabetic retinopathy in type II DM [7]. The potential complications posed due to free radicals, glycation end products, and hypercholesteremia necessitate the surge for a therapeutic agent with an established multipurpose role. The widespread belief in the potential of medicinal plants to offer remedies for health issues, characterized by minimal side effects, affordability, and ease of accessibility, prevails within various communities [8]. Hence, a substantial proportion of diabetic patients (80–85%) turn to herbal remedies for managing their condition. Ethnobotanical studies have cataloged the usage of over 1,200 medicinal plants for addressing DM. Plant-derived medicines hold promise in ameliorating metabolic issues and retarding the onset of diabetes-related complications [9]. Throughout history, plants have served as therapeutic agents for humans and other species, administered in various dosages of crude extracts. Herbal medicine has played a significant role in primary care facilities across developing and developed nations and current anti-diabetic drugs strive to achieve optimal hyperglycemia control with minimal side effects and easy availability, but realizing this objective remains a formidable challenge.

Aloe armatissima Lavr & Collen belongs to the family Aloaceae [10] where a number of species including *A. vera*, *A. perryi*, *A. arborescens*, and *A. ferox* find widespread applications for addressing diverse ailments. The folkloric use of aloe leaves has been witnessed to combat microbial infections, digestive disorders, and inflammatory conditions. The applications of aloe plants extend even to the food, beverages, and cosmetic sectors. The literature evidence reveals a potential role for the aloe plant in the treatment of gastrointestinal, inflammatory, and detoxification conditions [11,12] however, none of the studies explored the antihyperglycemic and anti-hypercholesteremic effects of *Aloe armatissima*. To the best of our knowledge, it is a first-time study to assess the potential for *Aloe armatissima* leaves (AAL) in diabetes and its related comorbidities. This study aims to establish a multidimensional role for AAL in combating free radicals, advanced glycated end products, and hypercholesteremia which may play a substantial role in developing type II DM and the associated complications.

2 Materials and methodology

2.1 Chemicals and reagents

Streptozotocin (STZ), ethanol, methanol, benzene, petroleum ether, ethyl acetate, glibenclamide, human serum albumin (HSA), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), and gallic acid, were all obtained for the research from SIGMA-Aldrich whereas, Lipid Profile kit was obtained from Human Diagnostica (Germany).

2.2 Instruments and equipment

Rota-Vapor® for evaporation and drying of samples (R-100, BUCHI Corporation); microplate reader (Multiskan Go, Thermo Scientific, USA); multi-plate reader (SpectraMax-384, Molecular Devices, USA); Gas Chromatography-Mass Spectrometry (GC-MS)

systems with specifications of GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with OPTIMA-5 column for separation of volatile components; HumaLyzer-3000 (Germany); Accu-Check Blood Glucose Meter with strips (Roche, Germany; Insulin assay kit Cisbio International, France.

2.3 Plant material collection and extraction

2.3.1 Plant identification and authentication

The plant species of *Aloe armatissima* was collected in April 2019 from Wadi Thee, Ghazal near Taif City, Saudi Arabia. The identification and authentication were carried out by Prof. A. Badar with a voucher specimen (2019/Folk/1) and submitted to the Laboratory of the Department of Pharmacognosy, Faculty of Pharmacy, Umm Al-Qura, Kingdom of Saudi Arabia.

2.3.2 Extraction from the AAL

For extraction, the previously reported method with slight modification was followed [13]. Briefly, fresh AAL were collected, cleaned with tap water, cut into small pieces, and subjected to lyophilization for drying. A hundred grams (100 g) of the dried leaves were weighed and macerated in 1L hydroalcoholic solvent of ET:water (70:30) for 3 days, followed by filtration (Whatman filter paper; 0.4 mm), and subsequent drying of the sample with the help of rotary evaporator. The AAL extract was weighed, the %yield calculated, and refrigerated till further use.

2.4 *In vitro* activities

2.4.1 DPPH free radical scavenging assay

The free radical scavenging activity for the AAL extract was evaluated using DPPH as reported [14]. A reaction mixture was prepared (95 µL of 300 µM DPPH with 5 µL of 0.5 mg/mL test solution), incubated in darkness (37°C for 30 min), and finally, the absorbance was measured at 517 nm using the multi-plate reader. The change in color of the solution (violet to pale yellow), due to the reduction reaction, indicative of the scavenging activity was noted, and the %radical scavenging potential was determined using the following formula:

$$\%\text{RSA} = 1 - \frac{\text{Abs of test}}{\text{Abs of control}} \times 100.$$

2.4.2 Anti-glycation activity

The anti-glycation activity was performed using a previously reported spectrophotometric method [14]. Briefly, 20 µL of AAL extract (2 mg/mL) along with 50 µL of HAS solution (10 mg/mL), 50 µL of fructose solution (500 mM), and 80 µL of phosphate buffer (100 mM; pH 7.4) were introduced in a 96-well microtiter plate. The microtiter plate was incubated (37°C for one week) followed by measurement of the fluorescence for the test sample (AAL), at excitation and emission wavelengths of 330 and 440 nm, respectively. The % inhibition was determined by the following formula:

$$\%\text{Inhibition} = 1 - \frac{\text{Fluorescence of test}}{\text{Fluorescence of control}} \times 100.$$

2.5 *In vivo* activity

2.5.1 Experimental animals

For the *in vivo* studies, Swiss Albino mice (male and female), weighing between 20 and 40 g, were procured from the Department of Biochemistry, College of medicine, Umm Al-Qura University, Kingdom of Saudi Arabia. The animals were grouped (n = 6), fed with a standard diet, and maintained in at standard laboratory environment (temperature = 23 ± 2°C; relative humidity = 55 ± 10%) with consecutive 12-h dark and light cycles. Prior to any laboratory experiment, the animals were properly acclimatized to the laboratory conditions for one week, and any experiment conducted was assured to follow the guidelines for national legislation on the use of animals for research [15]. The study was issued the ethical approval for experimental handling procedures, approved by the Animal Ethics Board of the College of Medicine, with ethical approval number: HAPO-02-K-012-2022-01-385.

2.5.2 Acute toxicity study

The limit test protocols from the Organization for Economic Cooperation and Development (OECD) guideline-425, were followed for the acute toxicity study utilizing the healthy mice [16]. In detail, five female mice (24–30 g) were subjected to fasting (3–4 h; with excess to water and restricted to food) before dosing and 2 h post-extract administration. The animals were administered with a single oral dose of the AAL

extract (2,000 mg/kg) with a close observation for any untoward reaction or symptoms for 24 h and with subsequent daily observation. The body weights of the animals were recorded on a daily basis followed by euthanizing of the animals on the 14th day. The internal organs were removed and thoroughly examined for any toxic effects or signs.

2.5.3 Antidiabetic animal models

2.5.3.1 Estimation of hypoglycemic activity for normoglycemic mice

The hypoglycemic activity of AAL extract was evaluated in normoglycemic mice [17]. A total of 30 animals were randomly allocated into five groups (n = 6) with an oral dose administration of 10 mL/kg distilled water for group-I (control group): AAL extracts of 100, 200, and 400 mg/kg, respectively, for groups II, III, and IV (treated groups): and 5 mg/kg GLB for group-V (standard drug group). The mice were subjected to fasting for 16 h with excess water only. For hypoglycemic activity evaluation, blood glucose levels were measured with the help of a glucometer, using a drop of blood from the animal's tail. The baseline Blood glucose level (BGL) (mg/dl: at 0 h) for the data was noted before animal dosing whereas, for the AAL extract effect, the samples from animal tail vein were collected at predetermined time points of 60, 120, and 240 min following treatment [17,18], and BGL was measured.

2.5.3.2 Effect of AAL on oral glucose tolerance test (OGTT)

The oral glucose tolerance assessment (OGTT) test was conducted following the reported experimental protocols [19]. The animals were grouped as previously with an overnight fasting of unrestricted access to water. Following the 30 min post-AAL dosing, the animals were orally administered a glucose solution of 2,000 mg/kg, and the blood samples were collected from the tail vein at 0, 30, 60, 120, and 240 mins in order to evaluate BGLs (mg/dL).

2.5.3.3 Induction of experimental diabetes

The reported method [19,20] with slight modifications was followed to induce diabetes in the experimental animals. Alike previous protocols, the animals were grouped and fasted overnight. Prior to inducing any diabetes, the blood sugar level and weights were recorded for all the animals. For the diabetes induction; streptozotocin (STZ) was diluted with 0.1M sodium citrate buffer (pH = 4.5) and was administered intraperitoneally (i.p.) as a single dose (150 mg/kg body weight). The mice were allowed access to food and water 30 min post-STZ injection whereas, the animals were administered with a 5% glucose solution (6 h post-STZ injection) in order to prevent hypoglycemia. The plasma BGL levels for the animals were evaluated (3 days post-STZ injection) via the tail vein using a glucometer. Any animal with FBG >200 mg/dL was declared diabetic and included for further antidiabetic evaluation.

2.5.3.4 Antidiabetic assessment for AAL extract

To evaluate the antidiabetic potential for AAL extract, the diabetic animals were categorized as previously. The oral doses were administered; distilled water for group-I (control animals), and group-II (diabetic control animals), GLB for group-III (positive control group) whereas group-IV, group-V, and group-VI received AAL extract of 100, 200, and 400 mg/kg, respectively. For BGL determination, the blood drops were obtained from the tail vein at time intervals of 0, 7, 14, and 21 days.

2.5.4 Assessment of the lipid profile and serum insulin levels

The diabetic mice used in the previous model of antidiabetic activity were used to determine the anti-hyperlipidemic activity of AAL extract. In order to determine the lipid profile, the blood was collected (21st day) from retro-orbital sinus of the animals and subjected to HumaLyzer for a complete blood profiling of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) [21].

The blood (21st day) from the AAL-administered diabetic mice was collected, and serum insulin levels were estimated with the help of a radioimmunoassay kit [22].

2.6 GC-MS profiling for the leaves

An Agilent GC-MS system with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with an OPTIMA-5 column was used to characterize the volatile components in the sample. The instrument conditions were as follows: Helium drift (1.0 mL/min), electron ionization (70 eV), OPTIMA-5 column for separation, and inline built NIST mass spectral database. However, the conditions for chromatography and mass determination consisted of an initial temperature of 50°C (3 min) with an incremental rise of 10°C/min for 20 min followed by an incremental increase of 10°C/min till 300°C. The temperature was

maintained for 25 min at 300°C, and the mass spectra were recorded. The compounds were detected by mass spectra comparison and similarity index of the compounds with the reference standards in the NIST mass spectral database [23].

2.7 Statistical analysis

The G*Power (V 3.1.9.2) and Statistical Package for Social Science Students (SPSS V21.0) were used for statistical analysis of the dataset. For one-way analysis of variance (*ANOVA*), effect size (f) is taken as 0.10, α-error probability at 0.05, and power at 0.80. The variables are displayed in the tables as mean ± SD whereas, the significance level was set at $P = 0.05$.

3 Results

3.1 Extract yield and %recovery

The extract yield for the AAL was 9 g which resulted in a yield of 9%.

3.2 DPPH activity

The *in vitro* free radical scavenging for AAL extract was determined by calculating the IC_{50} values. Gallic acid was used as a reference standard. The IC_{50} values (μg/mL) for AAL and gallic acid were 116 ± 0.66 and 2.6 ± 1.0, respectively. The %inhibition and IC_{50} values are shown in Table 1.

Table 1: *In vitro* free radical scavenging via DPPH and antiglycation activity for AAL extract

Sample	%Inhibition	IC_{50} value
DPPH radical scavenging activity (IC_{50} = μg/mL)		
AAL	82.5	116 ± 0.66
Gallic acid	97.3	2.6 ± 1.0
Antiglycation activity (IC_{50} = mg/mL)		
AAL	83.14	0.21 ± 0.009
Rutin	99.5	0.02 ± 0.01

3.3 Anti-glycation HSA assay

The AAL extract significant glycation inhibitory effect of 83.14% as compared to the reference drug rutin (99.50%). The IC_{50} values (mg/mL) for the AAL extract and rutin observed were 0.21 ± 0.009 and 0.02 ± 0.01, respectively. The data for antiglycation activity are shown in Table 1.

3.4 Acute oral toxicity test

The AAL extract showed no mortality and pathological changes at a dose of 2,000 mg/kg. The toxicity results at 2 h, 24 h, and 14 days showed a lack of any adverse effects on the gross behavioral pattern of the animals. Moreover, no significant differences were noted regarding food intake and body weight as compared to the control group.

3.5 Hypoglycemic activity of AAL extract in normoglycemic mice

For the antidiabetic effect in normoglycemic mice, the BGLs were measured immediately prior to any treatment (0 h) and subsequently at 1, 2, 3, and 4 h after treatment. No significant differences were observed for the control group ($P > 0.05$); however, significant BGL changes ($P < 0.05$) were observed after administration of glibenclamide and AAL extract (100, 200, and 400 mg/kg). The ANOVA test value showed a statistical significance with $F_{(24,125)} = 23.95$ at $P = 0.00$ and an effect size of 1.95, compared to the control (0 h). For the normoglycemic mice groups, the treatment with glibenclamide and AAL extract revealed a significant decrease for BGLs at 2, 3, and 4 h at different doses; 100 mg/kg ($P < 0.05$), 200 mg/kg ($P < 0.01$), 400 mg/kg ($P < 0.05$), and glibenclamide ($P < 0.001$).

With regard to the intra-group comparisons; AAL extract (400 mg/kg) exhibited pronounced reduction ($P < 0.0001$) in BGLs at 2 h (22%), 3 h (37%), and 4 h (44%) as compared to the baseline BGLs. The reference drug glibenclamide showed reductions of 14%, 22%, and 31% at the 2 h, 3 h, and 4 h, respectively. The reduction in BGLs at 4 h post-AAL treatment (400 mg/kg: 72.83 ± 7.55) was more pronounced compared to glibenclamide (87.83 ± 4.02).

In terms of inter-group comparisons, glibenclamide and AAL extract (200 and 400 mg/kg) demonstrated a significant reduction in BGLs at 4 h when compared to these values at 2 h of post-treatment. The control group (group 1: DW) exhibited non-significant changes in BGLs throughout the experiment at time 0–4 h. The data for AAL extract effect in normoglycemic mice are shown in Table 2.

Table 2: Effect of AAL extract on BGLs of normoglycemic, postprandial non-diabetic, and diabetic mice

Hypoglycemic activity for AAL extract in normoglycemic mice					
Group	**0 h**	**1 h**	**2 h**	**3 h**	**4 h**
Control	122.67 ± 3.93	124.17 ± 3.49	122.33 ± 4.97	119.83 ± 4.07	118.83 ± 4.72
Glibenclamide	127.50 ± 9.12	112.50 ± 3.56	109.67 ± 10.95*	99.17 ± 3.66***	87.83 ± 4.02***$^{\#}$
AAL 100 mg/kg	122.17 ± 6.27	116.67 ± 9.69	103.50 ± 13.41*	97.33 ± 8.98***	92.33 ± 7.45***
AAL 200 mg/kg	125.50 ± 9.40	119.83 ± 7.*57	104.17 ± 12.69*	90.67 ± 13.72***	83.50 ± 13.91***$^{\#}$
AAL 400 mg/kg	129.17 ± 3.97	117.17 ± 4.07	100.17 ± 4.62*	80.17 ± 9.77***$^{\#}$	72.83 ± 7.55***$^{\#}$
$F_{(24,\ 125)}$ = 23.95, *P = 0.001, effect size = 1.95, power = 0.99					
Significant decrease in BGL compared to their control value (at 0 h), *P < 0.05, ***P < 0.001					
Significant decrease in BGL compared to their value at 2 h $^{\#}P$ < 0.05					

Hypoglycemic activity for AAL extract in glucose-loaded mice (OGTT)							
Group	**0 h**	**0.5 h**	**0.5 h (% increase BGL)**	**1 h**	**2 h**	**4 h**	**% Decrease (at 4 h compared to 0.5 h)**
Control	105.67 ± 5.79	166.50 ± 8.67*	58%	154.50 ± 6.80*	142.5 ± 8.78*$^{@}$	129.33 ± 7.79*$^{@}$	22%
Glibenclamide	115.17 ± 5.31	162.67 ± 4.80*	41%	116.83 ± 5.31$^{@}$	93.67 ± 4.93$^{\#@}$	85.17 ± 4.83$^{\#@}$	47%
Extract (100 mg/kg)	118.67 ± 7.74	171.50 ± 6.89*	44%	125.83 ± 3.76$^{@}$	96.33 ± 6.68$^{\#@}$	89.33 ± 6.25$^{\#@}$	47%
Extract (200 mg/kg)	117.17 ± 3.87	168.17 ± 5.19*	43%	119.33 ± 12.09$^{@}$	94.17 ± 3.60$^{\#@}$	83.17 ± 6.37$^{\#@}$	49%
Extract (400 mg/kg)	119.33 ± 5.82	165.33 ± 3.20*	38%	106.33 ± 4.41$^{@}$	75.67 ± 7.31$^{\#@b}$	67.83 ± 6.05$^{\#@b}$	59%
$F_{(24,\ 125)}$ = 143.21, *P = 0.001, effect size = 4.79, power = 0.99							
Significantly increased as compared to their control value (at 0 h), *P < 0.001							
Significantly decreased as compared to their value (at 30 min), $^{@}P$ < 0.001							
Significantly decreased as compared to their value (at 1 h), $^{\#}P$ < 0.001							
Significantly decreased as compared to positive value ^{b}P < 0.001							

Antihyperglycemic activity for AAL extract on BGL in STZ-induced diabetic mice					
Group	**Pre-STZ**	**0 Day**	**7 Days**	**14 Days**	**21 Days**
Normal control	118.50 ± 1.87	120.167 ± 1.472	118.33 ± 4.97	114.50 ± 1.871	115.167 ± 2.37
Diabetic control	127.33 ± 15.56	228.83 ± 5.64*	236.17 ± 6.91*	250.50 ± 10.80*	261.67 ± 11.04*$^{\#}$
Glibenclamide	123.33 ± 8.09	232.17 ± 7.55*	198.00 ± 12.66*$^{\#}$	182.17 ± 9.95*$^{\#}$	147.67 ± 13.56$^{\#@}$
Extract 100 mg/kg	125.67 ± 20.50	221.50 ± 9.48*	206.50 ± 9.01*	190.00 ± 7.77*$^{\#}$	174.33 ± 7.20*$^{\#@}$
Extract 200 mg/kg	126.78 ± 9.85	226.50 ± 9.85*	197.83 ± 13.61*$^{\#}$	175.67 ± 12.21*$^{\#}$	164.00 ± 12.71*$^{\#@}$
Extract 400 mg/kg	129.33 ± 6.89	221.17 ± 16.07*	182.67 ± 5.72*$^{\#}$	154.67 ± 11.36*$^{\#@}$	142.67 ± 15.29$^{\#@}$
$F_{(24,125)}$ = 82.69, *P = 0.001, effect size = 3.6, power = 0.99					
Significantly increased as compared to their control value (before STZ) *P < 0.001					
Significantly decreased as compared to the value at 0 day, $^{\#}P$ < 0.001					
Significantly decreased as compared to the value on 7th day, $^{@}P$ < 0.001					

Results are expressed in mean ± SD, n = 6, Control = distilled water (10 mL/kg); glibenclamide = 5 mg/kg.

3.6 Effects of AAL extract in glucose-loaded/post-prandial mice

The AAL extract and glibenclamide revealed significant ($P < 0.05$) effects in glucose-loaded mice with statistical values of $F_{(24,125)} = 143.21$, $P = 0.000$, and effect size of 4.79. Following glucose administration, a significant increase in glucose level was observed in all the groups producing hyperglycemia at 0.5 h with a pronounced increase in the negative control group (58%). The positive control group of glibenclamide and AAL extract groups exhibited non-significant ($P > 0.05$) differences at any of the tested doses following post-30 min glucose loading.

The AAL extract (100, 200, and 400 mg/kg) and glibenclamide (5 mg/kg) significantly reduced the BGLs compared to the control group ($P < 0.001$) at 1 h and onwards. The AAL effect (400 mg/kg) showed a significant rise in glucose level with the passage of time, particularly at 2 h, with a notable decline at 2 h compared to the control and glibenclamide groups ($P < 0.001$). This effect remained sustained till the end of the experiment. The details for the AAL effect in the postprandial mice model are shown in Table 2.

3.7 Anti-diabetic effect of AAL in STZ-induced diabetic mice

The ANOVA for STZ-induced diabetes in mice revealed a significant result with $F_{(24,125)} = 82.69$, $P = 0.000$, and an effect size of 3.6. The BGLs for STZ-induced diabetic mice exhibited profound differences ($P < 0.001$) compared to the control group. The BGL level for the diabetic control significantly increased starting at day 0 till day 21 ($P < 0.001$). As compared to the diabetic control, all the doses for AAL extracts revealed a dose-dependent reduction for BGLs on days 7, 14, and 21. Likewise, glibenclamide-treated groups exhibited a significant decrease in BGLs (days 7, 14, and 21) however, no significant differences were observed for AAL extracts and glibenclamide. This indicates a potential anti-diabetic role for AAL extract. The data for AAL extracts in the diabetic mice model are shown in Table 2.

3.8 AAL effect on body weight of normal and diabetic mice

The statistical analysis for the AAL extract and glibenclamide revealed a non-significant result for the effect on body weight in STZ-induced diabetic mice, with $F_{(23,120)} = 1.28$ and $P = 0.193$ ($P > 0.05$). The body weight for the diabetic control mice group showed a decline throughout the stud whereas, administration of the AAL extract prevented the weight loss. However, the result was non-significant for AAL and glibenclamide across all the tested doses (100, 200, and 400 mg/kg). The data for the mice models are shown in Table 3.

3.9 AAL effect on lipid profile and serum insulin level

The STZ increased the serum total cholesterol, triglycerides, and LDL cholesterol for diabetic mice as compared to the normal mice; however, low levels of HDL and serum insulin levels were observed ($P < 0.05$). The administration of AAL extract produced a significant decrease in the serum level of total cholesterol ($P < 0.001$), triglyceride ($P < 0.01$), and low-density lipoprotein-cholesterol ($P < 0.001$).

On the contrary, the administration of AAL extract at 400 mg/kg resulted in a significant increase in serum insulin level and high-density lipoprotein-cholesterol ($P < 0.01$).

Table 3: Effect of AAL extract on body weight of normal and diabetic mice

Group	0 day	7 days	14 days	21 days
Normal control	26.733 ± 1.05	26.71 ± 0.85	26.86 ± 0.73	27.08 ± 1.65
Diabetic control	28.25 ± 0.95	27.81 ± 2.31	26.61 ± 1.82	25.82 ± 1.64*
Glibenclamide	27.63 ± 1.23	27.7 ± 1.32	28.41 ± 0.86	29.61 ± 1.49
Extract (100 mg/kg)	27.33 ± 1.57	27.68 ± 1.51	28.11 ± 2.02	28.38 ± 2.32
Extract (200 mg/kg)	27.61 ± 1.02	27.83 ± 2.41	28.3 ± 1.24	28.66 ± 2.01
Extract (400 mg/kg)	28.15 ± 2.05	28.5 ± 1.14	29 ± 1.87	29.66 ± 1.38
		$F_{(23,120)} = 1.28$, $P = 0.193$		

Results are expressed in mean ± SD, $n = 6$, distilled water (10 mL/kg), glibenclamide (5 mg/kg), AAL; as compared to their control value (before STZ)
*$P < 0.05$.

Table 4: Effect of the AAL extract on serum insulin level and lipid profiles

Group	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Serum insulin (μU/mL)
Normal control	91.17 ± 5.15	94.67 ± 3.83	37.00 ± 1.41	32.83 ± 2.04	18.83 ± 5.8
Diabetic control	187 ± 4.13*	177.67 ± 3.01*	26.16 ± 1.72*	129.33 ± 5.01*	10.45 ± 1.22*
Glibenclamide	96.50 ± 1.87#	106.00 ± 3.35#	36.50 ± 1.87	37.83 ± 4.26#	18.60 ± 3.45#
Extract (100 mg/kg)	138.6 ± 2.16#	155.17 ± 3.54#	29.66 ± 1.03#	96.67 ± 2.94#	13.33 ± 3.70
Extract (200 mg/kg)	120.33 ± 3.5#	148.33 ± 4.79#	33.33 ± 2.60#	86.50 ± 7.94#	15.12 ± 3.22
Extract (400 mg/kg)	109.67 ± 3.01#	127.66 ± 1.82#	37.33 ± 2.16#	45.33 ± 4.46#	17.45 ± 1.77#
	$F_{(5,30)}$ = 618.2, *P = 0.001, effect size = 5.3, power = 0.99	$F_{(5,30)}$ = 479.2, *P = 0.001, effect size = 4.7, power = 0.99	$F_{(5,30)}$ = 36.79, *P = 0.001, effect size = 1.38, power = 0.99	$F_{(5,30)}$ = 389.5, *P = 0.001, effect size = 4.43, power = 0.99	$F_{(5,30)}$ = 5.29, *P = 0.001, effect size = 0.5, power = 0.99

Results are expressed as mean ± SD, n = 6, NC = normal control, distilled water (10 mL/kg), DC = diabetic control, glibenclamide (5 mg/kg), significantly increased as compared to their control value *P < 0.001, significantly decreased as compared to their positive control value (diabetic), #P < 0.001.

The reference drug glibenclamide also improved the lipid profile and insulin levels in diabetic mice (P < 0.05). The data for insulin level and lipid profile are shown in Table 4.

3.10 GC-MS analysis of AAL extract

The GCMS analysis indicated the presence of eleven compounds in the AAL extract as shown in Table 5. These compounds (on the basis of peak area) may be arranged in the descending order of its occurrence; tetratetracontane (100%) > β-sitosterol (27.76%) > vitamin-E (18.68%) > squalene (18.32) > palmitic acid (16.76%) > lupeol (13.89%) > stearic acid (13.06%) > ethyl linolenate (7.7%) > 3,7,11,15-tetramethyl-2-hexadecen-1-ol (7.12) > γ-tocopherol (6.62%) > phytol (6.26%). The details regarding the retention time, molecular weight, and peak area for the eleven compounds are shown in Table 5.

4 Discussion

Diabetes is a widespread global concern with far-reaching implications for human health. The primary culprits behind diabetes-related issues are endogenous advanced glycation end-product (AGE) formations. The glycation process, a key driver of diabetic symptoms, involves a spontaneous reaction between proteins and reducing sugars where the synthesis of endogenous AGEs plays a pivotal role in initiating a majority of diabetic complications. Current scientific investigation aims to evaluate the *in vitro* and *in vivo* antioxidant and antiglycation activity for the AAL extract, to develop a potential source for combating these conditions. To assure the efficacy of the ALL extract in diabetes, *in vivo* diabetic models in mice were developed to investigate the effect of AAL in normoglycemic, glucose-loaded, and diabetic mice. Diabetes is accompanied by comorbidities of the cardiovascular system and blood disorders. Herein, the effect of AAL extract on blood serum insulin, weight loss, and lipid profile was evaluated in order to harness the pathogenesis or treat diabetes and its related complications.

The AAL extract demonstrated a noteworthy antioxidative property in the DPPH *in vitro* model of free radicals. The %inhibition was comparable to the standard drug gallic acid. Free radical scavenging plays a pivotal role in safeguarding against free radical-induced damage in various ailments, including hyperglycemia and this phenomenon has been observed for *Aloe vera* gel [24]. There is no report on the antioxidant potential of AAL extract

Table 5: Volatile profile for AAL extract based on GCMS analysis

S#	Compound	RT	Molecular formula	Molecular weight	Peak area (%)
1	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	25.5	$C_{20}H_{40}O$	296	7.12
2	Palmitic acid	27.32	$C_{16}H_{32}O_2$	256	16.76
3	Stearic acid	27.84	$C_{18}H_{36}O_2$	284	13.06
4	Phytol	30.31	$C_{20}H_{40}O$	296	6.26
5	Ethyl linolenate	31.76	$C_{20}H_{34}O_2$	306	7.7
6	Squalene	60.61	$C_{30}H_{50}$	410	18.32
7	γ-Tocopherol	63.42	$C_{28}H_{48}O_2$	416	6.62
8	Tetratetracontane	64.23	$C_{44}H_{90}$	618	100
9	Vitamin-E	64.53	$C_{29}H_{50}O_2$	430	18.68
10	β-Sitosterol	66.4	$C_{29}H_{50}O$	414	27.76
11	Lupeol	67.27	$C_{30}H_{50}O$	426	13.89

however, earlier investigations have underscored the antioxidant potential for a number of *Aloe* species. The results from this study are in line with these reports [25,26]. An effective antiglycation activity was observed for AAL extract where the %inhibition was comparable to the standard drug rutin. Albeit, the in-depth phytochemical investigation is needed to elaborate the phytochemical composition of AAL extract, previous studies suggest potent antioxidant and antiglycation activity for plants enriched with phenolics and flavonoids [27]. In addition, the hydroethanolic extract for the other species of Aloe revealed significant anti-glycation potential which supports the finding in the current study [27]. This suggests that AAL is a rich source of phenolic and flavonoid compounds.

For the safety profile of the AAL extract, the *in vivo* model showed no signs of mortality, adverse effect, or organ toxicity. The acute toxicity result is self-explanatory evidence for the safe oral administration of the AAL hydroethanolic extract in selected doses however, further chronic or long-term toxicity studies may help reveal the toxicity profile at large doses. This study developed *in vivo* diabetic mice models where the AAL effect was evaluated in normoglycemic, postprandial, and STZ-induced diabetic mice. The STZ-induced diabetic model carries the advantages of hyperglycemia induction which closely mimics human diabetes, [20,28] has extended half-life, low risk of ketosis, and lower mortality rate [29]. For the normoglycemic and postprandial mice models, a dose-dependent antihyperglycemic activity was observed where a profound effect was observed at 400 mg/kg of the AAL extract. For the STZ-induced mice model, the AAL extract demonstrated a significant ($P < 0.01$) dose-dependent reduction for BGLs as compared to the diabetic control group. The effect on BGLs was observed for all the doses of AAL, particularly starting from day 7 till day 21. Yet again, it is a first-time report regarding the antihyperglycemic effect of AAL extract in an *in vivo* mice model. The previous evidences suggest the potent hypoglycemic, antihyperglycemic, and glucose-suppressing effects in plants attributed to the presence of phenolics, flavonoids, saponins, and alkaloids [30]. These phytochemicals have the potential to safeguard the pancreatic β-cells, enhance insulin release and synthesis [70], and produce an anti-hyperglycemic effect [31]. Herein, the AAL extract also revealed an increase in serum insulin level which may be linked to the antihyperglycemic effect of AAL extract in normoglycemic mice. This suggests a significant link for the presence of one or more of these naturally occurring phenolics, flavonoids, saponins, and alkaloidal compounds. The findings in this study align with the previous literature reporting the antihyperglycemic effects for aloe species via stimulation of insulin release [18,29,32]. With regard to clinical and research context, oral glucose tolerance is considered a primary approach for assessing insulin secretion and resistance hence, an OGTT test was employed to validate the antihyperglycemic effect of AAL extract. The results declared a noteworthy enhancement for glucose tolerance at 100, 200, and 400 mg/kg of the AAL doses following 1, 2, and 4 h post-administration. This further supports the effect of AAL extract on the beta cells of the pancreas, rendering them more responsive to insulin, probably through the stimulation of PPAR-gamma or via extra-pancreatic mechanisms that enhance peripheral glucose consumption [33]. Further studies at molecular and receptor levels may help clarify the pharmacological and therapeutic basis for the antihyperglycemic mechanism of AAL extract and its phytochemicals.

The STZ-induced diabetic mice were subjected to weight loss activity. The effect on weight loss was assessed for 21 days where the AAL extract exhibited improved blood sugar control and safeguarded against weight reduction. The diabetic mice administered with AAL extract maintained their weight throughout the tested days as compared to the diabetic control mice. Keeping in mind the fact that STZ-triggered diabetes development results in heightened muscle wasting and protein depletion from tissues ultimately leading towards significant weight loss, the observed non-significant changes in weight may be attributed to the effect of AAL extract. The advancement in research elaborated on the state of diabetic dyslipidemia in diabetic patients, therefore this study included the antihyperlipidemic effects of AAL extract on the lipid profile of the diabetic mice. The results showed the potential for the AAL extract to maintain the BGLs along with a solid impact on the blood lipid profile in diabetic mice. Such phenomenon has been reported in numerous previous studies for Aloe species and our data is in agreement with these reports [34]. Interestingly, these studies also reported the presence of phenolics, flavonoids, and alkaloids responsible for the aforementioned activities. The current study used the leaves extract where the volatile components were expected hence, GCMS profiling was performed for the AAL extract. A total of eleven compounds were identified via GCMS; 3,7,11,15-tetramethyl-2-hexadecen-1-ol, palmitic acid, stearic acid, phytol, ethyl linolenate, squalene, γ-tocopherol, tetratetracontane, vitamin-E, β-sitosterol, and lupeol. Literature reports indicate alike volatile components in Aloe species such as palmitic acid, ethyl linolenate, and squalene [35,36]. It is noteworthy to mention the antihyperglycemic and antioxidant activity of phytol, and 3,7,11,15-tetramethyl-2-hexadecen-1-ol [37], palmitic acid [38], squalene [39], and lupeol [40]. The GCMS data for the AAL extract presented similar compounds in significant concentration, suggesting the correlation and possible role of these compounds in the antioxidant, antiglycation, anti-hypercholesteremic, and antihyperglycemic activities of AAL extract.

The data generated and analysed in this research proves the antioxidant, antiglycation, anti-hypercholesteremic, and antihyperglycemic effects of AAL extract in different *in vitro* and *in vivo* models due to the presence of volatile components. The potential applications for AAL extract urge the need for an in-depth phytochemical and metabolomic exploration to isolate the novel active moieties with mechanistic activities at the molecular level in order to establish the pharmacological role of these novel molecules.

5 Conclusion

The outcomes of this study demonstrate the effective use of AAL in improving oral glucose tolerance, hypoglycemia, and body weight. This corroborates the traditional uses of AAL for treating various ailments. As this species is yet to be explored, particularly in terms of phytochemistry, further research holds promise in establishing a robust foundation for an in-depth exploration in order to comprehensively grasp the mechanisms underlying the phytochemical in AAL. To harness these potentials effectively, the subsequent investigation is essential for isolating and identifying the bioactive compounds responsible for pharmacological activities. This may lead towards novel drug discovery with less adverse effects and possibly more therapeutic activity at low doses in diabetes.

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