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Methanolic extract of *Tillandsia recurvata* reduces blood glucose, triglycerides and cholesterol levels

Abraham H. García-Campoy[1], Rosa M. Pérez Gutiérrez[2], Efrén V. García Báez,[3] and Alethia Muñiz-Ramirez[4*]

[1] Facultad de Ciencias-Universidad Autónoma de San Luis Potosí, San Luis Potosí, Mexico.
[2] Laboratorio de Investigación de Productos Naturales, Escuela Superior de Ingeniería Química e Industrias Extractivas, Unidad Profesional Adolfo López Mateos, Instituto Politécnico Nacional, Mexico.
[3] Laboratorio de Química Supramolecular y Nanociencias, Instituto Politécnico Nacional, Ciudad de México, Mexico.
[4] CONAHCYT-IPICYT/División de Materiales Avanzados, San Luis Potosí, Mexico.
*Corresponding author: alethia.muniz@ipicyt.edu.mx

Abstract

Background: *Tillandsia recurvata* collected in San Luis Potosí does not have studies focused on its use as an adjuvant in treating diabetes mellitus.

Questions and / or Hypotheses: Will *Tillandsia recurvata L*. (Bromeliaceae) have antidiabetic activity *in vitro* and *in vivo*?

Studied species: *Tillandsia recurvata* L. (Bromeliaceae)

Study site and dates: *T. recurvata* was collected in Guadalcázar municipality, San Luis Potosí, Mexico, in December 2021.

Methods: The antidiabetic potential of *Tillandsia recurvata* methanol extract (TRM) was evaluated using *in vitro* and *in vivo* models, and its secondary metabolite content was analyzed using Gas chromatography-mass spectrometry.

Results: Results demonstrate that extract reduces blood glucose, triglyceride, and cholesterol levels *in vivo*. In addition, *in vitro* tests showed that extract diminished the formation of advanced glycation end products, methylglyoxal concentrations, and glycosylated hemoglobin levels. Gas chromatography-mass spectrometry analysis identified several compounds in the extract, including 2-methylbenzaldehyde, 4-hydroxy-2-methylacetophenone, 3',5' dimethoxyacetophenone, pentanoic acid, palmitic acid, linoleic acid, phytol, margaric acid, oleamide, cis-11-eicosenamide, stearic acid, 13-docosenamide, (Z), campesterol, and β-sitosterol.

Conclusions: These results highlight the potential of *T. recurvata* collected in San Luis Potosi as an adjuvant in treatment of diabetes mellitus.

Keywords: Advanced glycation end products, antidiabetic effect, *Tillandsia recurvata*, zebrafish.

Resumen

Antecedentes: *Tillandsia recurvata* colectada en San Luis Potosí, no cuenta con estudios enfocados a su uso como coadyuvante en el tratamiento de la diabetes mellitus.

Preguntas y / o Hipótesis: ¿*Tillandsia recurvata* L. (Bromeliaceae), tendrá actividad antidiabética *in vitro* e *in vivo*?

Especies de estudio: *Tillandsia recurvata* L. (Bromeliaceae)

Sitio y años de estudio: *T. recurvata* fue colectada en el municipio de Guadalcazar, San Luis Potosí, México, en diciembre 2021.

Métodos: Se evaluó el potencial antidiabético del extracto de metanol de *Tillandsia recurvata* (TRM) en modelos *in vitro* e *in vivo* y se realizó un perfilamiento de los metabolitos secundarios presentes mediante cromatografía de gases acoplada a espectrometría de masas.

Resultados: Los resultados demuestran que el extracto reduce eficazmente los niveles de glucosa, triglicéridos y colesterol en sangre *in vivo*. Además, las pruebas *in vitro* indican que el extracto reduce la formación de productos finales de glicación avanzada, así como la concentración de metilglioxal y los niveles de hemoglobina glicosilada. El análisis por cromatografía de gases acoplada a espectrometría de masas identificó varios compuestos presentes en el extracto, incluidos: 2-metilbenzaldehído, 4-hidroxi-2-metilacetofenona, 3',5'-dimetoxiacetofenona, ácido pentanóico, ácido palmítico, ácido linoleico, fitol, ácido margárico, oleamida, cis- 11-Eicosenamida, ácido esteárico, 13-Docosenamida, (Z), campesterol y β-sitosterol.

Conclusiones: Los resultados obtenidos resaltan el potencial de *T. recurvata* recolectada en San Luis Potosí como coadyuvante en el tratamiento de la diabetes mellitus, debido a sus propiedades antidiabéticas.

Palabras clave: Efecto antidiabético, productos finales de glicación avanzada, *Tillandsia recurvata,* pez cebra.

Diabetes mellitus (DM), more commonly known as diabetes, can result from the pancreas not producing enough insulin, an insulin deficiency (type 1), the cells not responding properly to insulin, or an insulin resistance (type 2). Its secondary conditions are related to arterial, cardiac, central nervous system, kidney, and eye problems, among others. Type 1 diabetes is a chronic condition; while type 2 diabetes is preventable and treatable, the latter being the most prevalent.

In 2021, there were 537 million diabetics and by 2045 that figure is projected to reach 783 million worldwide (International Diabetes Federation 2019). It is estimated to affect 90-95 % of mainly adult patients, although in recent years the number of cases in infants has also increased. Increases in type 2 diabetes cases are primarily associated with lifestyle choices, poor diet, and a sedentary lifestyle (Saeedi *et al*. 2019). DM is caused by a decreased, resistant, or null production of insulin caused by a malfunction of the pancreas, which in turn causes the appearance of hyperglycemia.

Increased blood glucose is responsible for the development of a series of affectations at the microvascular and macrovascular level of organs, as well as inducing the formation of Advanced Glycation End products (AGEs). These are a series of compounds formed by the interaction of proteins, lipids and/or nucleic acids with the sugars present in the body's blood plasma (Dariya & Puranchandra 2020).

This type of molecules is generated from non-enzymatic reactions of glycation and oxidation called Maillard reactions, and their accumulation is related to a developing diabetic pathology (atherosclerosis, cardiovascular, nephropathy, neuropathy and retinopathy) (Piarulli *et al*. 2013). The formation of AGEs can be divided into three stages: early, intermediate, and final.

In the early stage, Shiff bases are formed, in the intermediate stage dicarbonyl compounds such as glyoxal and methylglyoxal (MG) are created, which are generated by crosslinking and alkylation reactions with proteins (Wu *et al*. 2011), these products are known as Amadori products, and in the final stage the AGEs are generated.

Evidence shows that overweight or obese people are prone to developing type 2 diabetes. In dyslipidemia, elevated levels of triglycerides (> 150 mg/dL) and cholesterol (> 200 mg/dL) are present, or hypertriglyceridemia (> 500 mg/dL) may also develop accompanied due to low cholesterol levels (< 40 mg/dL), a condition known as atherogenic dyslipidemia (Núñez-Cortés & Pedro-Botet 2021).

On the other hand, the development and investigation of the use of new and innovative treatments for the care and control of diabetes is in a constant process of exploration, which has led to the use of model organisms (mice, rabbits, rats, fruit fly, etc.) that allow evaluating the effects on biological processes (Veldman & Lin 2008). Among them is the zebrafish (*Danio rerio*); which can be reproduced in the laboratory, maintenance is easy and low cost, it is considered ideal for investigating genetic, immunological, nutritional, physiological, and behavioral alterations.

Likewise, it has been implemented as a model for the study of acute lymphoblastic leukemia, polycystic kidney disease, Duchenne muscular dystrophy, human melanoma, nephronophthisis, acute kidney injury, Parkinson's disease, Huntington's disease, Alzheimer's disease, myocardial infarction, and some metabolic diseases (Teame *et al*. 2019) such as diabetes mellitus, since it presents physiological processes and functions similar to the pancreas and glucose regulation (Zang *et al*. 2018) Likewise, it is estimated that about 70 % of zebrafish genes have similarities with humans (Russo *et al*. 2022).

Regarding therapeutic treatments for diabetes control other than insulin, there is a wide variety of oral drugs (metformin, thiazolidinediones, glucagon-like peptide-1, receptor agonists, dipeptidyl peptidase-4, sodium glucose cotransporter 2, inhibitors, amylin analogs, α-glucosidase inhibitors, sulfonylureas, and meglitinides) (Pappachan *et al*. 2019), which can be used individually or in combination to help mitigate the disease. Additionally, it is common for diabetics to use herbal medicine and ethnobotany as an alternative remedy to help control DM.

The use of traditional medicine has a long tradition in diverse cultures. The knowledge, exploitation and use of natural products has contributed to consider it an alternative source in the search for new therapeutic agents that are beneficial to health. For this reason, there are many publications where studies and results of the therapeutic activities of natural extracts or bioactive molecules capable of providing a beneficial effect in the regulation of diabetic complications have been reported (Mollica *et al*. 2018, Salas-Salvadó *et al*. 2019, Pop *et al*. 2021).

T. recurvata is a plant that belongs to the *Tillandsia* genus of the Bromeliaceae family and is characterized by a high tolerance to drought. It grows in the aerial zones of trees and in extreme places and has a high adaptation capacity and a physiological resistance, which has allowed it to grow in urban, rural, and wild areas. Its size is between 10 to 12 cm in diameter, and it is a competitive plant (Piazzetta *et al*. 2019).

T. recurvata can be found from the north of Mexico to the south of Latin America and is popularly known as heno, heno chico, heno motita, gallinita, viejito, clavel del aire, or ball moss. In traditional medicine it is used as an ocular antimicrobial, antispasmodic, and antipyretic problems, as well as an antiinflammatory and headache treatment (Manetti *et al.* 2009). Likewise, its anticancer (Lowe *et al*. 2020), antiinflammatory and antitumor (Lowe 2010). Its antimicrobial activity has also been evaluated, showing effectiveness against *Pseudomonas aeruginosa* (Paz *et al*. 1995). Furthermore, its antidiabetic activity has been reported in rats fed with extracts of this species, in which a significant reduction in fasting glucose, fructosamine, and insulin levels was observed (Lowe *et al.* 2014). Regarding phytochemical information, it is known that the *Tillandsia* genus presents a wide variety of phytoconstituents, including flavonoids, steroids, terpenes, triterpenes, cinnamic acid derivatives and phenolic compounds (de Vasconcelos *et al*. 2013).

Cabrera & Seldes 1995 isolated the 25-hydroperoxycycloart-23-en-3ß-ol, and an epimeric mixture of 24-hydroperoxycycloart-25-en-3ß-ol, from the ethanolic extract of *T. recurvata,* and identified known compounds such as cycloartanone, cycloartenone, 24-methylenecycloartanone, cycloartanol, cycloartenol, lanostenol, lanosterol, and 24-ethylcholest-4-en-3-one and identified known compounds such as cycloartanone, cycloartenone, 24-methylenecycloartanone, cycloartanol, cycloartenol, 24-methylenecycloartanol, lanostenol, lanosterol, and 24-ethylcholest-4-en-3-one. Compounds such as 5,39-Dihydroxy-6,7,8,49-tetramethoxyflavanone, 1,3-di-O-cinnamoylglycerol and the ethyl ester of caffeic acid have also been isolated (de Queiroga *et al.* 2004)

The objective of this work is to evaluate the hypoglycemic, antiglycation and antidyslipidemic activity of TRM *in vitro* and *in vivo* models.

Materials and methods

Conditioning of raw material and obtaining of Tillandsia recurvata *methanol extract* (*TRM). T. recurvata* was collected in the municipality of Guadalcázar, San Luis Potosí (22° 37′ 0″ N, 100° 24′ 0″ W), a sample of the specimens was analyzed of Beatriz González in the herbarium of the Universidad Autónoma Metropolitana (specimen number 4983).

The plant was dried in an air current oven at 37 °C, then macerated for 7 days, using 1 kg of plant in 3 L of methanol. After extraction, the solution was filtered, and the solvent was completely removed with a rotary evaporator (Buchi R-100) under reduced pressure, obtaining 200 g of extract. TRM was refrigerated at 4 °C until use (Franco *et al.* 2018).

Protein glycation using the bovine serum albumin (BSA) -glucose model. The method reported by Huang *et al.* was used with some modifications (Huang *et al.* 2019). 30 mg/mL of glucose (20 mM), phosphate buffer (pH 7.0), 1 mM sodium azide and TRM were placed in a glass tube at different concentrations (0.30, 0.60, 1.2, 2.5 and 5 mg/mL). The mixture was incubated at 37 °C for 1, 2, 3, and 4 weeks, aminoguanidine (5 mg/mL) was used as a pharmacological control.

The formation of glycated BSA was determined in a fluorometer at an excitation wavelength of 355 nm and emission of 460 nm (GENios, TECAN). Assays were performed in triplicate.

BSA-methylglyoxal assay. The Justino method was used, with some modifications (Justino *et al.* 2018) MG (29 µmol/mL), sodium azide (1 mM/mL), TRM (0, 0.30, 0.60, 1.2, 2.5 and 5 mg/mL), a buffer solution of phosphates (pH 7.0), and 50 mg/ mL of BSA were placed in a test tube. The mixture was incubated for 7 days at 37 °C. Subsequently, 20 % trichloroacetic acid was added to the mixture and it was centrifuged at 10,000 rpm for 10 minutes. The pellet was resuspended in phosphate buffer and fluorescence intensity was measured (excitation wavelength of 355 nm and emission of 460 nm) (GENios, TECAN). Aminoguanidine (5 mg/mL) was used as a pharmacological control. Assays were performed in triplicate.

Glycation of hemoglobin. This test is used as a marker that reflects average glucose levels, and it is currently recognized as the most reliable indicator for the evaluation of retrospective glycemic control and the planning of clinical treatment (Selvaraj *et al*. 2008). Was using the method of Kazemi *et al.* (2019) with some modifications by adding to a test tube: hemoglobin (10 mg/mL), glucose (40 mM; as glycating agent) in phosphate buffer (50) mM to pH 7.4. Also TRM was used in different concentrations (0, 0.30, 0.60, 1.2, 2.5, and 5 mg/mL) as an antiglycating agent. The samples were incubated at 37 °C and 40 RPM for 5 weeks. Aminoguanidine (5 mg/mL) was used as a pharmacological control Assays were performed in triplicate.

Zebrafish acclimatization. Adult zebrafish (*Danio rerio*) of both sexes were acclimatized for 15 days in 60 L tanks at a temperature of 26 ± 2 °C; they were maintained with constant biological, mechanical, and chemical filtration, aeration (7.20 mg O_2/L) and a light/dark photoperiod of 14 h/10 h. The fish were fed three times a day with commercial pellets, then randomly selected to conduct the different experiments (Oyelaja-Akinsipo *et al*. 2020).

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Potosino Institute for Scientific and Technological Research (protocol code 11). Efforts were made to minimize suffering and reduce the number of animals used in experiments.

Induction of hyperglycemia. Five batches were formed with 18 fish each placed in 6 L tanks containing 111 mM glucose, for 14 days at a temperature of 26 ± 2 °C and were maintained under the same conditions as described in the previous section. Additionally, glucose solutions were changed every third day to avoid changes in concentration and contamination by microorganisms. The fish were monitored during the development of the trial (Capiotti *et al.* 2014).

Administration of TRM. To perform the test, five groups were formed, each consisting of fifteen diabetic fish housed in 6-liter tanks. Groups 1 and 2 were administered TRM at concentrations of 30 mg/L and 60 mg/L, respectively. Group 3 received glibenclamide at a concentration of 5 mg/L. Group 4 consisted of diabetic fish without any added treatment, while Group 5 was a normoglycemic control group without any added treatment. Each treatment was added daily to the tank of the corresponding group, with the medium being changed every 24 hours. The assay lasted 14 days after which the fish were sacrificed for the analysis of different biochemical parameters (Pérez Gutiérrez e*t al.* 2021).

Evaluation of glucose, triglycerides, and cholesterol in blood. To determine the different biochemical parameters, the fish were placed in tanks with water without glucose for 15 minutes. To reduce the variability of the analyses, each specimen was induced to hypothermia (water at 4°C), and then the tail was cut with a scalpel to obtain blood, which was placed on test strips and read with a glucometer (Accu-Chek). To measure the levels of triglycerides and cholesterol, Accutrend Plus Roche equipment was used. (Pérez Gutiérrez *et al.* 2021).

Evaluation of AGE in vivo. The eyes of the sacrificed fish were removed and placed in a 1 mL phosphate buffer (pH 7.0). The ocular organs were homogenized and centrifuged for 15 minutes at 9,000 rpm. The supernatant was placed in a 96 well microplate and read at an emission wavelength of 460 nm and an excitation wavelength of 355 nm using a fluorometer (GENios, TECAN) (Gleeson *et al*. 2007). Results are reported as fluorescence intensity.

Gas chromatography - mass spectrometry conditions. Sample was analyzed by a Gas Chromatograph coupled to a Mass Spectrometry detector 7820 A/5977E System (Agilent Technologies) with an automatic injector 7683 Series (Agilent Technologies) using a capillary column HP5-MS (Agilent Technologies) 30m long, 0.25 mm diameter and 0.25 µm-film thickness. The injector temperature was 250 °C, in splitless mode. Ultrapure helium was used as gas carrier at 1mL/min flow. The temperature program started at 50 °C for 1 min, and it was increased at a rate of 30 °C/min until 280 °C, then increased at a rate of 15 °C/min to 300 °C and held for 4 min. The ionization potential was 70 eV and a scan function with 35-400 m/z range for identification. Compounds were identified by comparing their mass spectra with those obtained in the NIST 14 library (Gaithersburg, MD, USA).

Statistical Analysis. All data are expressed as mean ± SD, through an ANOVA analysis. Values of $P \leq 0.05$ were considered statistically significant. Statistical analysis was performed using the GraphPad Prism 7 software (GraphPad Software Inc., San Diego, CA, USA). The experiments were performed in triplicate. Statistical significance was represented as *, **, ***and **** when $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$ when compared to diabetic group.

Results

Protein Glycation using the BSA-Glucose Model. The inhibitory effect of TRM on the formation of AGEs during a period of four weeks is shown in Figure 1. When administering TRM during the experimentation period, the fluorescence decreased, obtaining a reduction of 48 % at a concentration of 5 mg/mL at the fourth week with respect to the positive control. This percentage is remarkably close to that of the pharmacological control (aminoguanidine) which showed a 50 % decrease in the same time interval.

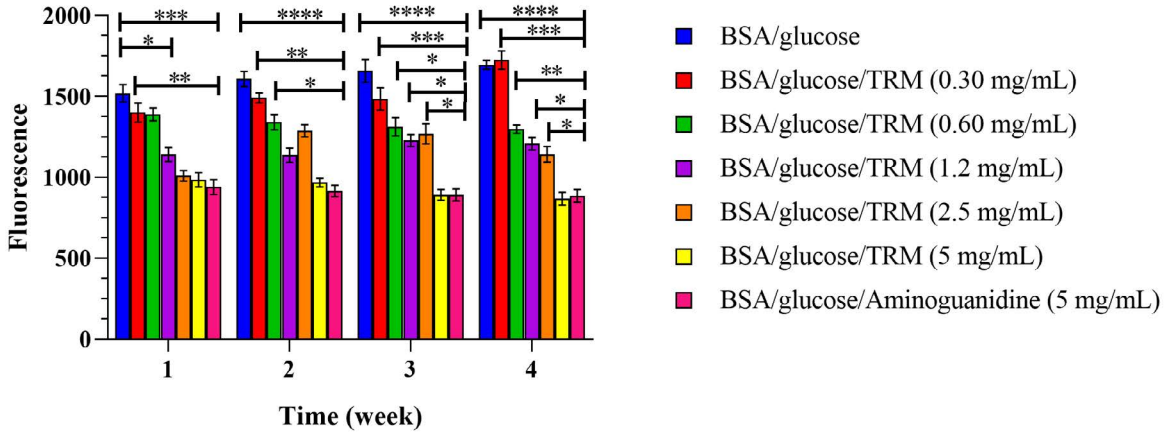

Figure 1. Effect of TRM on the formation of AGEs during a period of 4 weeks using the BSA/glucose model *in vitro*. Results are expressed as mean ± SD. Statistical significance was represented as *, **, ***and **** when $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.0001$ when compared to diabetic group.

BSA-methylglyoxal assay. Figure 2 shows the fluorescence values for the MG assay. At the end of the experiment the treatment at a concentration of 5 mg/mL of TRM presented a value of 875, which is remarkably like what was observed in the pharmacological control (aminoguanidine) with a value of 900 at the same concentration. As for the positive control, it showed a fluorescence of 1,710 at the end of the test.

Glycation of hemoglobin. The results of glycosylated hemoglobin (HbA1c) are shown in Figure 3. It is observed that the negative control and the pharmacological control showed HbA1c values of 11.2 and 15.8, respectively. Regarding TRM at the different concentrations, it is observed that as its quantity increased, the HbA1c data decreased at a concentration of 5 mg/mL of TRM it had a value of 13.1, a lower result than that obtained in the pharmacological control.

Hypoglycemic effect of TRM. Figure 4 shows the blood glucose levels of diabetic zebrafish treated with TRM at different concentrations. It shows that the greatest decrease in blood glucose in the treated batches was at a concentration of 60 mg/L, which presented a concentration of 60 mg/ dL blood glucose, compared to the normoglycemic control, which recorded a value of 47 mg/dL. In contrast, the pharmacological control and the diabetic control registered a blood glucose concentration of 114 and 136 mg/dL, respectively.

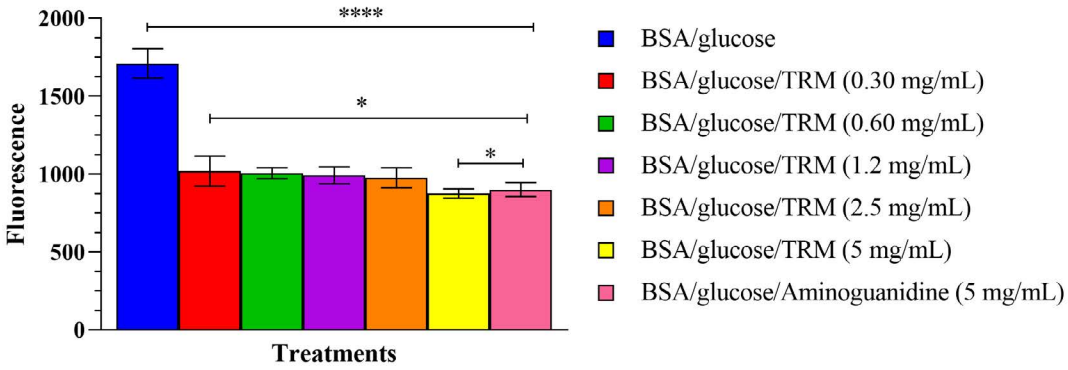

Figure 2. Effect of TRM on MG formation *in vitro*. Results are expressed as mean ± SD. Statistical significance was represented as * and *** when $P < 0.05$ and $P < 0.001$ when compared to diabetic group. AG; Aminoguanidine.

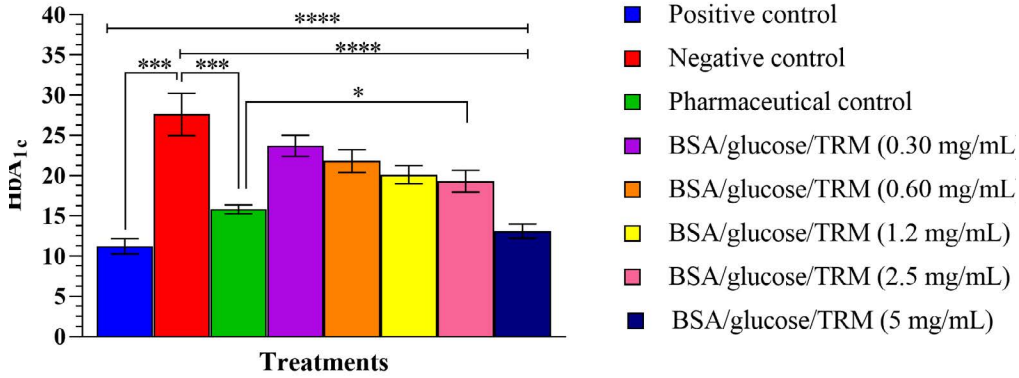

Figure 3. Effect of TRM on HbA1c *in vitro.* Positive control: Incubation with hemoglobin (10 mg/mL), negative control: Incubation with hemoglobin (10 mg/mL) + glucose (40 mM). Results are expressed as mean ± SD. Statistical significance was represented as *, *** and **** when $P < 0.05$, $P < 0.001$ and $P < 0.0001$ when compared to diabetic group.

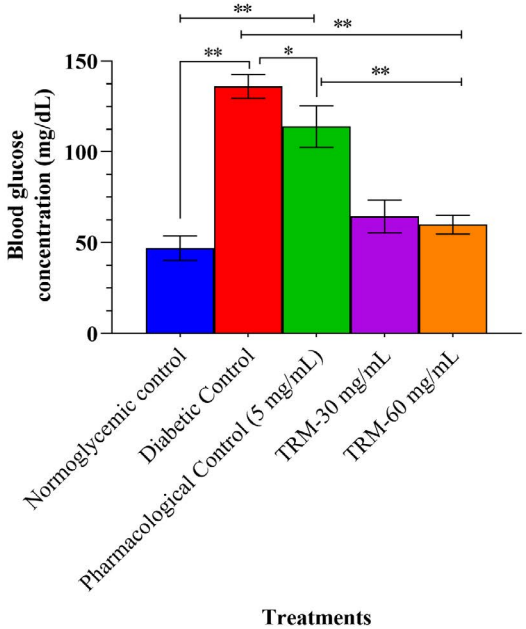

Figure 4. Blood glucose levels in diabetic zebrafish treated with TRM. Results are expressed as mean ± SD. Statistical significance was represented as *, ** and *** and when $P < 0.05$, $P < 0.01$ and $P < 0.001$ when compared to diabetic group.

Effect of TRM on triglyceride and cholesterol levels. Figure 5 shows cholesterol and triglyceride levels when TRM is administered at different concentrations. In the case of cholesterol at a concentration of 60 mg/L, a value of 191 mg/dL was obtained; this concentration is comparable to that recorded with the normoglycemic control (188 mg/dL) with both readings within normal limits. Regarding the diabetic control, it showed a value of 241 mg/dL (Figure 5A).

On the other hand, in the results of triglycerides (Figure 5B), it is observed that at the concentration of TRM at 60 mg/L there was a value of 185.6 mg/dL compared to the diabetic control, which showed values of 268 mg/dL, while the normoglycemic control recorded triglyceride values of 134 mg/dL.

Effect of TRM on protein glycation in vivo. Figure 6 shows the fluorescence values of the different batches. The normoglycemic control and the group treated with TRM at 60 mg/mL present values of 26,121 and 26,500 respectively, while the diabetic control and the pharmacological one showed values of 41,088 and 31,513 correspondingly.

Analysis GC-MS of TRM. The TRM chromatogram is shown in Figure 7, in which 5 relevant signals for the present study are observed, and are identified in Table 1.

Discussion

DM is generally treated with oral hypoglycemic agents, which exert their antidiabetic potential through various mechanisms (Hui *et al*. 2005, de Souza *et al*. 2018). These mechanisms include delayed intestinal carbohydrate absorption by α-glucosidase and biguanide-mediated reduction of hepatic gluconeogenesis, meglitinide and sulfonylurea mediated stimulation of insulin secretion and increased peripheral glucose absorption by thiazolidinediones and biguanides (Gupta *et al*. 2017).

In recent years, herbal medicines have attracted interest due to their low cost, minimal side effects, but above all therapeutic effect. It is estimated that 72.8 % of diabetic patients use medicinal plants, as well as dietary supplements to treat the disease (Pang *et al*. 2019). Research reports that various plant-based medications have high efficacy in the treatment of DM through different mechanisms, acting as antioxidants, anti-inflammatories, hypoglycemic agents, regulating the metabolism of lipids in the blood, among others (Kooti *et al*. 2016). Studies report that the antidiabetic effect is due to the secondary metabolites present in plants such as tannins, flavonoids, phenols, and alkaloids, which decrease the intestinal absorption of glucose or increase insulin secretion (Kooti *et al*. 2016). Our results show

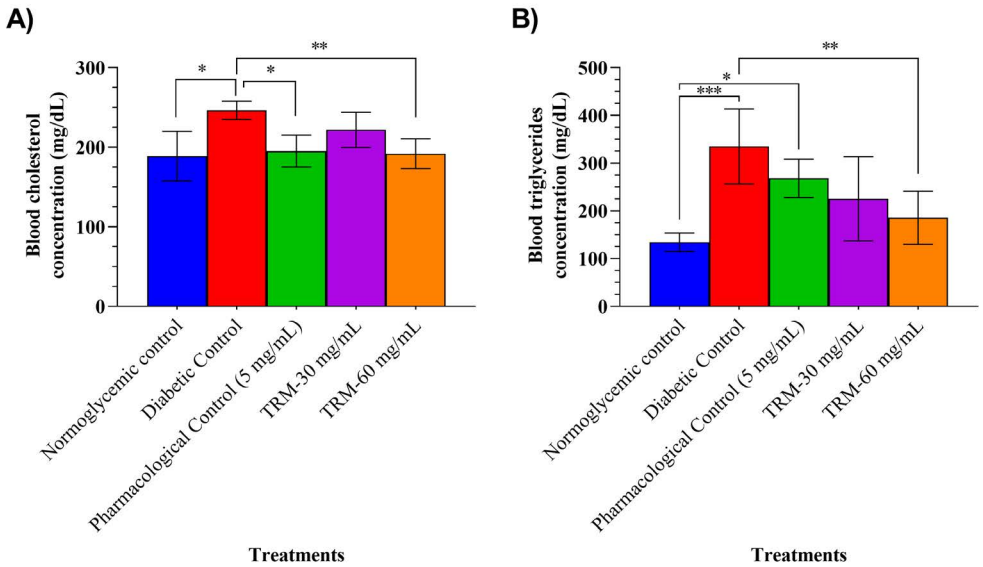

Figure 5. Cholesterol (A) and triglyceride (B) levels in diabetic zebrafish treated with TRM. Results are expressed as mean ± SD. Statistical significance was represented as *, **, and *** when $P < 0.05$, $P < 0.01$, $P < 0.001$ when compared to diabetic group.

that TRM reduces blood glucose levels, these results agree with what was reported by Lowe who mentions that the methanol extract of *Tillandsia recurvata* reduces the glycemic index, the concentration of fructosamine, as well as the concentration of insulin in mice (Lowe *et al.* 2014). In addition, previous studies conducted on *T. usneoides* report that the species exhibits hypoglycemic activity, which is attributed to the presence of flavones (Miranda-Nuñez *et al*. 2021). Studies have shown that the protein glycation reaction followed by the formation of AGE's are part of the main causes of different complications that occur in diabetes (Bose *et al*. 2013, Aydın *et al*. 2022). Therefore, in the glycemic control and diagnosis of DM, HBA1c is analyzed, since it is associated with chronic complications in diabetic patients (Bunn *et al*. 1979, Saudek *et al*. 2006 Sen *et al.* 2005). Studies have reported that both glucose and fructose cause hemoglobin to be modified due to the Maillard reaction, promoting oxidation reactions by free radicals and leading to oxidative stress in diabetics (Ramasamy *et al*. 2005). Due to erythrocytes being permeable to glucose, the formation of HBA1c depends on glucose concentrations as well as the exposure time of erythrocytes. As erythrocytes age, they slowly lose their ability to metabolize glucose, which is why the intracellular glucose concentration mirrors the extracellular or plasma glucose concentration (Banerjee 2021).

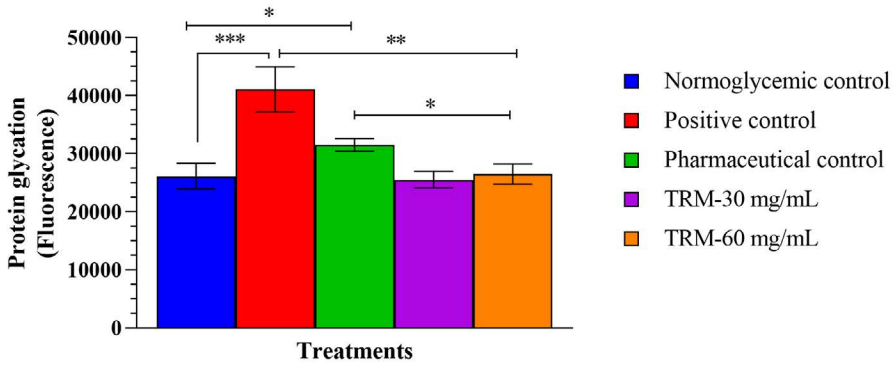

Figure 6. Effect of TRM on the formation of AGEs *in vivo*. Results are expressed as mean ± SD. Statistical significance was represented as *, ** and *** when $P < 0.05$, $P < 0.01$, $P < 0.001$ when compared to diabetic group.

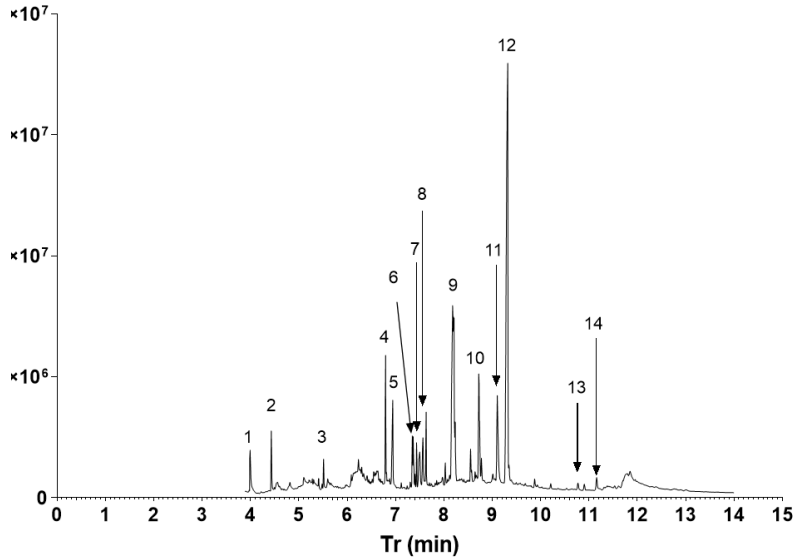

Figure 7. GC-MS chromatogram de TRM, (1) 2-methylbenzaldehyde, (2) 4-hydroxy-2-methylacetophenone, (3) 3',5'-dimethoxyacetophenone, (4) pentanoic acid, (5) palmitic acid, (6) linoleic acid, (7) phytol, (8) margaric acid, (9) oleamide, (10) cis-11-eicosenamide, (11) stearic acid, (12) 13-docosenamide, (Z) o erucamide, (13) campesterol, (14) β-sitosterol.

The glycosylation reaction of hemoglobin is irreversible, meaning a HBA1c molecule will remain as such until the end of its useful life. The assays to determine HBA1c, evaluate young erythrocytes and red blood cells glycosylated, which has made the HBA1c proof the standard test to conduct glycemic control in diabetic patients (Banerjee 2021). Considering that the glycation of the N-terminal valine residues of hemoglobin is conducted slowly, and that this reaction comes from Schiff base, it gradually undergoes a rearrangement to form Amadori compounds, which are irreversible (Ramasamy *et al.* 2006). According to the results obtained, TRM could reduce the formation of Amadori compounds. This was reflected in the decrease of hemoglobin glycation and the formation of AGEs. AGEs are related to diabetic complications and other pathological conditions. They modify the key structure of cells, damaging cell permeability and motility, which derives from the crosslinking of the basement and structural membranes of cells (Dyer *et al.* 1993). Based on the evidence that AGEs and MG are the cause of various complications in diabetes, bioactives capable of inhibiting or reducing their formation are being sought. MG is a highly reactive α-oxoaldehyde produced during glycolysis; it is formed in cells from triose phosphate, glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (Thornalley 1993, Kumar *et al*. 2004). The presence of hyperglycemia, uremia, aging, inflammation and oxidative stress increases MG production, accelerating protein and DNA modifications, resulting in the generation of AGEs (Oya *et al.* 1999). Dicarbonyl compounds such as glyoxal and MG are the main source of AGE formation in the lens (Cai *et al.* 2016), damaging the α-crystallin protein, which promotes the formation of cataracts, these being the main cause of blindness in the world (Stratton *et al*. 2000, Griffith *et al*. 2014, Duan *et al*. 2017). Studies have found elevated concentrations of MG in the lens compared to any other tissue or plasma in diabetic patients (Banz *et al*. 2007, Saba *et al*. 2016). The results obtained show that TRM reduces MG levels *in vitro*, in addition to decreasing AGE formation *in vivo*. As MG is a precursor of AGEs (Giovannoni *et al*. 2005), by reducing MG formation, the formation of heterogeneous AGEs and protein cross-linking was reduced (Duan *et al.* 2017), which may delay the formation of cataracts and other AGE mediated diabetic pathologies.

Table 1. Phytochemical components of TRM analyzed by GC-MS.

No.	Name	RT (min)	Molecular weight (g/mol)	Molecular Formula	CAS #:
1	2-methylbenzaldehyde	3.9937	120.2	C_8H_8O	529204
2	4-hydroxy-2-methylacetophenone	4.4317	150.2	$C_9H_{10}O_2$	876028
3	3',5'-dimethoxyacetophenone	5.5114	180.2	$C_{10}H_{12}O_3$	39151194
4	pentanoic acid	6.7939	102.1	$C_5H_{10}O_2$	109524
5	palmitic acid	6.9411	256.4	$C_{16}H_{32}O_2$	57103
6	linoleic acid	7.3462	280.5	$C_{18}H_{32}O_2$	60333
7	phytol	7.4034	296.5	$C_{20}H_{40}O$	150867
8	margaric acid	7.4357	270.5	$C_{17}H_{34}O_2$	506127
9	oleamide	8.1823	281.5	$C_{18}H_{35}NO$	301020
10	cis-11-eicosenamide	8.724	309.5	$C_{20}H_{39}NO$	10436085
11	stearic acid	9.1042	284.5	$C_{18}H_{36}O_2$	57114
12	13-docosenamide, (Z)	9.3176	337.6	$C_{22}H_{43}NO$	112845
13	campesterol	10.7723	400.7	$C_{28}H_{48}O$	474624
14	β-sitosterol	11.1578	414.7	$C_{29}H_{50}O$	83465

The alteration of lipid metabolism (lipotoxicity) that occurs in DM causes or aggravates insulin resistance and malfunction of pancreatic cells; therefore, lipid metabolism plays a key role in the pathogenesis of type 2 DM (Kooti *et al*. 2016). Previous tests have reported that herbal medicine contributes to lowering levels of triglycerides, low density lipoproteins, and total cholesterol (Delaporte *et al*. 2006, Estrella-Parra *et al*. 2019). The rise of free fatty acids (FFA) increases the levels of triglycerides in the blood (Wyne 2003). Additionally, reports show that plant-based extracts can inhibit acetyl coenzyme A carboxylase (ACC), which is involved in fatty acid metabolism, so that ACC inhibition can decrease blood FFA (de Souza *et al*. 2018). Due to this, secondary metabolites capable of inhibiting ACC have immense potential in the treatment of diabetes (Yang *et al*. 2020).

In the case of high cholesterol levels there are reports showing that bioactives from plants have the ability to decrease the activity of acetyl coenzyme A (CoA) and acetyl transferase 2 (ACAT2) (Islam *et al*. 2015, Babu & Jayaraman 2020). ACAT2 is an enzyme that participates in the generation and metabolism of total cholesterol, therefore the inhibition of ACAT2 can reduce hypercholesterolemia (Cheng *et al.* 2010). The results obtained show that TRM is capable of reducing triglyceride and cholesterol levels in a diabetic zebrafish model, which is probably due to the fact that TRM is capable of reducing the activity of ACC and ACAT2; however, more studies are needed to elucidate its mechanism of action.

The *Tillandsia* genus exhibits different secondary metabolites with diverse biological activities such as flavonoids, triterpenes, sterols, cyanic acid and glycosides (Prasad *et al*. 2022, Muzahid *et al*. 2023).

By analyzing the phytochemical components of TRM by GC-MS, compounds with antioxidant activity were identified (5), (6), (7), (14) (Islam *et al*. 2015, Lowe *et al*. 2017, Yang *et al*. 2020), inhibitors of the alpha glucosidase enzyme (5) and (6) (Lowe *et al*. 2017), antidiabetic (7) and (14) (Islam *et al*. 2015,Yang *et al*. 2020), lipid lowering (7), (9), (14) (Islam *et al*. 2015, Babu & Jayaraman 2020, Yang *et al*. 2020), hypocholesterolemic (5) (Cheng *et al.* 2010), insulin secretion (6) (Muzahid *et al*. 2023) and a compound that improves insulin resistance (13) (Prasad *et al*., 2022), these metabolites could be related to the reported antidiabetic activities; however, more studies are necessary to determine the mechanisms of action involved.

In conclusion, the results of our experimental study clearly demonstrate the promising potential of *Tillandsia recurvata* methanol extract (TRM) as an effective adjuvant in the treatment of diabetes mellitus. Both *in vivo* and *in vitro* experiments revealed significant reductions in key markers associated with diabetes, including blood glucose, triglycerides, cholesterol levels, advanced glycation end products, methylglyoxal concentrations, and glycosylated hemoglobin levels. The identification of specific bioactive compounds within TRM, such as 2-methylbenzaldehyde, 4-hydroxy-2-methylacetophenone, and various fatty acids, provides valuable insights into the underlying possible mechanisms of its antidiabetic properties.

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