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Original Research Article

Antidiabetic Activity and *in silico* Molecular Docking of GC-MS- Identified Compounds in Chromatographic Fractions of *Tephrosia bracteolata* Guill. & Perr. (Fabaceae) Leaves

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ABSTRACT

Recent efforts for the complementary treatment of diabetes have focused on medicinal plants and their bioactive compounds. This study evaluated the antidiabetic activity of chromatographic subfractions of ethylacetate fraction (EAF) of *Tephrosia bracteolata* leaves. Also, to carry out molecular docking of some GC-MS-identified compounds in the subfraction with the highest antidiabetic activity against key targets in the pathophysiology of diabetes. The EAF (5 g) was subjected to silica gel column chromatography using gradient elution with hexane, chloroform, ethyl acetate, methanol and water. Approximately 100 fractions (100 mL each) were collected and monitored via TLC, resulting in 7 major sub-fractions (SF1-SF7). The antidiabetic activity of the sub-fractions was evaluated (at doses of 200 and 400 mg/kg) against alloxan-induced diabetes in mice. GC-MS analysis was carried out on the subfraction with the highest activity. Subsequently, some of the identified active compounds were docked against key targets in the pathology of diabetes using AutoDock Vina. Phytochemical analysis revealed the presence of several phytochemicals in varying proportions in the subfractions. SF5 (400 mg/kg) produced the most significant ($p< 0.05$) decrease in FBS (35.29 and 38.37 %) at 12 h and 24 h- post-treatment respectively. GC-MS analysis of SF5 revealed the presence of 36 compounds among which are Mome inositol, 2-methoxy-4-vinylphenol, 1-D-thio-glucitol and 4-Piperidinone. Molecular docking showed that Mome inositol and 1-D-thio-glucitol have a strong affinity for alpha-glucosidase and sodium-glucose co-transporter 2 (SGLT 2). These findings suggest that the antidiabetic activity of the extract maybe attributed to the bioactive compounds identified in SF5.

Keywords: Antidiabetic activity, Molecular docking, *Tephrosia bracteolata*, Diabetes, Chromatographic fraction.

Introduction

Diabetes mellitus (DM) is a cluster of metabolic conditions identified and characterized by the occurrence of elevated levels of glucose in the blood caused by defects in insulin secretion and insulin resistance.[1] The gradual progression of this disease affects other organs of the body, and serious complications appear after the onset of diabetes. It is the most prevalent and rapid-growing worldwide problem and arises as a huge health and socioeconomic burden.[2] In 2017, 425 million adults were diabetic globally and these figures are predicted to reach 629 million by 2045.

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The developing economies of Africa and Asia contribute a significant fraction of this figure. The prevalence of diabetes mellitus in Nigeria was 2.2 % in 1997 and it is predicted to reach 3.9 % by 2025.[3] Alloxan is a widely used chemical model for studying diabetes in animals. [4] Alloxan, a toxic glucose analogue when administered to vertebrates leads to the destruction of pancreatic β-cells. Consequently, an insulin-dependent type of diabetes mellitus (Type 1) develops in the animals. Alloxan is selectively toxic to insulin-producing pancreatic β-cells because it preferentially accumulates in β-cells through uptake via glucose transporter-2 (GLUT2).[4] Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The β-cell toxic action of alloxan is initiated by free radicals formed in this redox reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells.[5] Administration of alloxan elevates serum glucose, which signifies induction of diabetes mellitus. Medicinal plants continue to play a key role in the healthcare system of a large number of countries of the world. This is particularly true in developing countries, where herbal medicine has a long and uninterrupted history of use. [6] Recognition and development of medicinal and economic benefits of these plants are on the increase in both developing and industrialized nations.[6] These plants are specifically screened for the presence of bioactive compounds which are known to possess therapeutic properties. [7, 8] The process of utilization of the bioactive compounds involves identification, isolation

and characterization for use in pharmaceutical and food industries. [9] *Tephrosia bracteolata* Guill. & Perr. (Fabaceae) is an erect, perennial shrub that typically grows to a height of 2-3 feet, but under favourable conditions, it may reach up to 8 feet. [10] In folkloric medicine, *Tephrosia bracteolata* has been employed for various therapeutic purposes. [11] The root is traditionally used in the management of venereal diseases, including the treatment of pregnant women with syphilis. [11] It has been reported to possess analgesic, anti-inflammatory and antipyretic properties. [12] Idakwoji *et al*. reported the *in vitro* antidiabetic properties of the ethanol extract of the leaves. [13] Similarly, a study by Egharevba *et al*. also reported the antidiabetic activity and revealed the presence of alkaloids, steroids, tannins, flavonoids and terpenoids in the hexane and ethylacetate extracts of the leaves of the plant. [14] In this study, we investigated the antidiabetic activity of the subfractions of ethyl acetate fraction of *Tephrosia bracteolata* and carried out *in silico* molecular docking of some of the GC-MS-identified compounds in the most active fraction against key molecular targets implicated in diabetes.

Materials and Methods

Collection and identification of plant material

Fresh leaves of *T. bracteolata* Guill. & Perr. (Fabaceae) were collected from their natural habitat along the River Niger, near the Old Market area of Lokoja, Kogi State, Nigeria, on 6th April 2017. The geographical coordinates of the collection site are approximately 7º47'49" N, 6º47'26" E. The plant sample was identified and authenticated at the Herbarium Unit of the Department of Biological Sciences, Federal University, Lokoja, Kogi State, Nigeria by Mr. Gbenga, an ethnobotanist, and was assigned a voucher number (FULH/0765) which was deposited at the herbarium for future reference.

Chemicals, reagents and drugs

Drangendorff's reagent, Wagner's reagent, Mayer's reagent, 5% Iron (III) chloride, 1% aluminium (III) chloride (BDH, England), bromine water, aqueous ammonia, concentrated tetraoxosulphate (VI) acid (BDH, England), concentrated Hydrogen chloride acid, naphthol and lead acetate solution, Ethanol (≥ 99.5 %), hexane (≥98.5 %), chloroform (≥98.5 %), ethylacetate (≥99.8 %) and methanol (≥99.8 %) (Sigma-Aldrich, UK), Pre-coated silica gel 60F_{254} on aluminium sheets, silica gel (60-120 mesh) for column chromatography (Sigma-Aldrich, UK), Alloxan monohydrate (Sigma-Aldrich, UK), metformin (Glucophage®-Bristol Myers Squibb Company) and distilled water.

Experimental animals

Adult Male Swiss mice weighing 25-30 g obtained from the Animal House Facility of Salem University, Lokoja, Kogi State, Nigeria, were used for the study. They were kept in well-ventilated stainless-steel cages under standard laboratory conditions guaranteed a 12 h dark/light cycle and were maintained on standard rodent feed and potable drinking water *ad libitum*. The animals were acclimatized to the laboratory environment for a period of 7 days before use and accorded humane care in line with the institutional ethical committee (Approval number: FVM-UNN-IACUC-2017-05/1038) and the International Guidelines for Handling of Laboratory Animals. [15]

Preparation and processing of plant material

The leaves of *T. bracteolata* were rinsed with distilled water to remove all debris, shade-dried for fifteen days (15) days and subsequently pulverized using an electric blender.

Crude extraction and solvent partitioning

A known weight, 2000 g of the pulverized leaves was subjected to cold-maceration in 5 L of 70% ethanol for 72 h. The resulting mixture was filtered using muslin cloth and Whatman No. 1 filter paper. The filtrate was then concentrated under reduced pressure using a rotary evaporator (Buchi) at 40 ºC and 100 mbar to yield the ethanol extract (EETB). The crude extract was subsequently fractionated using the method previously described by Uzor et al.[16] A measured quantity (200 g) of the EETB was thoroughly triturated with 400 g of silica gel (mesh size 60-120) to enhance surface area and facilitate effective adsorption. The resulting mixture was then subjected to successive partitioning using ethyl acetate to obtain the ethyl acetate-soluble fraction (EAF), following the procedure outlined in our previous study. [17]

Fractionation of the EAF

As guided by findings from an earlier study, [17] the EAF was subjected to further fractionation by column chromatography, following the method described by Cannell. [18] Five grams (5 g) of EAF were dissolved in chloroform and adsorbed onto 30 g of silica gel. The slurry was allowed to dry into a free-flowing powder and then introduced into a glass column (75 cm x 3.5 cm) packed with silica gel in hexane. The column was eluted using hexane: chloroform (100:0, 80:20, 60:40, 40:60, 20:80, 0:100), chloroform: ethyl acetate (100:0, 80:20, 60:40, 40:60, 20:80, 0:100), Ethyl acetate: methanol (100:0, 80:20, 60:40, 40:60, 20:80, 0:100) and methanol: water (100:0, 80:20, 60:40, 40:60, 20:80, 0:100). Approximately 100 fractions of 100 ml each was collected in flasks. These were monitored using TLC on pre-coated silica gel 60G F_{254} plates (Merck), visualized under UV light, and sprayed with 50 % H_2SO_4, followed by heating at 110 ºC. Based on the TLC profiles, the fractions were pooled into 7 major sub-fractions, labelled SF1-SF7.

Phytochemical analysis of sub-fractions

Phytochemical analysis of the EAF and the sub-fractions were determined by the methods of Trease and Evans [19] and Harborne. [20]

Investigation of antidiabetic property of the sub-fractions in mice

Induction of diabetes in mice: Adult mice were fasted for 12 h and subsequently injected intraperitoneally with alloxan solution at a dose of 150 mg/kg b.w. Mice with blood glucose levels of 200 mg/dl or more after 48 h were selected for the study.

Experimental design

Induction of experimental diabetes: Mice were fasted overnight with free access to water before the induction of diabetes. Diabetes was induced via a single intraperitoneal injection of Alloxan monohydrate at a dose of 100 mg/kg body weight, dissolved in 0.9 % cold normal saline solution.[21] Given that Alloxan can induce fatal hypoglycaemia due to a massive release of insulin from the pancreas, the mice were administered 20 % glucose solution orally 6 h post-injection. Subsequently, a 5 % glucose solution was provided in their drinking bottles for the following 24 hours to prevent hypoglycaemia. [22] After 48 h of Alloxan administration, blood samples were collected from the tail vein, and blood glucose levels were measured using the glucose oxidase method of Beach and Turner [23] with Fine Test® glucometer and compatible test strips (Fine Test® Milpitas, USA). Mice exhibiting fasting blood glucose levels ≥ 200 mg/dl were considered diabetic and selected for the study.[24]

Grouping of mice and treatment: A total of 102 (96 diabetic and 6 non-diabetic) adult male Swiss mice (25- 30 g) were used for the study. Group 1 consisted of the 6 non-diabetic mice and served as normal control. The 96 diabetic mice were randomly distributed into an additional 16 groups (groups 2-17) of six mice each. Group 2 served as diabetic control and was administered 1 ml/kg b.w of normal saline. Group 3 served as standard control and was administered 150 mg/kg b.w Metformin hydrochloride. Groups 4 and 5 were administered 200 and 400 mg/kg SF1 respectively. Groups 6 and 7 were administered 200 and 400 mg/kg SF2 respectively. Groups 8 and 9 were administered 200 and 400 mg/kg SF3 respectively. Groups 10 and 11 were administered 200 and 400 mg/kg SF4 respectively. Groups 12 and 13 were administered 200 and 400 mg/kg SF5 respectively. Groups 14 and 15 were administered 200 and 400 mg/kg SF6, respectively, while groups 16 and 17 were administered 200 and 400 mg/kg SF7, respectively.

Determination of fasting blood glucose concentration

All blood samples were collected from the tail vein of the rats at intervals of 0 h, 3 h, 6 h, 12 h and 24 h post-treatment. Fasting blood glucose levels were determined by the glucose oxidase method of Beach and Turner, [23] using a digital glucometer (Fine Test glucometer and test

strips, Fine Test® Milpitas, U.S.A), and the results were expressed as mg/dl.[25]

Principle: Glucose in the sample reacts with glucose oxidase in the glucose electrode strips, oxidizing glucose to gluconic acid and temporary transfer of two electrons from the glucose molecule to the enzyme. The reduced enzyme reacts with the mediator, transferring a single electron to each of the two mediator ions, thus returning to its original state. At the electrode surface, the reduced mediator is re-oxidized, providing amperometric signal whose magnitude is proportional to the glucose concentration in the sample.

Procedure: The data chip from the strip pack was inserted into the chip holder beside the device. The glucometer test strip was inserted into the strip holder of the device (just in front of the device). The tail of the mice was punctured using sterile lancet, and blood samples from the animal were allowed to drop (one drop) on the sensor part of the glucometer strip in front of the device. The results were displayed on the device screen and recorded accordingly.

Identification of active principles
The fraction with the highest antidiabetic action (SF5) was selected and subjected to GC-MS for identification of active principles.

GC-MS analysis of the SF5
The chemical composition of the SF5 was determined by GC/MS-QP-2010 plus Ultra (Shimadzu, Kyoto, Japan) using a DB-5 MS fused silica capillary column (30 × 0.25 m internal diameter, film thickness 0.25 µm). For GC-MS detection an electron ionization system with ionization energy of 70 eV was used. The carrier gas was Helium gas; the flow rate was 1.2 mL/ min. Injector and MS transfer line temperatures were set at 260 ºC and 270 ºC, respectively. The oven temperature was initially maintained for 2 min and then increased to 210 ºC at a rate of 8 ºC/min to 280 ºC; the hold time was 10 min. Samples were completely dissolved in absolute ethanol, and 0.3 µL was injected through auto-sampler in the split mode; the split ratio was 1:100. Relative percentage of each constituent was expressed as percentages by peak area normalization. Each component was identified based on its column retention time relative to computer-based matching of mass spectra with those of standards (NIST and Wiley libraries for GC-MS system).

In silico studies
Alpa-glucosidase (PDB ID: 2JKP) and SGLT2 (PDB ID: 7VSI) were obtained from the Protein Data Bank. Preparation of the proteins before molecular docking was performed using AutoDock tools which involved the addition of charges, the addition of polar hydrogen and grid set-up. The 3D structure of 1-Thio-D-Glucitol and Mome inositol in SDF format were downloaded from PubChem. These SDF files were then converted to PDB files using PyMOL software (PyMOL v2.5). [26] A site- specific molecular docking was performed targeting Glutamate (GLU) 532 in Alpha-glucosidase and Serine (Ser) 287 in SGLT2 due to their role in the catalytic mechanism of the target proteins using AutoDock Vina Software [27] and their binding affinities/energy reported in Kcal/mol. The renderings for the receptor-surface view (3D view) were computed using PyMOL software (PyMOL v2.5) [26] while the 2D diagrams of the interactions were computed using BOVIA-Discovery Studio Visualizer 4.5. All analyses were conducted using a computer system running a 64-bit Windows operating system.

Statistical analysis
All data were expressed as mean ± standard deviation and statistical differences between means were determined by one-way ANOVA followed by Duncan's post-hoc test for multiple comparison tests using SPSS version 20. Values were considered significant at $P \leq 0.05$.

Results and Discussion

Phytochemical composition of the ethylacetate fraction (EAF) and its sub-fractions
In our previous study, [17] the ethylacetate fraction (EAF) of the extract of *T. bracteolata* showed a more promising antidiabetic activity compared to n-hexane, chloroform, methanol and aqueous fractions. Based on this, the EAF was subjected to further fractionation using the column chromatographic technique. The fractionation afforded one hundred (100) fractions which were pooled into seven (7) sub-fractions (SF1- SF7) based on similarities in TLC mobility profiles (Table 1). Phytochemical analysis of EAF and its fractions revealed the presence of phenols, terpenes, saponins, steroids, glycosides, flavonoids, tannins and alkaloids in varying proportions. All the phytochemicals tested were detected in EAF and SF5. Terpenoids were also detected in all the sub-fractions except SF6 and SF7. Alkaloids were detected in all the sub-fractions except SF2. Steroids were detected in only SF1, SF2, SF3 and SF5 while tannins were detected only in SF4 and SF5 (Table 2).

Table 1: TLC profiles of sub-fractions of the EAF

Sub-fraction	Number of major spots	R_f values
1	4	0.19, 0.44, 0.63, 0.76
2	2	0.81, 0.89
3	3	0.22, 0.43, 0.88
4	3	0.49, 0.74, 0.82
5	2	0.39, 0.75
6	2	0.73, 0.88
7	2	0.69, 0.79

Rf: Retention factor

Effect of sub-fractions of the ethylacetate fraction (EAF) on 24 h fasting blood sugar levels of diabetic mice
The results of the antidiabetic studies carried out in mice with the sub-fractions of EAF (SF1-SF7) are summarized in Table 3. The sub-fractions at doses of 200 and 400 mg/kg significantly ($p< 0.05$) reduced FBS of rats at 12 and 24 h post-treatment compared to the diabetic control. However, the results clearly showed that the antidiabetic activity resides mostly with the SF5. SF5 at 400 mg/kg afforded the best FBS reduction of 35.29 and 38.37% at 12 and 24 h post-treatment, respectively, relative to the diabetic control. This reduction produced by SF5 was comparable to that of the standard- metformin (150 mg/kg), which produced an FBS reduction of 27.82 and 35.95% at 12 and 24h post-treatment, respectively. SF4 at a dose of 200 mg/kg, also produced a significant ($p< 0.05$) reduction in FBS of the mice after 24 h. However, other sub-fractions did not seem to produce remarkable blood sugar reduction.

Phytochemicals such as terpenoids, saponins, alkaloids, glycosides, phenols and flavonoids are usually responsible for the antidiabetic activity of medicinal plants. In this study, these phytochemicals were found in higher concentrations in EAF and SF5 (Table 2). The observed presence of these phytochemicals could be responsible for the high antidiabetic activity produced by SF5. Several reports have shown that flavonoids, steroids, terpenoids or phenolic acids are known to be bioactive antidiabetic principles. [28, 29, 30, 31] Flavonoids have been noted as one of the most numerous and widespread groups of phenolic compounds in higher plants. [32] Some of them, due to their phenolic structure, are known to be involved in the healing process of free-radical mediated diseases including diabetes. [33] Flavonoids are known to regenerate the damaged *β*-cells in alloxan-induced diabetic mice and act as insulin secretagogues. [34]

Table 2: Phytochemical composition of the EAF and its sub-fractions

Phytochemicals	EAF	SF1	SF2	SF3	SF4	SF5	SF6	SF7
Phenols	+	-	-	+	+	+	+	-
Terpenoids	+	+	+	+	+	+	-	-
Saponins	+	-	-	+	-	+	-	-
Steroids	+	+	+	-	-	+	+	+
Glycosides	+	-	-	-	-	+	+	+
Flavonoids	+	-	+	+	+	+	+	-
Tannins	+	-	-	-	+	+	-	-
Alkaloids	+	+	-	+	+	+	+	+

Key: + (present), - (not detected), SF (sub-fraction)

Table 3: Effect of sub-fractions of the EAF on 24 h fasting blood sugar levels of diabetic mice

Treatment	0h	3h	6h	12h	24h
Normal control	72.3±5.01^{a}	72.7±3.61^{a}	73.0±2.28^{a}	71.2±4.88^{a}	72.5±4.89^{a}
Diabetic control	326.7±15.23^{b}	367.5±12.34^{c}	393.2±24.16^{c}	456.7±26.16^{d}	451.8±27.21^{d}
150 mg/kg b.w met	323.2±13.17^{b}	343.5±20.11^{c}	327.2±15.24^{c}	233.3±10.12^{b}	207.0±12.89^{ab}
200 mg/kg b.w SF1	323.0±10.08^{b}	331.0±13.14^{c}	329.8±17.13^{c}	351.2±19.21^{c}	332.7±17.73^{c}
400 mg/kg b.w SF1	321.0±17.23^{b}	335.0±11.21^{c}	334.5±21.27^{c}	345.2±16.21^{c}	330.2±16.44^{c}
200 mg/kg b.w SF2	318.2±12.11	327.3±10.14^{c}	323.8±22.16^{c}	339.7±12.14^{b,}	320.8±12.35^{c}
400 mg/kg b.w SF2	320.4±14.25^{b}	323.8±17.26^{c}	323.1±24.06^{c}	320.1±23.99^{c}	320.8±15.16^{c}
200 mg/kg b.w SF3	324.1±11.61^{b}	327.5±10.15^{c}	320.5±17.21^{c}	343.8±17.26^{c}	322.2±17.08^{c}
400 mg/kg b.w SF3	321.2±17.24^{b}	321.4±18.99^{c}	320.2±21.07^{c}	328.8±14.18^{c}	320.5±19.51^{c}
200 mg/kg b.w SF4	322.2±15.08^{b}	307.8±23.82^{bc}	302.7±18.20^{bc}	310.7±19.43^{b}	308.5±16.31^{bc,}
400 mg/kg b.w SF4	324.5±10.23^{b}	312.4±21.10^{bc}	301.8±11.41^{bc}	301.6±11.17^{b}	298.2±10.45^{b,}
200 mg/kg b.w SF5	320.0±14.21^{b}	298.5±19.21^{b}	293.8±15.21^{b}	228.0±15.28^{ab}	204.3±09.26^{ab}
400 mg/kg b.w SF5	325.0±20.21^{b}	291.4±25.41^{b}	283.6±12.77^{b}	210.3±09.33^{ab}	200.3±07.19^{ab}
200 mg/kg b.w SF6	320.0±17.56^{b}	320.3±18.26^{c}	317.7±15.89^{bc}	339.5±16.28^{c}	330.8±21.17^{c}
400 mg/kg b.w SF6	320.0±11.05^{b}	316.3±27.15^{bc}	315.2±22.13^{bc}	314.3±11.36^{bc}	310.3±17.80^{c}
200 mg/kg b.w SF7	324.2±20.21^{b}	317.3±17.26^{bc}	304.7±18.16^{bc}	324.3±17.40^{b}	328.5±12.39^{c}
400 mg/kg b.w SF7	324.2±17.14^{b}	315.3±20.19^{bc}	310.4±19.21^{bc}	300.4±25.88^{bc}	297.6±10.61^{c}

Data are presented as mean ± SD. (n=6). Mean values having different lowercase alphabets as superscripts down the columns are considered significant ($p< 0.05$).

Thus, the antidiabetic activity of the ethanol leaf extract of *T. bracteolata* may reside with any of the constituents of SF5 or a combination of these constituents. It was on this note that SF5 was selected and subjected to GC-MS analysis for identification of its active principles.

The GC-MS analysis of sub-fraction 5 (SF5)

The GC-MS analysis of SF5 revealed several bioactive compounds with reported direct or indirect antidiabetic properties (Table 4 and Figure 1). Hence, these compounds could be responsible for the antidiabetic activity observed with SF5. Some of the compounds are highlighted below

Mome inositol: Inositol is a cyclitol present in animal and plant cells. It can be present in nine distinct stereoisomers, myo-inositol being the most represented. D-chiro-inositol is an inositol isoform derived from myo-inositol through an epimerization process, and this reaction is insulin-dependent.[35] Both myo- and d-chiro-inositol showed insulin-mimetic effects in animal models of insulin resistance. [36,37] Inositol has been mainly used as a supplement in treating several pathologies such as gestational diabetes (GDM). [38] Inositol plays a preventive role in GDM onset. [39] In addition, inositol has been studied as a therapeutic option for the treatment of GDM. [40, 41] The main effect of inositol, when used in pregnancy, is decreasing the level of insulin resistance. Consequently, a potential role of inositol as a treatment option could be hypothesized for type-2 diabetes which is characterized by insulin resistance and deficiency of insulin secretion from the pancreas. [42] It showed for the first time a direct beneficial effect of the supplementation with the association of myo- and d-chiro-inositol on glycemic parameters of subjects with T2DM. Particularly, a significant reduction in blood glucose and HbA1c levels was registered with inositol supplementation. [43]

1-thio-D-glucitol: Luseogliflozin, a novel and orally bioavailable drug is a 1-thio-D-glucitol derivative. It is a highly selective inhibitor of SGLT 2. [44] The sodium-glucose cotransporter 2 (SGLT 2) inhibitors are gaining attention as a new drug class for treatment of diabetes mellitus. Inhibition of SGLT 2 promotes urinary glucose excretion by preventing the reuptake of filtered glucose in the proximal tubules of the kidney, consequently lowering plasma glucose levels. [44, 45]

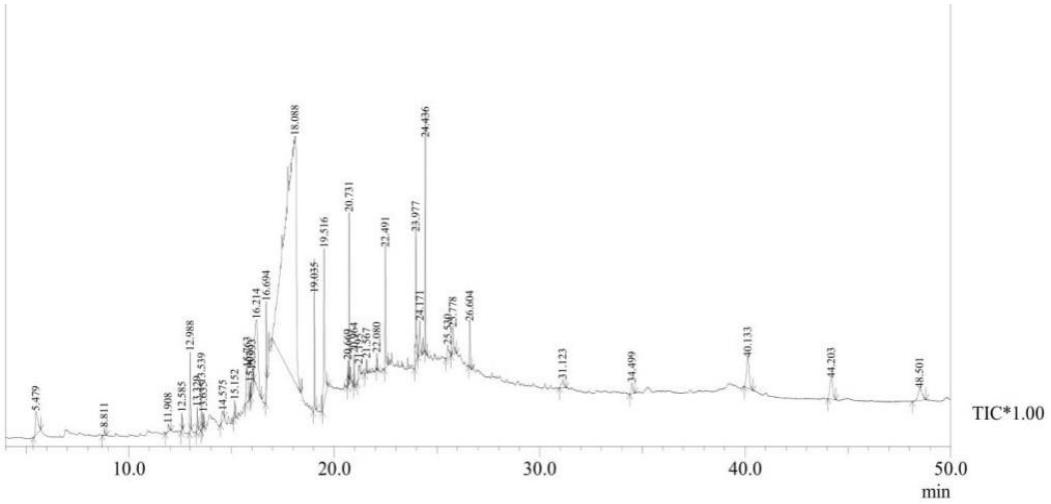


Figure 1: GC-MS Chromatogram of Sub-Fraction 5

Table 4: Identification of bioactive compounds in SF5

Peak No.	R. Time	Area	Area%	Name of compound
1	5.479	53822793	1.35	2-cyclopenten-1-one, 2-hydroxy-
2	8.811	441042	0.11	4-piperidinone, 2,2,6,6-tetramethyl-
3	11.908	841673	0.21	2-methoxy-4-vinylphenol
4	12.585	761111	0.19	2-propanone, 1-(3,5,5-trimethyl-2-cyclohexane-1-ylidene)-
5	12.988	3888243	0.98	1-(3,6,6-trimethyl-1,6,7,7 a-tetrahydrocyclo
6	13.329	1251207	0.31	Ethanone, 1-(2,3-dihydro-1,1-dimethyl-1h-inden-4-yl)-
7	13.539	2621869	0.66	1-(2-[2n-2-methyl-furan-2-yl)-propyl]-cyclopropyl
8	13.635	693935	0.17	3,3-dimethyl-4-phenyl-4-penten-2-one
9	14.575	1779787	0.45	D-allose
10	15.152	1241100	0.31	3-(tert-butyl)-4-methoxyphenyl acetate
11	15.763	6421829	1.62	alpha. -d-glucopyranoside, methyl
12	15.907	614813	0.15	Megastigmatrienone 2
13	15.993	2413208	0.61	1-s-hexyl-1-thio-d-glucitol
14	16.214	18677393	4.70	1,3,4,5-tetrahydroxy-cyclohexanecarboxy
15	16.694	4071548	1.02	Hexanoic acid, tridec-2-ynyl ester
16	18.088	252100743	63.43	Mome inositol
17	19.035	6544023	1.65	Hexadecanoic acid, methyl ester
18	19.516	15934297	4.01	N-hexadecanoic acid
19	20.669	1013280	0.25	9,12-octadecadienoic acid (z, z)-, methyl ester
20	20.731	6397519	1.61	9-octadecenoic acid (z)-, methyl ester
21	20.964	1204762	0.30	Methyl stearate
22	21.195	2015839	0.51	9-octadecenoic acid (z)-
23	21.567	1083136	0.27	Octadecanoic acid, ethyl ester
24	22.080	602604	0.15	Oxacyclohexadecan-2-one, 16-methyl-13-ni’
25	22.491	6540877	1.65	Glycidyl palmitate
26	23.977	9357771	2.35	Glycidyloleate
27	24.171	3980523	1.00	Glycidyl palmitate
28	24.436	10885630	2.74	Bis(2-ethylhexyl) phthalate
29	25.530	1911035	0.48	9-octadecenoic acid (z)-, 2,3-dihydroxypropyl ester

30	25.778	3309520	0.83	Oleoyl chloride
31	26.604	2826684	0.71	Decanedioic acid, bis(2-ethylhexyl) ester
32	31.123	1564235	0.39	Spirostan-6-ol, 3-amino-, (3. beta.,5. alpha.,6. alpha.,25r)
33	34.449	1680824	0.42	16-hentriacontanone
34	40.133	7523042	1.89	Cyclodocosane, ethyl-
35	44.203	5493173	1.38	No hit compound
36	48.501	4351466	1.09	Neronine, 4. beta,5-dihydro-

Since SGLT 2 inhibitors have an insulin-independent mechanism of action, these are expected to improve glycemic control with a low risk of major hypoglycemic events. [46] Luseogliflozin lowered glucose levels by promoting urinary glucose excretion in animal models. [47] Results from previous clinical studies have shown that a daily dose of luseogliflozin leads to significant improvements in HbA1c as well as other glycemic parameters. [48] In addition, the drug was found to be well tolerated, with a favourable safety profile. [48, 49]

Molecular docking of the most abundant compounds in sub-fraction 5 (SF5)

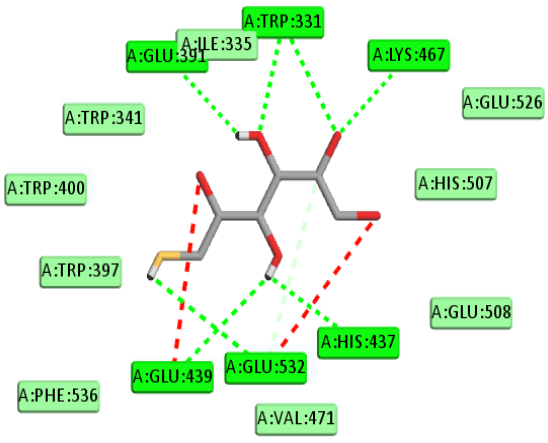
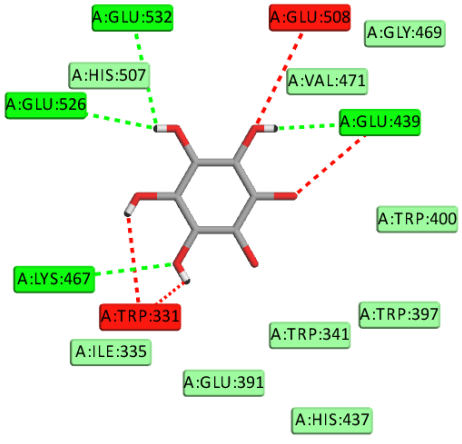
Molecular docking of the most abundant compounds in SF5- Mome inositol and 1-thio-d-glucitol against two key targets in the pathophysiology of diabetes- alpha-glucosidase and SGLT 2 revealed strong affinity between the compounds and the protein targets (Tables 5 and 6). The docking between mome-inositol, 1-D-thio-glucitol and alpha-glucosidase showed that mome inositol had a stronger affinity for the enzyme.

Similarly, for sodium-glucose cotransporter 2 (SGLT 2), mome inositol had a stronger affinity than 1-D-thio-glucitol (Table 5). This observation provides insight into the possible mechanism of action of SF5. The possible inhibition of alpha-glucosidase by SF5 could be useful in blunting post-prandial hyperglycaemia. Inhibition of SGLT 2 is a useful way of reducing hyperglycaemia by preventing the reuptake of filtered glucose in the proximal tubules of the kidney back into circulation. The inhibition of these two key targets by the most abundant compounds in SF5 is a pointer to the therapeutic potential of SF5. These two compounds, therefore, can serve as lead for the discovery of new drugs.

Table 5: Binding Affinity of Ligands against target proteins

Target Proteins	1-thio-d-Glucitol	Mome inositol
Alpa-glucosidase (PDB ID: 2JKP)	-6.1 Kcal/mol	-6.7 Kcal/mol
SGLT2 (PDB ID: 7VSI)	-5.7 Kcal/mol	-6.5 Kcal/mol

Table 6: 2-D diagram of protein-Ligand Interactions

Target Proteins	1-thio-d-Glucitol	Mome inositol
Alpa-glucosidase (PDB ID: 2JKP)		

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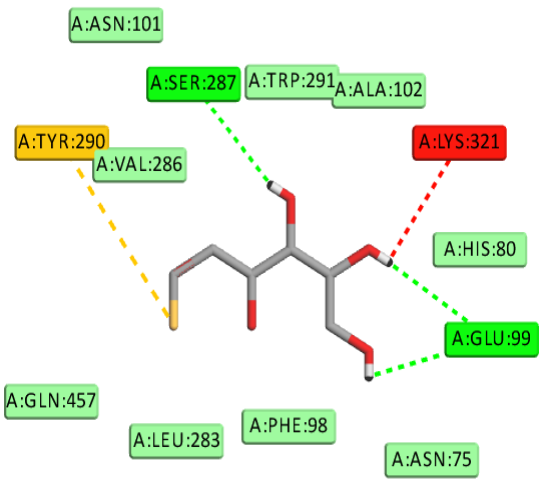
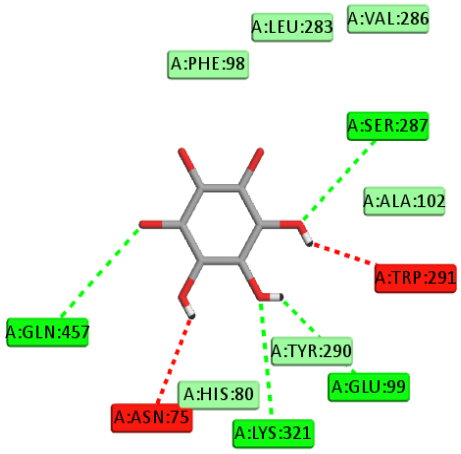
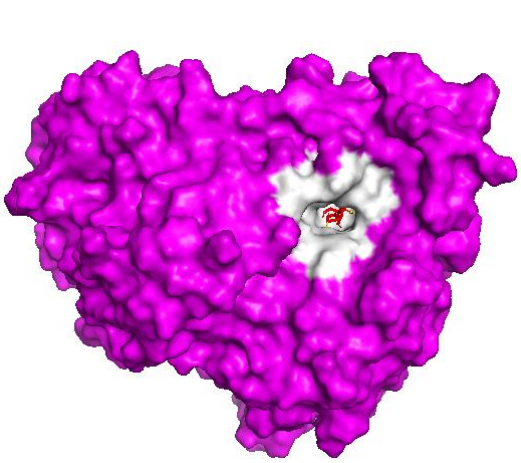
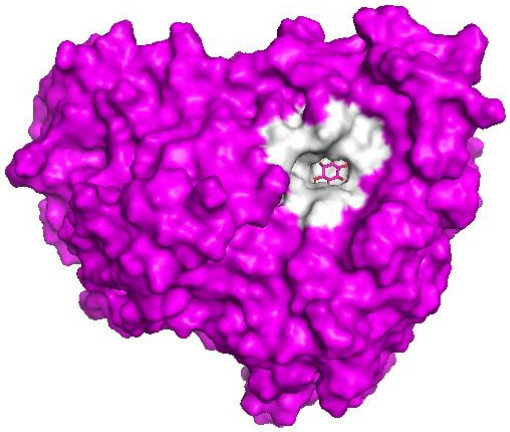
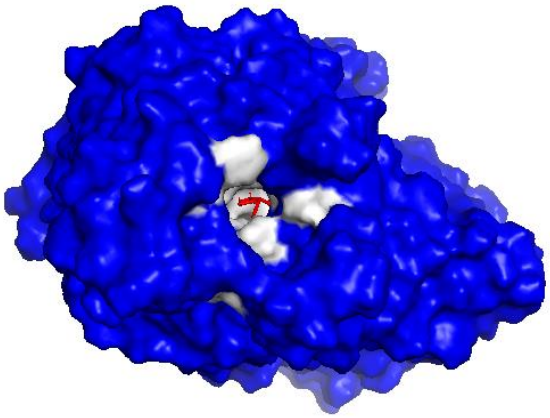
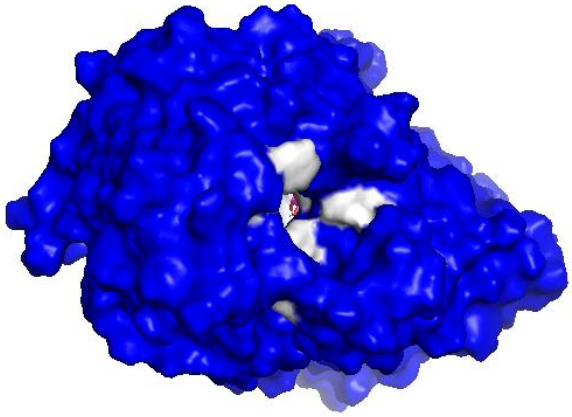
SGLT2 (PDB ID: 7VSI)		

Table 7: 3-D diagram of protein-Ligand Interactions

Target Proteins	**1-thio-d-Glucitol**	**Mome inositol**
Alpa-glucosidase (PDB ID: 2JKP)		
SGLT2 (PDB ID: 7VSI)		

Conclusion

The present study demonstrated that sub- fraction 5 (SF5) obtained from the ethyl acetate fraction of the crude extract of *Tephrosia bracteolata* leaves through column chromatography possesses significant antidiabetic activity against alloxan-induced diabetes mellitus. This was evident in its ability to lower the FBS of diabetic mice at various time intervals. The antidiabetic effect of SF5 may be attributed to the presence of mome-inositol, 2-methoxy-4-vinylphenol, 1-thio-D-glucitol, Hexadecanoic acid, 4-Piperidinone and D-allose, whose presence was confirmed by GC-MS analysis. The most abundant of these compounds (mome-inositol and 1-thio-D-glucitol) showed a high binding affinity for two molecular targets in the pathophysiology of diabetes. This study, therefore, shows that there is a prospective future in the use of *T. bracteolata* leaves as a source of lead molecules for the synthesis of antidiabetic drugs.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. American Diabetes Association Professional Practice Committee. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2022. Diabetes Care. 2022; 45(Supplment_1): S17–S38. https://doi.org/10.2337/dc22-S002.
2. Basit A, Fawwad A, Qureshi H, Shera AS, NDSP Members. Prevalence of diabetes, pre-diabetes and associated risk factors: second National Diabetes Survey of Pakistan (NDSP), 2016–2017. BMJ Open. 2018; 8(8): e020961. https://doi.org/10.1136/bmjopen-2017-020961.
3. Ansari P, Akther S, Hannan JMA, Seidel V, Nujat NJ, Abdel-Wahab YHA. Pharmacologically Active Phytomolecules Isolated from Traditional Antidiabetic Plants and Their Therapeutic Role for the Management of Diabetes Mellitus. Molecules. 2022; 27(13): 4278. https://doi.org/10.3390/molecules27134278.
4. Asante DB, Wiafe GA. Therapeutic Benefit of *Vernonia amygdalina* in the Treatment of Diabetes and Its Associated Complications in Preclinical Studies. J. Diabetes Res. 2023; 1: 3159352. https://doi.org/10.1155/2023/3159352
5. Ning C Jiao, Y, Wang Y, Li W, Zhou J, Lee YC. Recent advances in the management of type 2 diabetes mellitus and natural hypoglycemic substances. Food Sci Hum Well. 2022; 11(5):1121–1133. https://doi.org/10.1016/j.fshw.2022.04.004.
6. Ansari P, Samia JF, Khan JT, Rafi MR, Rahman MS, Rahman AB, Abdel-Wahab YHA, Seidel V. Protective Effects of Medicinal Plant-Based Foods Against Diabetes: A Review on Pharmacology, Phytochemistry and Molecular Mechanisms. Nutr. 2023; 15(14): 3266. https://doi.org/10.3390/nu15143266.
7. Bidulka P, Lugo-Palacios DG, Carroll O, O'Neill S, Adler AI, Basu A, Silverwood RJ, Bartlett JW, Nitsch D, Charlton P. Comparative effectiveness of second-line oral antidiabetic treatments among people with type 2 diabetes mellitus: Emulation of a target trial using routinely collected health data. BMJ Open. 2024; 385: e077097. https://doi.org/10.1136/bmj-2023-077097.
8. Chhabria S, Mathur S, Vadakan S, Sahoo DK, Mishra P, Paital B. A Review on Phytochemical and Pharmacological Facets of Tropical Ethnomedicinal Plants as Reformed DPP-IV Inhibitors to Regulate Incretin Activity. Front. Endocrinol (Lausanne). 2022; 13: 1027237. https://doi.org/10.3389/fendo.2022.1027237.
9. Karigidi KO, Olaiya CO. Antidiabetic activity of corn steep liquor extract of *Curculigo pilosa* and its solvent fractions in streptozotocin-induced diabetic rats. J. Traditional. Complem. Med. 2019; 10(6): 555–564. https://doi.org/10.1016/j.jtcme.2019.06.005.
10. Dalziel JM. *The Useful Plants of West Tropical Africa*. The Crown Agents for the Colonies, London. 1937; Pp 52-560. https://doi.org/10.12691/ajbr-2-4-1.
11. Burkill HM. *The Useful Plants of West Tropical Africa*. Royal Botanical Gardens, Kew, 1985 1:319.
12. Onaolapo MAO, Nzelibe HC, Aduadi AO, Ayo JO. Toxicity and antipyretic studies of the crude extract of *Tephrosia bracteolata* leaves. JOPAT. 2004; 9: 15-19. https://doi.org/10.4314/jopat.v9i1.48589.
13. Idakwoji PA, Oguche M, Sule FA, Oniwon WO, Shaibu IE, Edogbo E, Onoja AO, Ukwubile II. *In vitro* Antidiabetic Activity and Sub-chronic Toxicity Profile of Ethanol Extract of *Tephrosia bracteolata* Leaves. Trop. J. Nat. Prod. Res. 2024; 8(4): 7012-7019. https://doi.org/10.26538/tjnpr/v8i4.37.
14. Egharevba GO, Dosumu OO, Oguntoye SO, Njinga NS, Dahunsi SO, Hamid AA, Anand A, Amtul Z, Ukkujuri P. Antidiabetic, antioxidant and antimicrobial activities of extracts of *Tephrosia bracteolata* leaves. Heliyon. 2019; 5:1-6. https://doi.org/10.1016/j.heliyon.2019.e02481.
15. Derrell C, Gerald FG, Janet CG, Michale EK, Dennis FK. The 1996 Guide for Care and Use of Laboratory Animals. ILAR Journal. 1997; 1:41-48. https://doi.org/10.1093/ilar.38.1.41.
16. Uzor PF, Osadebe PO, Omeje EO, Agbo MO. Bioassay Guided Isolation and Evaluation of the Antidiabetic Principles of *Combretum dolichopetalum* Root, Br. J. Pharm. Res. 2014; 18: 2155-2171.
17. Idakwoji PA, Ekpo DE, Joshua PE, Njoku OU, Nwodo OFC. Ethanol Extract of *Tephrosia bracteolata* Leaves & its Fractions Ameliorates Alloxan-induced Diabetes and its Complications in Wistar Rat Model. Int. J. Diabetes Dev. Ctries. 2021; 41(3): 456–468. https://doi.org/10.1007/s13410-020-00900-w.
18. Cannell RJP. *Methods in Biotechnology, Natural products isolation*. Human Press, New Jersey. Pp 14-36.
19. Trease GE, Evans MC. *Pharmacognosy*. 13th Edn. Bailiere, Tindall. 2000; pp. 200- 775.
20. Harborne JB. *Phytochemical Methods. A Guide to Modern Technology of Plant Analysis*. 3rd Edn. Chapman and Hall, New York. 1998; pp. 88-185.
21. Drake T, Landsteiner A, Langsetmo L, MacDonald R, Anthony M, Kalinowski C, Ullman K, Billington CJ, Kaka A, Sultan S. Newer Pharmacologic Treatments in Adults with Type 2 Diabetes: A Systematic Review and Network Meta-analysis for the American College of Physicians. Ann. Intern. Med. 2024; 177: 618–632. https://doi.org/10.7326/M23-1490.
22. Natarajan V, Smith AA, Vishwanath BA. Antidiabetic Activity of Phenolic Compounds from *Drega volubilis* (Benth) Leaves in Streptozotocin-induced Diabetic Rats. Global J. Pharmacol. 2020; 14:1- 7.
23. Beach EF, Turner JJ. An enzymatic method for glucose determination uptake in body fluids. Clin. Chem. 1958; 4: 462-468.
24. Stanley MP, Venugopal MP. Anti-oxidant action of *Tinospora cordifolia* root extract in alloxan-induced Diabetic rats. Phytother Res. 2001; 15:213-218.
25. Rheney CC, Kirk KK. Performance of three blood glucose meters. Ann Pharmacother. 2001; 34: 317-321.
26. Delano WL. The PyMOL Molecular Graphics System. Delano Scientific, San Carlos. 2002
27. Trott O, Olson AJ. AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading. J. of Comp. Chem. 2010; 31: 455–461. https://doi.org/10.1002/jcc.
28. Akter S, Ali H, Shati AA, Alfaifi MY, Elbehairi SEI, Sayyed RZ, Yeasmin T. Antidiabetic Activity of Methanolic Extract of *Hibiscus sabdariffa* Linn. Fruit in Alloxan-induced Swiss Albino Diabetic Mice. Open Agric. 2024; 9:2022-2043.
29. Momoh S, Yusuf OW, Adamu MM, Agwu COC, Atanu FO. Evaluation of the phytochemical composition and hypoglycaemic activity of methanolic leaves extract of *Costusafer* in albino rats. Br. J. Pharm. Res. 2011; 1:1-8.

30. Oliver-Bever B. Medicinal Plants in Tropical West Africa. London: Cambridge University Press.1986: pp 6. http://dx.doi.org/10.1017/cbo9780511753114.
31. Roni WS, Omri SS, Saar D, Itamar R. Overview of Oxidative Stress and Inflammation in Diabetes. J. Diabetes. 2024; 16: e70014. https://doi.org/10.1111/1753-0407.70014.
32. Idakwoji PA, Joshua PE, Asamodu RO, Njoku OU, Nwodo OFC. Antidiabetic activity, phytochemical and proximate compositions of different extracts of *Tephrosia bracteolata* leaves. Asian J. Plant Sci. 2021; 20:291-299.
33. Furman BL, Candasamy M, Bhattamisra SK, Veettil SK. Reduction of blood glucose by plant extracts and their use in the treatment of diabetes mellitus; discrepancies in effectiveness between animal and human studies. J. Ethnopharmacol. 2020; 247:1122-1164.
34. Roy S, Ghosh A, Majie A, Karmakar V, Das S, Dinda SC, Bose A, Gorain B. Terpenoids as potential phytoconstituent in the treatment of diabetes: From preclinical to clinical advancement. Phytomedicine. 2024; 129:155638.
35. Pak Y, Huang LC, Lilley KJ, Larner J. "In vivo conversion of [3H] myoinositol to [3H] Chiroinositol in rat tissues," J. Biol. Chem. 1992; 267: 16904–16910.
36. Kamalesh, DR, Geetha KM. Role of dietary myo-inositol in diabetes and diabetic complication. J. Chem. Health Risks. 2025; 15:737–750. https://jchr.org/index.php/JCHR/article/view/7530.
37. Quaresima P, Myers SH, Pintaudi B, D'Anna R, Morelli M, Unfer V. Gestational diabetes mellitus and polycystic ovary syndrome, a position statement from EGOI-PCOS. Front. Endocrinol. 2025; 16:1501110. https://doi.org/10.3389/fendo.2025.1501110.
38. Romeo P, D'Anna R. Myoinositol and metformin in the prevention of gestational diabetes in high-risk patients: A narrative review. J. Clin. Med. 2024; 13: 53-87. https://doi.org/10.3390/jcm13185387.
39. Li C, Shi H. Inositol supplementation for the prevention and treatment of gestational diabetes mellitus: A meta-analysis of randomized controlled trials. Arch. Gynecol. Obs. 2024; 309: 1959-1969. https://doi.org/10.1007/s00404-023-07100-x.
40. Corrado FD, Anna R, di Vieste G. "The effect of myoinositol supplementation on insulin resistance in patients with gestational diabetes," Diabet Med. 2011; 28: 972–975.
41. Lubin V, Shojai R, Darmon P, Cosson E. "A pilot study of gestational diabetes mellitus not controlled by diet alone: first line medical treatment with myoinositol may limit the need for insulin," Diabetes Metab. 2016; 42: 192–195.
42. Chand S, Tripathi AS, Dewani AP, Sheikh NWA. Molecular targets for management of diabetes: Remodelling of white adipose to brown adipose tissue, Life Sci. 2024; 345: 122607. https://doi.org/10.1016/j.lfs.2024.122607.
43. Basilio P, Giacoma Di V, Matteo B. The Effectiveness of Myo-Inositol and D-Chiro Inositol Treatment in Type 2 Diabetes. Int. J. Endocrinol. 2016; 16: 1-5.
44. Baigent C, Emberson J, Haynes R, Herrington WG, Judge P, Landray MJ, Mayne KJ, Ng SY, Preiss D, Roddick AJ. Impact of diabetes on the effects of sodium-glucose co-transporter-2 inhibitors on kidney outcomes: Collaborative meta-analysis of large placebo-controlled trials. Lancet. 2022; 400: 1788-1801. https://doi.org/10.1016/S0140-6736(22)02074-8.
45. Palmer SC, Tendal B, Mustafa RA, Vandvik PO, Li S, Hao Q, Tunnicliffe D, Ruospo M, Natale P, Saglimbene V. Sodium-glucose cotransporter protein-2 (SGLT-2) inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists for type 2 diabetes: Systematic review and network meta-analysis of randomized controlled trials. BMJ. 2021; 372: m4573. https://doi.org/10.1136/bmj.m4573.
46. Kurosaki E, Ogasawara H. Ipragliflozin and other sodium-glucose cotransporter- 2 (SGLT2) inhibitors in the treatment of type 2 diabetes: preclinical and clinical data. Pharmacol. Ther. 2013; 139: 51-59. https://doi.org/10.1016/j.pharmthera.2013.04.003.
47. Kakinuma H, Oi T, Hashimoto-Tsuchiya Y. (1S)-1,5-anhydro-1-[5-(4- methoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol (TS-071) is a potent, selective sodium-dependent glucose cotransporter 2 (SGLT 2) inhibitor for type 2 diabetes treatment. J. Med. Chem. 2010; 53: 3247-61.
48. American Diabetes Association Professional Practice Committee. 9. Pharmacologic Approaches to Glycemic Treatment: Standards of Care in Diabetes- 2025. Diabetes Care. 2025; 48: S181–S206. https://doi.org/10.2337/dc25-S009.
49. Seino Y, Sasaki T, Fukatsu A. Dose-finding study of luseogliflozin in Japanese patients with type 2 diabetes mellitus: a 12-week, randomized, double-blind, placebo-controlled, phase II study. Curr. Med. Res. Opin. 2014; 30:1238-1245.