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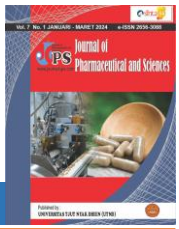

Integrating GC–MS Phytochemical Profiling and In Vivo Pharmacological Evaluation to Reveal the Antidiabetic Potential of the Ethanolic Extract of *Scleria sumatrensis* Retz. in Alloxan-Induced Diabetic Rats.

Integrasi Profil Fitokimia Berbasis GC–MS dan Evaluasi Farmakologis In Vivo dalam Menilai Potensi Antidiabetes Ekstrak Etanol *Scleria sumatrensis* Retz. pada Model Tikus Diabetes Induksi Aloksan.

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Abstract

This study evaluated the antidiabetic activity and phytochemical profile of the ethanolic extract of Scleria sumatrensis Retz. Using an alloxan-induced diabetic rat model. Diabetes was induced by a single intraperitoneal injection of alloxan (150 mg/kg BW) after a 12-hour fasting period, and fasting blood glucose was measured at three standardized time points: GD1 (baseline), GD2 (72 hours post-induction), and GD3 (day 14 post-treatment). GC–MS analysis revealed several major constituents, including ethyl α-D-glucopyranoside, ethyl linoleate, ethyl linolenate, phytol, tocopherol, and β-sitosterol, which are associated with improved insulin sensitivity, modulation of PPAR-γ–related pathways, antioxidant protection of pancreatic β-cells, and reduced intestinal carbohydrate digestion. Rats were assigned to negative control (vehicle), positive control (metformin 45 mg/kg BW), and extract-treated groups (75, 150, and 300 mg/kg BW). Percentage reduction from GD2 to GD3 was analyzed using one-way ANOVA followed by Tukey's post-hoc test. The extract produced a significant and dose-dependent decrease in fasting glucose ($p < 0.001$). All extract doses differed significantly from the negative control, and the 300 mg/kg dose demonstrated glucose-lowering efficacy comparable to metformin. These findings indicate that Scleria sumatrensis possesses vigorous antihyperglycemic activity consistent with its lipophilic phytochemical composition. Further studies are required to verify the underlying mechanisms and identify the most active constituents.

***Keywords*: Scleria sumatrensis; Antidiabetic Activity; Alloxan-Induced Diabetes; GC–MS Analysis; Phytochemical Profiling.**

Abstrak

Penelitian ini mengevaluasi aktivitas antidiabetes dan profil fitokimia ekstrak etanol *Scleria sumatrensis* Retz. menggunakan model tikus diabetes induksi aloksan. Diabetes diinduksi dengan injeksi intraperitoneal aloksan (150 mg/kgBB) setelah puasa 12 jam, dan kadar glukosa darah puasa diukur pada GD1 (sebelum induksi), GD2 (72 jam setelah induksi), dan GD3 (hari ke-14 perlakuan). Analisis GC–MS mengidentifikasi beberapa senyawa utama, termasuk ethyl α-D-glucopyranoside, ethyl linoleate, ethyl linolenate, phytol, tokoferol, dan β-sitosterol, yang diketahui berperan dalam peningkatan sensitivitas insulin, modulasi jalur PPAR-γ, perlindungan antioksidan terhadap sel β pankreas, serta penghambatan pencernaan karbohidrat. Tikus dibagi menjadi kelompok kontrol negatif (vehicle), kontrol positif (metformin 45 mg/kgBB), dan perlakuan ekstrak (75, 150, dan 300 mg/kgBB). Persentase penurunan glukosa dari GD2 ke GD3 dianalisis

menggunakan ANOVA satu arah dan uji lanjut Tukey. Ekstrak menunjukkan penurunan glukosa yang signifikan dan bergantung dosis ($p < 0.001$). Semua dosis ekstrak berbeda signifikan dari kontrol negatif, dan dosis 300 mg/kgBB menunjukkan efektivitas sebanding dengan metformin. Hasil ini menunjukkan bahwa *Scleria sumatrensis* memiliki aktivitas antihiperglikemia yang kuat, konsisten dengan komposisi fitokimianya. Studi lanjutan diperlukan untuk memverifikasi mekanisme dan mengidentifikasi senyawa aktif utamanya.

Kata Kunci: Scleria sumatrensis; Aktivitas Antidiabetes; Diabetes Aloksan; Analisis GC–MS; Profil Fitokimia.

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Introduction

Diabetes mellitus (DM) is a metabolic disorder with a rising global prevalence, presenting a significant rise to 643 million by 2030[2]. In Indonesia, DM is the third leading cause of death, with prevalence growing annually; the 2018 Basic Health Research (Riskesdas) indicated a diabetes prevalence of 10.9%, which rose to 13.5% in 2021[3]. The high costs of diabetes management place considerable strain on the healthcare system, emphasizing the urgent need for safer, more effective, and affordable treatment options.

Medicinal plants like Scleria sumatrensis Retz. from the Cyperaceae family offer promising antidiabetic agents due to their bioactive compounds. This plant has been traditionally used for various health issues, including diabetes, in West and North Kalimantan[4], vaginal discharge in Central Kalimantan[5], and other conditions across Indonesia. Qualitative phytochemical screenings have identified phenolic compounds, flavonoids, tannins, alkaloids, steroids, and terpenoids, along with antioxidant, antibacterial, and antifungal activities[6][7]. However, a comprehensive quantitative phytochemical analysis using Gas Chromatography-Mass Spectrometry (GC-MS) to identify secondary metabolites has not been conducted.

The hypoglycemic activity of S. sumatrensis has not been evaluated in vivo using diabetic animal models. This study aims to investigate the antidiabetic potential of S. sumatrensis through scientific methods, promoting the development of herbal medicines based on local knowledge. Phytochemical screening will be performed using GC-MS, a precise technique for identifying organic compounds, allowing separation based on volatility and identification of chemical structures[8]. By employing GC-MS, we aim to accurately identify secondary metabolites with potential antidiabetic properties.

Previous ethnobotanical studies have confirmed the use of S. sumatrensis for the treatment of diabetes and other health conditions. However, quantitative phytochemical screening using GC-MS and in vivo hypoglycemic activity testing are limited in Indonesia. Therefore, this study was conducted to bridge this gap by integrating GC–MS phytochemical profiling with in vivo antidiabetic evaluation of the ethanolic extract of Scleria sumatrensis. The findings are expected to contribute significantly to public health challenges[1]. The International Diabetes Federation (IDF) reported that the number of adults diagnosed with diabetes increased from 537 million in 2021 to 547 million in 2022, with projections suggesting the development of new herbal medicines for diabetes management and supporting the preservation of local healthcare traditions.

Experimental Section

This research is an experimental study conducted in a laboratory using a Completely Randomized Design (CRD) method. A total of 15 male Wistar rats (Rattus norvegicus), weighing 180–220 g, were used in this study. They were used as test animals, and all procedures obtained ethical approval from the Animal

Ethics Commission of Sam Ratulangi University (KEH UNSRAT). The study included five treatment groups: one positive control, one negative control, and three groups given extracts, each with three repetitions. The entire research series was carried out in three main stages. The negative control group consisted of diabetic rats receiving only the vehicle solution. The vehicle used to solubilize the extract was distilled water, administered at the same volume as in the treatment groups. The positive control group received metformin (45 mg/kg BW), while the treatment groups received the ethanolic extract of Scleria sumatrensis at doses of 75, 150, and 300 mg/kg BW. The absence of a normal control group is acknowledged as a limitation of this study.

Research instruments and materials

Rotary Evaporator, Measuring Cup, Blood Sugar Strips, Dispo, Wing Needle, Glucometer (Autocheck), Volumetric Flask, Analytical Balance, Mortar and Pestle, Pipette, Jar, Spatula, Filter Paper, Separatory Funnel, Stationery. *Scleria Sumatrensis* Retz. Plants collected from Kiawa 2 Village, Minahasa, Alloxan, white mice, NaCl, Metformin, 95% Ethanol, Aquadest.

First Stage:

a. Sample Preparation

Samples of *Scleria sumatrensis* Retz grass were collected in Kiawa Dua Village, Minahasa Regency, North Sulawesi. All parts of *S. sumatrensis* Retz (roots, stems, fruit, and leaves) were cleaned of dirt, washed in running water, air-dried, and then chopped. The chopped leaves were then weighed, and the sample was extracted using the maceration method.[7].

b. Extraction of Bioactive Compounds

Approximately 800 grams of the crude drug was weighed, placed in a jar, and macerated using 95% ethanol for 3 x 24 hours, stirring occasionally. Remaceration was then carried out for 2 x 24 hours, stirring occasionally. The maceration results from both filtrates were then filtered using filter paper to obtain the macerate. The extract was then evaporated in a rotary evaporator at 40ºC to remove the solvent, resulting in a viscous extract[7].

Second Stage:

a. Phytochemical Analysis with GC-MS

Before GC-MS analysis, the extract was prepared through derivatization using BSTFA (N, O-bis(trimethylsilyl)trifluoroacetamide) to increase the volatility of non-volatile compounds. This process was carried out by adding BSTFA to the extract and incubating it at 60 °C for 30 minutes. The sample was then injected into the GC-MS system using a non-polar capillary column measuring 30 m × 0.25 mm × 0.25 µm. Operating conditions included an initial temperature gradient of 50°C (hold for 2 minutes), increasing at 10°C/minute to 250°C (hold for 5 minutes). Helium gas was used as the carrier gas with a flow rate of 1 mL/min, and sample injection was performed in splitless mode with a volume of 1 µL. The MS detector was operated in scan mode with an m/z range of 50–500 to detect the mass spectrum of each compound. GC-MS results comprehensively identified the chemical composition of the extract, including key compounds such as flavonoids, alkaloids, phenolics, and steroids that have potential antidiabetic activity[9][10][11].

b. Hypoglycemic Activity Test

Rats were fasted for 12 hours before alloxan induction, with free access to water. Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate at 150 mg/kg BW, freshly prepared in cold distilled water immediately before administration, and protected from light to minimize degradation. Fasting blood glucose was measured 72 hours post-induction (GD2). Only rats with fasting glucose levels above 140 mg/dL were included in the study. No animals were excluded or died during the induction process.

Scleria sumatrensis Retz extract was administered orally using a tube at doses of 75 mg/kgBW (P3), 150 mg/kgBW (P4), and 300 mg/kgBW (P5). The positive control (P2) used metformin at a dose of 500 mg/kg BW, administered orally via a gastric tube. Scleria sumatrensis Retz extract was administered orally using a tube at doses of 75 mg/kgBW (P3), 150 mg/kgBW (P4), and 300 mg/kgBW (P5). The positive control (P2) used metformin at a dose of 500 mg/kgBW orally using a gastric tube[12].

Blood glucose measurement

Blood glucose levels were measured 3 days after the injection. Blood was drawn through the tail by cleaning the tip of the tail with ethanol. Blood was then drawn intravenously by inserting a wing needle into

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a vein in the rat's tail, which had been cleaned and massaged to induce blood flow. After blood flow was measured, blood glucose was measured using a glucometer with a blood glucose stick. The monitor screen would light up when the blood glucose stick was inserted, indicating it was ready for injection. The blood glucose test was performed by touching the tip of the strip to the blood drop, which would then enter the test area. Once the test area was filled, the meter would beep and calculate the blood glucose level, and the Reading would be obtained within 5 seconds.

The first blood glucose level measurement was performed after the animal had fasted for 8 hours, or before alloxan administration (Gd1). The second measurement was performed after the alloxan injection on the 3rd day (Gd2), and then on the 14th day after treatment (Gd3)[12].

Calculation of blood glucose level reduction

The percentage reduction in blood sugar levels is calculated using the following formula: Percentage reduction in blood sugar levels $= = \frac{Gd2-Gd3}{Gd3} x100\%$

Description:
Gd2: Blood sugar after administration of alloxan preparation.
Gd3: Blood sugar after administration of test preparation.[12]

Monitoring of Body Weight and Food/Water Intake

Body weight, food intake, and water intake of all rats were monitored throughout the 14-day experimental period. Measurements were recorded on Day 1 (baseline GD1), Day 3 (post-alloxan GD2), on the 7th day (to assess the trend in decreasing blood sugar levels), and on Day 14 (GD3). Monitoring these parameters ensured that no confounding physiological changes, such as dehydration, excessive weight loss, or abnormal feeding behavior, interfered with the interpretation of blood glucose outcomes.

Stage Three:

a. Phytochemical Data Analysis:

GC-MS results were analyzed using NIST Library software to identify compounds. Compound profiles are presented in a table with compound name, retention time, and relative area percentage.[13]

b. Statistical Analysis

All data were presented as mean ± SD or mean ± SEM. A paired-samples t-test was used to compare blood glucose levels between day 3 (PRE_KGDH3) and day 14 (POST_KGDH14) to evaluate the overall effect of treatment following alloxan induction. Homogeneity of variance was assessed using Levene's test before performing one-way ANOVA to compare fasting blood glucose among treatment groups at day 14. When the ANOVA showed significant differences, Tukey's HSD post-hoc test was applied to identify pairwise group differences. A significance level of $p < 0.05$ was used for all analyses.[12].

Results and Discussion

Phytochemical Profile of Scleria sumatrensis Extract Based on GC–MS Analysis

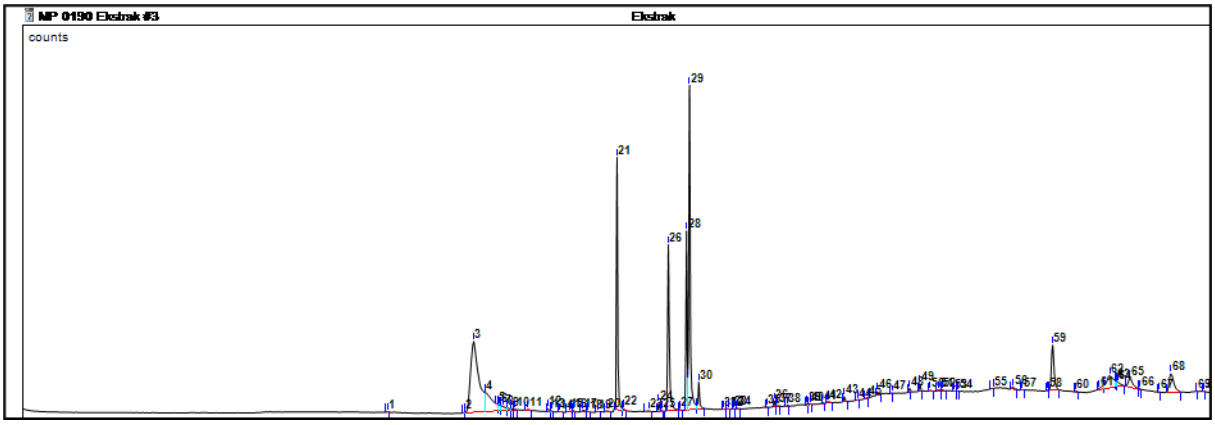

Figure 1. Chromatogram GC-MS

GC–MS analysis of the ethanol extract of Scleria sumatrensis identified thirteen major compounds with peak areas ≥ 0.5%, consisting of glycosides, saturated and unsaturated fatty acids, terpenoids, tocopherols,

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and phytosterols. The compound with the highest concentration was ethyl α-D-glucopyranoside (21.92%), followed by ethyl 9,12,15-octadecatrienoate (19.27%), hexadecanoic acid ethyl ester (12.21%), phytol (10.36%), and ethyl linoleate (7.70%). Other components, such as vitamin E (4.54%), β-sitosterol (3.41%), and several ethyl iso-allocholate derivatives (~5.5%), also contributed to the extract's phytochemical profile.

The presence of unsaturated fatty acid esters, particularly ethyl linoleate and ethyl linolenate, has pharmacological relevance because these two compounds belong to the extract, which contains polyunsaturated fatty acids (PUFAs), known to modulate insulin sensitivity, reduce inflammation, and influence the GLUT-4 expression pathway[14][15]. These components are plausible contributors to the extract's antidiabetic potential.

Table 1. Major Compounds of the Ethanolic Extract of *Scleria sumatrensis* Identified by GC–MS

RT	Name	Formula	MW	% Area	Area
14.119	Ethyl a-d-glucopyranoside	C8H16O6	208.0	21.916	13209326
14.374	Ethyl a-d-glucopyranoside	C8H16O6	208.0	5.69	3429335
14.792	d-Mannose	C6H12O6	180.0	0.749	451523
17.34	Hexadecanoic acid, ethyl ester	C18H36O2	284.0	12.212	7360306
18.489	Phytol	C20H40O	296.0	10.36	6244366
18.897	9,12-Octadecadienoic acid, ethyl ester	C20H36O2	308.0	7.697	4639085
18.965	Ethyl 9,12,15-octadecatrienoate	C20H34O2	306.0	19.267	11612447
19.173	Heptadecanoic acid, 15-methyl-, ethyl ester	C20H40O2	312.0	1.56	940495
27.118	Vitamin E	C29H50O2	430.0	4.544	2738561
28.4	Ethyl iso-allocholate	C26H44O5	436.0	3.355	2022016
28.57	Ethyl iso-allocholate	C26H44O5	436.0	0.569	342714
28.856	Ethyl iso-allocholate	C26H44O5	436.0	1.61	970642
29.774	β-Sitosterol	C29H50O	414.0	3.412	2056185

Phytol, a linear diterpenoid present at 10.36%, has been reported to possess antioxidant properties.[16][17][18] and hepatoprotective activity[19][20], as well as the ability to suppress gluconeogenesis by modulating key carbohydrate metabolism enzymes. Meanwhile, vitamin E (tocopherol) acts as a potent lipophilic antioxidant, protecting against oxidative stress in pancreatic β-cells[21]A mechanism highly relevant in alloxan-based diabetes models.

Phytosterols such as β-sitosterol and its derivative ethyl iso-allocholate play a key role in antihyperglycemic activity through several pathways: inhibition of α-glucosidase, reduction of intestinal glucose absorption, and increased insulin sensitivity in peripheral tissues[22][23]. This activity has been supported by in vivo studies in various animal models. The combination of PUFAs, phytosterols, and terpenoids gives S. sumatrensis extract a chemical profile consistent with plants with antidiabetic activity. Chemotaxonomically, the detected metabolite pattern resembles that of several Scleria species and other members of the Cyperaceae family, which are reported to be rich in sterols, PUFAs, and terpenoids. This strengthens S. sumatrensis's position as a previously understudied species with significant phytopharmacological potential.

The GC–MS profile of Scleria sumatrensis extract revealed the presence of bioactive compounds relevant to antidiabetic activity, including PUFA esters, terpenoids, tocopherols, and phytosterols. Therefore, in vivo testing was conducted to determine the extent to which these phytochemicals improve hyperglycemia in an alloxan-induced rat model.

Antidiabetic activity of Scleria sumatrensis extract in alloxan-treated diabetic rats.

Alloxan induction successfully induced a stable hyperglycemic state in all test animals. This was demonstrated by a sharp increase in blood glucose levels from expected values (GD1: 80–100 mg/dL) to 150–400 mg/dL on the third day (GD2). The negative control group, which received only distilled water, remained in severe hyperglycemia (100 → 407 → 377 → 465 mg/dL), even increasing on day 14, indicating that alloxan caused sustained damage to pancreatic β-cells.

In contrast, the positive control group, which received metformin, showed a highly significant decrease in glucose (276 → 97 mg/dL), with a reduction of 64.85%, thereby confirming the validity of the animal model and the response to standard medication.

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Administration of Scleria sumatrensis ethanol extract showed a dose-response pattern of glucose reduction. At a dose of 75 mg/kgBW, the glucose reduction was relatively small (156 → 122 → 142 mg/dL) and unstable until day 14, with a total reduction of only 8.97%. This indicates that this dose is insufficient to provide a significant pharmacological effect on alloxan-induced hyperglycemia.

Table 2. Fasting Blood Glucose Levels of Experimental Groups (mg/dL)

Group	GD1(Day 1)	GD2(Day 3)	GD3(Day 14)	Mean ± SD	% Reduction
Extract 75 mg/kg	88	156	142	142 ± 18.2	8.97%
Extract 150 mg/kg	87	196	115	115 ± 21.5	41.32%
Extract 300 mg/kg	101	297	116	116 ± 12.9	60.94%
Positive Control	91	276	97	97 ± 15.2	64.85%
Negative Control	100	407	465	465 ± 18.5	-14.25%

The 150 mg/kgBW dose showed a more pronounced response, with a consistent reduction from 196 mg/dL to 115 mg/dL on day 14, representing a 41.32% reduction. This response indicates that at moderate doses, the extract begins to exhibit relevant biological potential. The highest efficacy was achieved at a dose of 300 mg/kgBW, which showed a 60.94% reduction in glucose despite this group having the highest initial hyperglycemia (297 mg/dL on GD2). This value is nearly equivalent to that of the metformin group (64.85%), indicating that Scleria sumatrensis extract at high doses exhibits vigorous antidiabetic activity.

Although ethyl α-D-glucopyranoside had the highest peak area in the chromatogram, GC–MS abundance does not necessarily reflect pharmacological potency. Literature indicates that PUFA esters (e.g., ethyl linoleate, ethyl linolenate) and phytosterols such as β-sitosterol may exert strong antihyperglycemic effects even at lower relative abundance. A basic drug-likeness assessment using Lipinski's criteria suggests that β-sitosterol has favorable physicochemical properties for oral activity. At the same time, PUFA esters exceed molecular weight thresholds but remain bioactive as lipid mediators. Thus, contributions of individual compounds remain inferential and would require further validation through molecular docking, fractionation, or enzyme-based assays. The PPAR-γ pathway, a key regulator of adipocyte differentiation and glucose homeostasis, thereby contributing to increased tissue glucose uptake and improved insulin response[30][31]. The overall combination of these mechanisms is very likely the pharmacological basis for the significant reduction in blood glucose, especially at a dose of 300 mg/kgBW, which shows effectiveness approaching that of metformin.

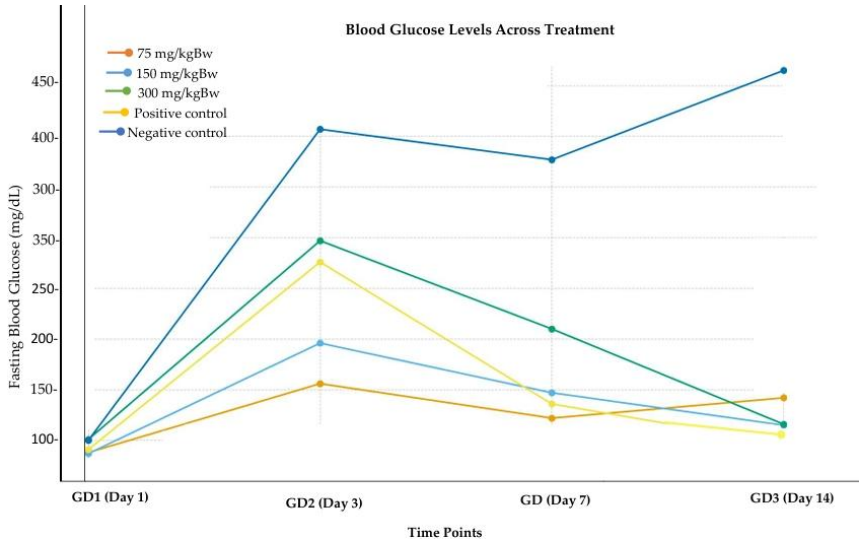

Figure 2. Blood Glucose Curve Across Treatment Groups

Throughout the study, body weight, food intake, and water intake remained relatively stable across all groups. Although a slight decrease in body weight was observed in diabetic rats after alloxan induction (GD3), values stabilized following treatment. No significant differences in food or water intake were observed between groups, indicating that changes in blood glucose were attributable to treatment effects rather than physiological disturbances.

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Comparative Analysis with Previous Studies on Cyperaceae or Related Species

The finding of the antidiabetic activity of Scleria sumatrensis extract is consistent with several previous reports on members of the Cyperaceae family that demonstrated pharmacological potential through similar mechanisms. Several studies on other species in the Scleria genus, such as Scleria lithosperma, revealed the presence of phytosterols, unsaturated fatty acids, and terpenoid compounds that contribute to anti-inflammatory and antioxidant activities. This phytochemical profile is similar to the main components detected in this study, including β-sitosterol, phytol, ethyl linoleate, and ethyl linolenate, which are known to be involved in mechanisms that improve insulin sensitivity, inhibit carbohydrate digestive enzymes, and protect β-cells[32][33]. Furthermore, several reports on other Cyperaceae species, such as Cyperus rotundus and Cyperus esculentus, have demonstrated antihyperglycemic activity in diabetic animal models, with mechanisms related to increased glucose uptake, PPAR-γ modulation, and reduced oxidative stress[34][35][36][37]. The similarity in lipophilic metabolite content patterns and biological responses between S. sumatrensis and other Cyperaceae species indicates a consistent chemotaxonomic basis, where this family tends to produce compounds relevant to the management of hyperglycemia. Thus, the results of this study not only complement the limited literature on the genus Scleria but also strengthen the hypothesis that members of the Cyperaceae family have potential as a source of phytochemical candidates for antidiabetic therapy.

Statistical Analysis

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	292723.067	4	73180.767	125.985	.000
Within Groups	5808.667	10	580.867		
Total	298531.733	14			

Treatment effects were instead analyzed using one-way ANOVA followed by Tukey's post-hoc test. The ANOVA demonstrated a highly significant treatment effect on GD3 glucose levels ($F(4,10) = 125.985$, $p < 0.001$). Tukey's post-hoc test showed statistically significant differences between the negative control and all extract-treated groups (75 mg/kg, 150 mg/kg, and 300 mg/kg), with p-values <0.001. The 300 mg/kg group did not differ significantly from the metformin group ($p > 0.05$), indicating comparable antihyperglycemic efficacy. The 75 mg/kg group differed substantially from the 300 mg/kg group ($p < 0.01$), confirming a precise dose–response relationship.

This study has several limitations. First, acute toxicity and safety assessments were not conducted, limiting conclusions regarding dose tolerability. Second, biomarkers such as serum insulin, lipid profile, oxidative stress markers, and liver enzymes were not measured, reducing mechanistic strength. Third, the alloxan-induced diabetes model represents an acute β-cell cytotoxic model and does not fully mimic type 2 diabetes. Future studies should employ chronic models, perform molecular docking, assess histopathology, and isolate or fractionate active compounds. This study has several limitations. First, acute toxicity and safety assessments were not conducted, limiting conclusions regarding dose tolerability. Second, biomarkers such as serum insulin, lipid profile, oxidative stress markers, and liver enzymes were not measured, reducing mechanistic strength. Third, the alloxan-induced diabetes model represents an acute β-cell cytotoxic model and does not fully mimic type 2 diabetes. Future studies should employ chronic models, perform molecular docking, assess histopathology, and isolate or fractionate active compounds.

Conclusions

The ethanolic extract of *Scleria sumatrensis* demonstrated significant antihyperglycemic activity in alloxan-induced diabetic rats. GC–MS profiling revealed bioactive constituents such as polyunsaturated fatty acid esters, phytol, tocopherol, and β-sitosterol, which collectively support mechanisms that improve insulin sensitivity, suppress intestinal carbohydrate digestion, provide antioxidant protection for pancreatic β-cells, and modulate glucose homeostasis pathways. In vivo evaluation showed an apparent dose-dependent effect, with the 300 mg/kgBW dose producing a glucose-lowering response comparable to metformin and the 150 mg/kgBW dose demonstrating moderate efficacy. These findings indicate that *Scleria sumatrensis* possesses strong potential as a natural source of antidiabetic agents and warrant further investigation through

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biomarker assays, histopathological evaluation, and fractionation studies to identify the most active chemical constituents.

Conflict of Interest

The authors declare that there are no financial, personal, or organizational conflicts of interest that could inappropriately influence the work reported in this manuscript. All experimental procedures, data analyses, and interpretations were conducted independently without external bias.

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