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Identification of bioactive antidiabetic compounds from *Teucrium polium* L. enhancing glucose uptake in HepG2 cells

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ABSTRACT

The global rise in diabetes prevalence has intensified the search for more effective glucose-lowering agents derived from natural sources. *Teucrium polium* L. (Lamiaceae), referred to as "golden germander," has been traditionally used for centuries in Middle Eastern and Mediterranean regions as a medicinal plant for treating of diabetes and other metabolic disorders. Although its antihyperglycemic properties have been previously reported in ethnobotanical and pharmacological studies, the specific bioactive components responsible for its antidiabetic properties remain inadequately characterized. The total ethanol/water (70:30) extract of *Teucrium polium* aerial parts was extracted sequentially using organic solvents (*n*-hexane, ethyl acetate, and chloroform). The glucose uptake potential of each fraction was evaluated in HepG2 cells using the fluorescent glucose analogue 2-NBDG. The most active extract (ethyl acetate) underwent bioassay-guided purification involving column chromatography and preparative high-performance liquid chromatography. Purified compounds were identified using high-resolution tandem mass spectrometry. The resazurin assay was employed to assess cell viability. The ethyl acetate extract demonstrated significant glucose uptake activity (~76 % efficacy relative to metformin positive control) without notable cytotoxicity. Bioassay-guided purification resulted in the identification of eight active compounds: Teupolin XII, Forsythoside H, Crisiliol, Cynaroside, Cosmosioside, Cirsilineol, 24-Methylenecycloartanol, and Moretenyl acetate. All purified compounds significantly enhanced glucose uptake (>70 %) and displayed minimal cytotoxic effects (>90 % cell viability at effective concentrations). The results highlight the capacity of *Teucrium polium* to yield bioactive compounds with therapeutic potential for diabetes, particularly by enhancing glucose uptake in HepG2 cells.

1. Introduction

As a persistent metabolic disorder, diabetes mellitus (DM) exerts a profound impact on global health by elevating the risk of numerous complications and contributing to high rates of illness and death. DM is a multifactorial disorders of metabolism defined by sustained hyperglycemia, which commonly result from insufficient insulin resistance, insulin secretion, or combination of both (Goyal et al., 2023). As of 2017, diabetes was estimated to affect approximately 451 million individuals worldwide, with projections indicating an increase to 693 million cases by 2045, driven by aging populations, sedentary lifestyles, and increasing rates of obesity (Ong et al., 2023). The diabetes results in intensified by chronic complications, such as cardiovascular disease, nephropathy, neuropathy, and retinopathy, which collectively reduce patients quality of life and contribute significantly to the financial strain on healthcare systems (Yang et al., 2025).

Conventional antidiabetic therapies primarily rely on synthetic glucose-lowering agents such as insulin, sulfonylureas, and metformin. Although effective in managing blood glucose levels (BGL), these agents are frequently linked to notable side effects, including hypoglycemia, gastrointestinal distress, and long-term risks of resistance or diminished effectiveness. These limitations, coupled with the growing prevalence of

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diabetes, have spurred interest in alternative, more sustainable therapeutic strategies (Dalal et al., 2023; Hammoud et al., 2025; Yang et al., 2025). Among available options, medicinal plants and derived compounds have emerged as a promising avenue due to affordability, accessibility, and lower side effect profiles (Balgoon and Alghamdi, 2024; Elzaiat et al., 2024; Mousavi et al., 2025; Subtain et al., 2023; Asghari et al., 2015). Traditional plant-based remedies are increasingly recognized as valuable sources of bioactive compounds with antidiabetic potential, and the mechanisms underlying their actions are being systematically investigated (Sknepnek et al., 2025; Uti et al., 2025).

T. polium L., a species within the Lamiaceae family, is recognized for its broad pharmacological potential, including anti-inflammatory, antioxidant, vasorelaxant, cognitive-enhancing, antidiabetic, and lipid-lowering activities (Asghari et al., 2020; Sadeghi et al., 2022). Also, *T. Polium* has anti-carcinogenic (Movahedi et al., 2014), anti-hypertensive (Mahmoudabady et al., 2014), antinociceptive (Baluchnejadmojarad et al., 2005), and cardioprotective properties (Mahmoudabady et al., 2018). Additionally, its antidiabetic activity is exerted through various molecular pathways, including attenuation of oxidative stress, stimulation of insulin secretion, regeneration of pancreatic β-cells, inhibition of α-amylase activity, and enhancing muscular glucose uptake (GU) by facilitating GLUT-4 translocation. (Bahramikia and Yazdanparast, 2012). Multiple *in vitro* and *in vivo* investigations have demonstrated the antidiabetic potential of *T. polium*, highlighting its antihyperglycemic ability and improve lipid profiles. In streptozotocin (STZ)-induced diabetic rats, *T. Polium* aqueous extracts substantially reduced BGLs following intravenous and intraperitoneal treatment, likely through effects on peripheral glucose metabolism rather than insulin release (Ardestani et al., 2008; Esmaeili and Yazdanparast, 2004; Gharaibeh et al., 1988). Oral treatment with *T. polium* extracts in STZ-induced diabetic rats consistently lowered serum glucose levels, restoring normoglycemia within a few days (Zal et al., 2001). Hydroalcoholic extracts were given orally over 14 days also improved lipid profiles by reducing cholesterol, triglycerides, and low-density lipoproteins while exhibiting hepatoprotective properties (Ayoubi et al., 2013; Sarkarizi et al., 2015; Tabatabaie and Yazdanparast, 2017). Crucially, no study to date has isolated and quantified the glucose uptake-enhancing activity of its individual components in hepatocyte-derived cell lines.

Phytochemical studies on *T. Polium* have revealed a rich composition of bioactives, with flavonoids, terpenoids, and iridoids, many of which exhibit strong antioxidant and insulinotropic activities (Bahramikia and Yazdanparast, 2012; Tepe et al., 2021; Sadeghi et al., 2022). While such studies have established its general antidiabetic potential, most research to date has focused on crude or partially fractionated extracts, without clearly identifying the specific compounds responsible for glucose-uptake effects. This gap in compound-level identification limits the development of standardized therapeutics derived from *T. polium*. To address this gap, a bioassay-guided fractionation strategy was implemented, integrating high-resolution mass spectrometry with chromatographic purification, to isolate and identify active constituents. The biological activity of the purified compounds was evaluated using -[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (2-NBDG) in HepG2 cells, and their cytocompatibility was assessed via the resazurin assay. This approach enables a functional linkage between phytochemical content and glucose uptake enhancement, offering novel insight into specific small molecules from *T. polium* with potential to modulate hepatic glucose metabolism through insulin-independent pathways.

2. Material and methods

2.1. Plant and chemical material

T. Polium arial parts were collected at full flowering stage (June 2019) from the Zagros Mountains around Yasooj, Kohgiluyeh and Boyer-Ahmad Province, Iran, and Dr. A. Sonboli identified it. At the herbarium of the Medicinal Plants and Drugs Research Institute of Shahid Beheshti University a voucher specimen was archived (MPH-3271).

In all cases, HPLC grade solvents were parched from Chem-Lab NV (Zedelgem, Belgium). The HepG2 cells were obtained from the KU Leuven University's zoology department's cell bank. The human insulin were purchased from Sigma-Aldrich (St. Louis, MO, USA); the fetal bovine serum (FBS), modified Eagle's medium (MEM), the PD98059 and palmitate were purchased from Hyclone (Logan, UT, USA). Additionally, water from a Milli-Q system (Millipore, Bedford, MA, USA) was obtained.

Table 1
HPLC condition applied for the active fractions.

Fraction	gradient
F61	0–5 min, 5 % B; 5–8 min, 5–15 % B; 8–38 min, 15 % B; 38–60 min, 15–100 % B; 60–65 min, 100 % B
F77	0–5 min, 5 % B; 5–8 min, 5–15 % B; 8–38 min, 15 % B; 38–85 min, 15–100 % B; 85–90 min, 100 % B

2.2. Extraction

The aerial parts (2.0 kg) were washed, dried in the laboratory without direct sunlight, ground into powder, and subjected to ethanol-water (70:30, v/v) extraction 4 times (10 L × 4). Using a rotary evaporation at 40 °C solvent was removed, yielding 150 g of dried extract, which was stored at 4°C for subsequent analysis. This was followed by a solvent-solvent fractionation. 500 mL of water were used to suspend the dried extract. This mixture was then extracted using different solvents, including *n*-hexane, chloroform, and ethyl acetate. Extraction was carried out in 6 cycles for each solvent with a solvent-to-suspension ratio of 3:1. Each solvent-derived extract was concentrated. As a result, 12 g of the *n*-hexane fraction, 100 g of the chloroform fraction, and 23 g of the ethyl acetate fraction were obtained from the initial hydroalcoholic extract.

2.3. Separation

2.3.1. Column chromatography separation

Silica gel column chromatography (ECOPLUS 35 × 500 mm, YMC, Kyoto, Japan) was employed to separate the constituents of the ethyl acetate fraction. In brief, 18 g of the extract was blended with 70 g of silica gel and subjected to preparative liquid chromatography using a Waters Delta 600 system (multi-solvent quaternary pump, detector 2487, controller 600; Waters, MA, USA). The mobile phase included of *n*-hexane (A), ethyl acetate (B), methanol (C), and 25 % acetic acid in methanol (D). At a flow rate of 40 mL/min a gradient elution was performed, starting from 95 % A and 5 % B and progressing to 100 % D in 5–20 % increments every 10 min. After collecting 215 fractions (1 fraction/minute), stocks for GU testing were prepared by drying 1 mL of every fraction and diluting with 100 μL DMSO.

2.3.2. HPLC separation

Two active fractions (F61 and F77) were selected relying on the GU assay results of 215 fractions. Separation was performed using HPLC (LC-20AT pumps with SPD-M20A detectors and DGU-20A3R degassers, SHIMADZU, Kyoto, Japan). For semi-preparative separation, the Polar-RP column (250 × 10 mm, 4 μm, Polar-RP, Synergi, Phenomenex) uses a mobile phase consisting of water (A) and acetonitrile (B), both containing 0.1 % Trifluoroacetic acid (TFA). For both fractions, the injection amount was 2 mg, the flow rate set at 3 mL/min for the semipreparative column, and subfractions (1/minute) were collected via gradient elution using the conditions listed in Table 1.

2.4. UHPLC-HRMS-MS condition

An Ultimate 3000 UHPLC system (Dionex Thermo Scientific) equipped with a C18 EasySpray column (50 μm × 15 cm, 2 μm, Thermo Scientific) and coupled to an Orbitrap Elite mass spectrometer (Thermo Scientific, USA) was used for analysis. The mobile phase included of water (solvent A) and acetonitrile (solvent B), both containing 0.1 % formic acid. A stepwise gradient elution was implemented accordingly: 5 % B from 0–3 min, ramping to 100 % B from 3–20 min, held at 100 % B for 3 min (20–23 min), followed by a return to 5 % B from 23–24 min and maintained until 34 min. The flow rate set at 0.3 μL/min, with a column temperature of 35 °C and 5 μL injection volume was used. The 20 most intense ions for MS/MS fragmentation over an *m/z* range of 50–2000 were selected. Key MS parameters such as capillary temperature, and ion source temperatures, spray voltage and RF amplitude for both ITMS and FTMS were optimized based on previously established protocols (Hu et al., 2021).

2.5. Cell culture

For HepG2 cells, 5 % CO_2 at 37 °C and 10 % FBS were used as the culture medium. Cells were incubated with penicillin and streptomycin. Cell culture media were changed every two days. After 48 h of incubation in 96-well plates, PBS was used to wash three times the cells. Subsequently, the cells were cultured in Minimum Essential Medium (MEM).

2.5.1. Cell viability

The resazurin assay was employed to assess cell viability. Cells were counted and the dilution was calculated before plating 2 × 10^5 cells per well. With different sample concentrations (2.5, 5, 7.5, 10, and 20 μg/mL) with 5 % CO_2 for 24 h at 37 °C cells were treated. In each well, added 10 μL of resazurin after 24 h. Incubation took place for 4 h at 37 °C. A FlexStation (Molecular Devices, USA) with wavelength excitation at 550 nm and emission wavelength at 590 nm was used for reading the results (Borra et al., 2009).

2.5.2. Glucose uptake measurement

Based on Yamamoto et al. a black, transparent-bottom 96-well plate was seeded with 2 × 10^5 HepG2 cells for 48 h for GU assay. Plates were cultured with MEM medium. Two washes were performed with serum-free, glucose-free MEM medium after 48 h. A volume of 200 μL culture medium was added per well. The plates were divided into three groups: a. Samples; B. positive control (metformin); c. negative control (DMSO). In all samples DMSO final concentration was less than 1.0 %. PBS was used to wash cells twice after 24 h to remove any remaining culture medium. Subsequently, 100 μL/well of 2-NBDG solution was added to each well. The plates were incubated at 37 °C for 30 min in an atmosphere containing 5 % CO_2. 2-NBDG is prevented by treating cells three times with ice-cold PBS. The FlexStation II spectrofluorometer (Molecular Devices, USA) measured fluorescence at wavelengths between 460 nm and 490 nm and 530 nm and 550 nm (Yamamoto et al., 2015).

2.6. Data processing and analyses

To ensure experimental reliability, all assays were conducted in triplicate. Inhibition percentages derived from dose–response analyses were calculated and statistically evaluated using GraphPad Prism. Mass spectrometric data were interpreted using multiple platforms, including Xcalibur 4.2 and Freestyle™ 1.5 (Thermo Scientific, USA), along with ACD/MS Workbook Suite 2021 integrated with MS Fragmenter and ChromGenius (ACD/Labs, Canada).

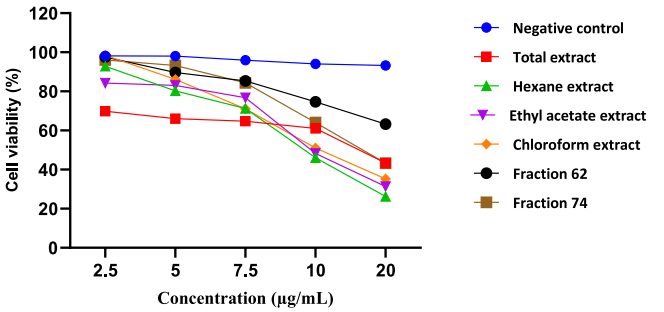


Fig. 1. Cell viability (%) of T. Polium extracts and fractions at different concentrations (2.5–20 μg/mL), determined by resazurin assay.

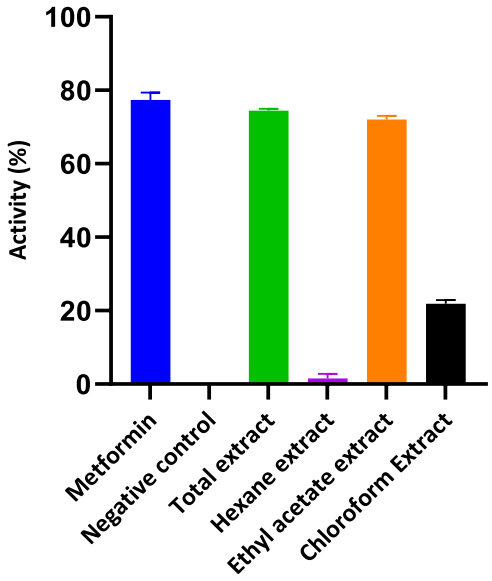


Fig. 2. GU activity (%) of HepG2 cells treated with different T. Polium extracts compared with metformin (positive control) and untreated negative control.

3. Result and discussion

3.1. Cell viability

The impact of *T. Polium* extracts on the viability of HepG2 cells was assessed to confirm that any observed enhancement in GU was not a result of cytotoxic stress. Cell viability assays conducted over a wide concentration range (1–20 μg/mL) indicated no significant toxicity associated with the crude ethanol/water extract or its solvent fractions at elevated doses, as cell survival remained above approximately 90 % at moderate concentrations (Fig. 1). This finding aligns with existing literature indicating that *T. Polium* demonstrates minimal toxicity in hepatic cells and may exhibit hepatoprotective effects at moderate doses (Rahmouni et al., 2021). In some literature, kidney tissue toxicity and *T. Polium* have also been demonstrated to cause liver toxicity. *T. Polium* has a minor side effect, according to Scognamiglio et al.; it can be safely used (Albadr et al., 2022; Scognamiglio et al., 2012). Some additional literature supported this statement. Al-Asmari et al. (2014) noted that due to its anti-inflammatory and antioxidant constituents, *T. polium* exhibited only mild toxicity at elevated concentrations in cultured hepatocytes (Al-Asmari et al., 2014). Similarly, Kulevanova et al. (2006) described a hepatoprotective effect associated with *T. polium*, further supporting its potential safety in therapeutic applications (Kulevanova et al., 2006).

3.2. Glucose uptake enhancement by extracts and fractions

Exposure of HepG2 cells to *T. polium* extracts resulted in a enhancement of GU compared to untreated controls. The hydroalcoholic extract (75 % ethanol) enhanced GU in HepG2 cells, demonstrating the intrinsic antidiabetic potential of the plant extract (Fig. 2). Among the solvent-extractions, the ethyl acetate extract exhibited the highest GU activity among all extracts. Notably, this fraction achieved approximately 73 % of the maximum glucose-lowering response observed with the positive control (metformin-treated cells), far outperforming the *n*-hexane and chloroform fractions. In contrast, those non-polar fractions showed little to no enhancement of GU. These findings support the hypothesis that *T. polium* contains compounds capable of enhancing hepatic glucose uptake through potentially insulin-independent mechanisms. The significant activity of the ethyl acetate fraction suggests that mid-polar phytoconstituents, such as flavonoids and phenylpropanoids, may underlie this effect. This aligns with therapeutic strategies that target hepatic glucose metabolism as a complementary route to classical insulin-centered approaches. The superior efficacy of the mid-polarity ethyl acetate fraction suggests that the key bioactive compounds are of intermediate polarity, which is in agreement with prior studies indicating that relatively polar extracts of *T. Polium* possess the strongest antidiabetic effects (Ardestani and Yazdanparast, 2007). This *in vitro* GU result aligns well with the plant's ethnomedicinal use for diabetes and provides a cellular basis for its efficacy. The findings dovetail with a body of *in vivo* evidence for *T. Polium*'s antihyperglycemic activity. Since the late 1980s, multiple studies have documented that *T. Polium* extracts reduce blood glucose in diabetic animal models (Albadr et al., 2022; Bahramikia et al., 2022). *T. polium* aqueous extract considerably lowered BGLs following both intravenous and intraperitoneal administration, with hypoglycemic effects observed in both streptozotocin (STZ)-induced diabetic and normal rats (Asghari et al., 2020). Moreover, chronic oral administration of *T. Polium* extract at 0.5 mg/kg for 30 days lowered significantly BGLs (p 0.0001) in STZ-induced diabetic rats compared with STZ-induced diabetic controls (Ardestani et al., 2008; Yazdanparast et al., 2005). There was no positive control in these studies, so further analysis may be needed to confirm the results. *T. Polium* extract administered by mouth to normoglycemic rats at 125 mg/kg demonstrated significant reductions after a single dose after 4 h in BGLs, superior to glibenclamide (Stefkov et al., 2011). These *in vivo* outcomes corroborate the cell-based results, implying that the GU-enhancing activity observed in HepG2 cells is translated to whole-body glucose homeostasis in animal models. *T. Polium* ability to improve glycemic control has been attributed not only to peripheral tissue effects (enhanced GU) but also to pancreatic action. Prior *ex vivo* studies on isolated rat pancreas showed that *T. Polium* extracts can stimulate glucose-stimulated insulin secretion (GSIS), an effect credited partly to flavonoid constituents like apigenin (Mirghazanfari et al., 2010). *T. Polium* hydroalcoholic extract effects at 1–100 mg/mL in glucose solutions 2 and 16 mmol/L was also reported by Yazdanparast et al. on rat pancreatic islets that were isolated and were able to stimulate ($p = 0.001$) GSIS. However, the effect was decreased at 1000 mg/mL (Yazdanparast et al., 2005). This effect was linked to the cytotoxic properties at higher concentrations. Furthermore, studies have demonstrated that treatment with *T. polium* in STZ-induced diabetic rats promoted regeneration of pancreatic islets, an effect potentially linked to the quercetin within the extract (Albadr et al., 2022).

The enhancement of GU by *T. Polium* extracts in HepG2 cells indicates an insulin-independent stimulation of glucose transporter activity. HepG2 is an insulin-insensitive hepatocellular model (largely relying on insulin-independent glucose transporters), so the observed increase in 2-NBDG uptake suggests that the extract's components likely activate post-receptor pathways to augment glucose entry into cells (Kasabri et al., 2012). One plausible mechanism is activation of AMP-activated protein kinase (AMPK) or related signaling pathways, ultimately enhancing the translocation of glucose transporters to the cell membrane. Indeed, natural compounds that activate AMPK can promote GU even in the absence of insulin. The results find support in a Kadan et al. study, demonstrated that *T. Polium* extracts in muscle tissue facilitate GLUT4 transporter translocation via both insulin-dependent and independent pathways. In that study, methanol and n-hexane extracts of *T. polium* enhanced glycemic control in diabetic mice by promoting GLUT4 translocation to the muscle cell membrane, thereby facilitating glucose clearance (Kadan et al., 2013). Although experiments did not directly measure GLUT transporter movements, the substantial uptake increase implies a similar results in HepG2 cells, potentially through AMPK activation or PI3K/Akt pathway modulation. Additionally, the known insulinotropic effect of *T. Polium* flavonoids might amplify insulin signaling even in non-pancreatic cells by indirectly enhancing glucose utilization (Martín and Ramos, 2021)(Martín and Ramos, 2021)(Martín and Ramos, 2021). The convergence of insulin secretion mechanism support and peripheral uptake facilitation, underlies the pronounced antihyperglycemic effect of *T. Polium* observed *in vivo* (Martín and Ramos, 2021)(Martín and Ramos, 2021)(Martín and Ramos, 2021).

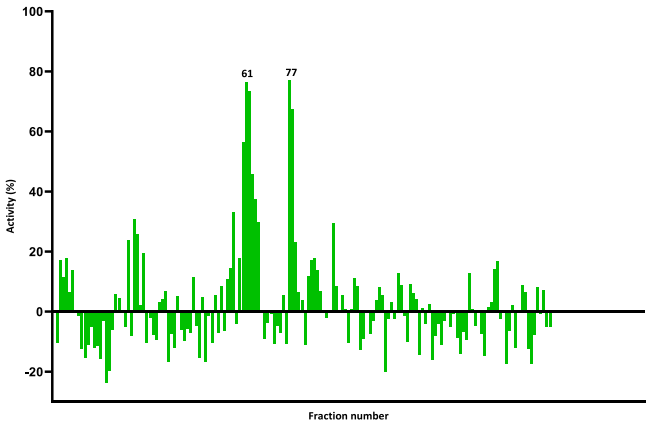


Fig. 3. GU activity (%) of HepG2 cells treated with fractions (1–215) obtained from bioassay-guided chromatography of the T. Polium ethyl acetate extract. Active fractions (F61, and F77) selected for further purification are indicated.

3.3. Bioassay-guided fractionation and activity profiling

A bioassay-guided isolation approach was employed to fractionate the active ethyl acetate extract using column chromatography, yielding 215 sub-fractions that were each tested for GU activity in HepG2 cells. The GU activity profile across these fractions exhibited two pronounced peaks of bioactivity (Fig. 3). One cluster of active fractions emerged around fraction F60–F65, and another distinct spike was observed around fraction F75–F80, indicating the presence of at least two separate groups of active constituents. In particular, fractions 61, and 77 showed consistently high GU stimulation in repeated assays, standing out as peak active fractions. All other fractions either showed baseline-level activity or only minor effects. The fact that the bioactivity concentrated in two relatively narrow fraction ranges suggests that a limited number of compounds (or synergistic co-eluting compounds) are responsible for the extract's overall effect. To ensure the reliability of these findings, each fraction was assayed in duplicate, and the reproducibility of the activity profile was confirmed – the same fraction ranges were active upon re-testing, ruling out false positives. Thus, guided by the activity profile, fractions 61, and 77 were selected as candidates for further purification since these two fractions represented the centers of the two major activity peaks.

3.4. Identified compounds via LC-HRMS/MS

HPLC separation was performed individually on the two most active

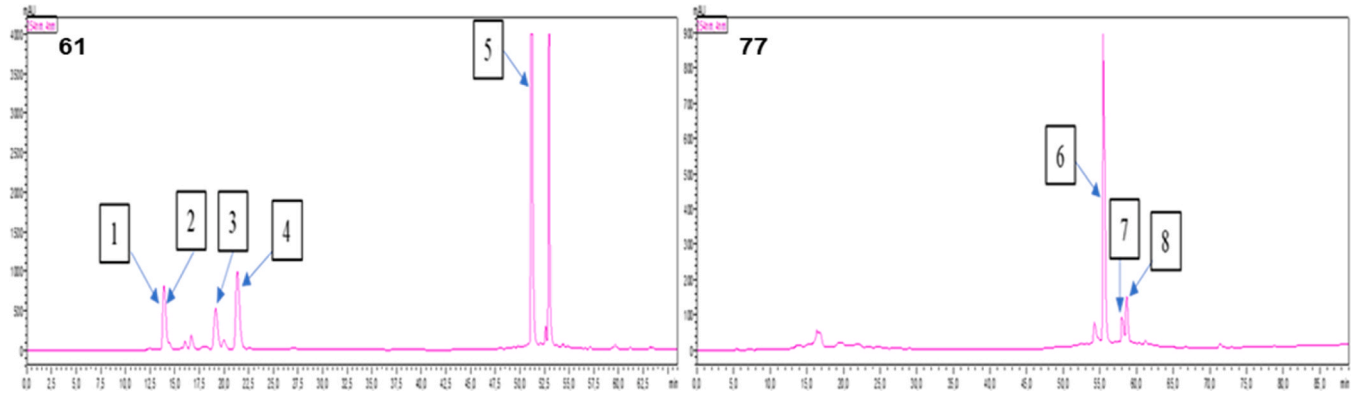


Fig. 4. HPLC–UV chromatograms of the active fractions (F61 and F77) from T. Polium ethyl acetate extract, with arrows indicating peaks corresponding to bioactive compounds selected for further analysis.

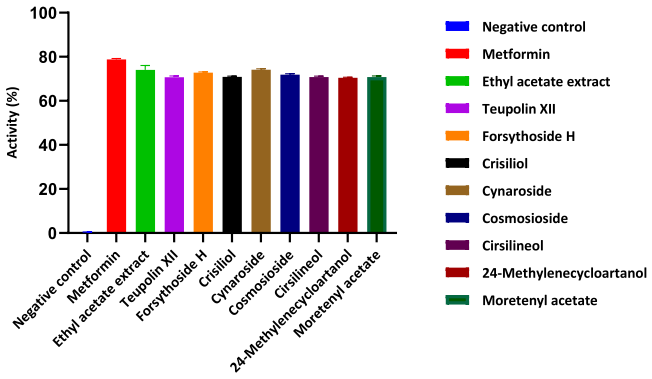


Fig. 5. GU activity (%) of isolated bioactives on HepG2 cells treated from T. polium. Metformin and negative control are included.

fractions (F61 and F77) to purify the constituent compounds.The HPLC–UV chromatograms of these fractions (Fig. 4) revealed multiple well-resolved peaks in each, indicating that each bioactive fraction contained a mixture of compounds. Each peak was collected and analyzed for activity, which allowed us to pinpoint the specific compounds contributing to the GU enhancement. In total, eight bioactive compounds were isolated from fractions F61 and F77.The HPLC peak shapes and retention times before and after isolation confirmed that the compounds were obtained in pure form without degradation or transformation during the purification process. Each isolated compound reproducibly increased GU in the bioassay (with each yielding ≥70 % of the maximal activity) (Fig. 5), confirming that compounds were underlying the biological effects of the parent fractions.

Bioassay-guided fractionation of ethyl acetate extract resulted in isolation of 8 compounds implicated in promoting GU. All eight peaks, along with several blank samples, in positive ion mode using a UHPLC-Orbitrap-Ion Trap mass spectrometer under optimized conditions were analyzed. Comparison with predicted fragmentation patterns using MS Fragmenter identified these compounds as: Teupolin XII, Forsythoside H, Crisiliol (Cirsiliol), Cynaroside (luteolin-7-O-glucoside), Cosmosioside (apigenin-7-O-glucoside), Cirsilineol, 24-methylenecycloartanol, and moretenyl acetate (Table 2, Fig. 6). The HRMS data for each compound showed an excellent match between the observed molecular ions and the theoretical masses (with mass errors on the order of 0.0–0.1 Da), and the MS/MS fragmentation patterns (Table 2) were consistent with literature or database spectra for these structures. Each isolated compound, tested individually, retained significant activity, with at least ~70 % of the maximal GU-stimulation observed for the parent fraction. These findings provide direct evidence that multiple constituents of *T. Polium* act in concert to produce the overall antidiabetic effect. Notably, the chemical diversity of the identified hits is wide, including clerodane diterpenoids (Teupolin XII), phenylethanoid glycosides (Forsythoside H), flavonoid aglycones (cirsiliol, cirsilineol), and flavonoid glycosides (cynaroside, cosmosioside), as well as pentacyclic triterpenes/sterols (24-methylenecycloartanol, and moretenyl acetate). This diversity suggests a multi-target pharmacological profile, where

Table 2
Identification and mass spectrometric characteristics of isolated compounds from the T. Polium extract using UHPLC-HRMS/MS analysis.

	Name	tR (min)	Formula	Mass	$[M+H]^+$	$[M-H]^-$	$M^{+\cdot}$	$M^{-\cdot}$	Mass Difference (Da)	MS/MS fragments
1	Teupolin XII	9.90	$C_{21}H_{28}O_6$	376.1886	377.1959	375.1813	376.188	376.189	0.055	302.174, 292.191, 209.119, 208.135
2	Forsythoside H	13.18	$C_{29}H_{36}O_{15}$	624.2054	625.2127	623.1981	624.205	624.206	0.000	629.044, 627.004, 625.998, 597.046, 595.053, 345, 212, 288.049, 285.045
3	Crisiliol	14.41	$C_{17}H_{14}O_7$	330.0740	331.0812	329.0667	330.073	330.075	0.007	699.092, 683.121, 369.027, 353.055, 351.540, 331.074, 315, 153
4	Luteolin−7-glucoside (Cynaroside)	14.51	$C_{21}H_{20}O_{11}$	448.1006	449.1078	447.0933	448.100	448.101	0.100	450.211, 449.208, 433.191, 432.201, 403.198, 402.191, 401.187
5	Apigenin−7-glucoside (Cosmosioside)	16.95	$C_{21}H_{20}O_{10}$	432.1056	433.1129	431.0984	432.105	432.106	0.023	433.113, 219.044, 370.105, 368.089, 261.112, 236.047
6	Anisomelin (Cirsilineol)	17.97	$C_{18}H_{16}O_7$	344.0896	345.0969	343.0823	344.089	344.090	0.025	399, 389, 319, 375, 247
7	24-Methylenecycloartanol	19.36	$C_{31}H_{52}O$	440.4018	441.4091	439.3945	440.401	440.402	0.004	522, 340, 300, 289,239, 123
8	Moretenyl acetate	20.91	$C_{32}H_{52}O_2$	468.3967	469.4040	467.3895	468.396	468.397	0.027	469.431, 468.396, 450, 423.415, 422.384, 368, 184

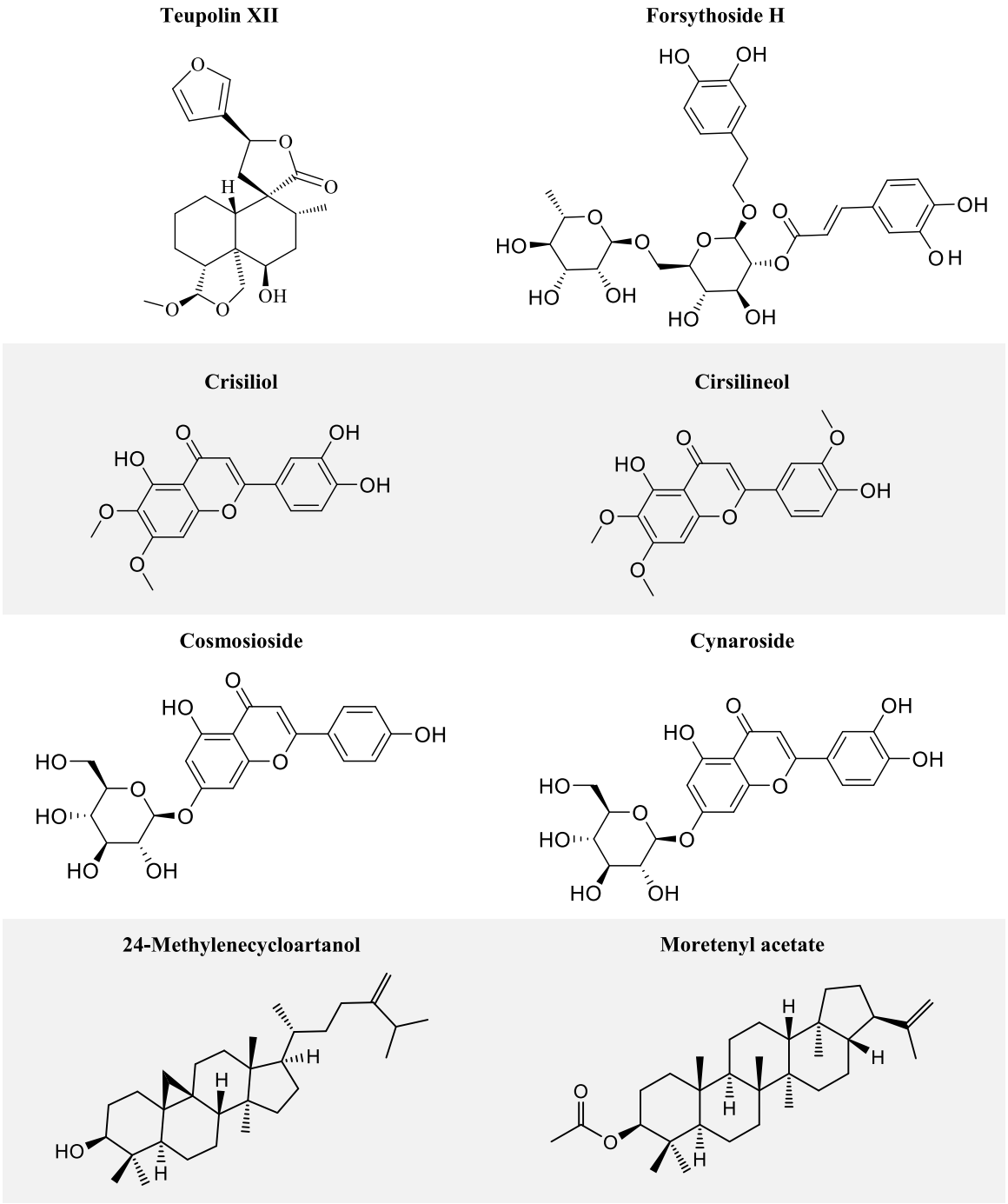


Fig. 6. Chemical structures of isolated compounds from the *T. polium* extract.

different compounds may modulate distinct yet complementary pathways of glucose homeostasis. To our knowledge, this is the first report that isolates and identifies *T. polium* compounds with quantifiable activity in a human hepatic cell GU-assay. Previous studies have primarily focused on *in vivo* models or crude extracts without delineating specific active principles. The identification of flavonoid glycosides such as cymaroside, cosmosioside, and terpenoids like moretenyl acetate, provides molecular insight into the mechanisms underlying the plant antidiabetic effects.

3.5. Cell viability of pure compounds

All eight purified compounds exhibited minimal cytotoxicity at their active concentrations (Fig. 7). The viability of HepG2 cells remained elevated (generally exceeding 90 %) when exposed to each isolated compound at a concentration of 5 μg/mL, utilized for GU assays. The absence of significant cytotoxicity, particularly in conjunction with notable glucose uptake activity, highlights the therapeutic promise of these compounds. Previous toxicological studies on components isolated from *T. polium* and related plant species support this paper findings. Among the compounds isolated, Teupolin XII, a neo-clerodane diterpenoid, was specifically studied by Fiorentino et al. (2011), who demonstrated that neo-clerodane diterpenes exhibited low cytotoxicity towards HepG2 cells even at concentrations as high as 200 μM, thus indicating a favorable safety profile at biologically relevant doses (Fiorentino et al., 2011). Forsythoside H,

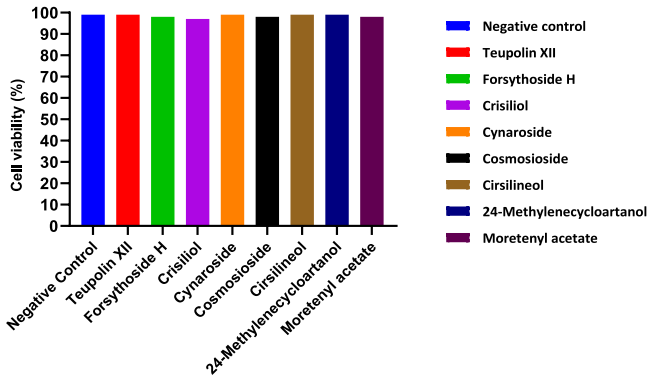


Fig. 7. Cell viability (%) of HepG2 cells treated with isolated bioactive compounds from T. Polium at 5 μg/mL, assessed using the resazurin assay.

a phenylethanoid glycoside, is another critical compound that was isolated. Phenylethanoid glycosides, such as forsythosides, have been extensively studied for their anti-inflammatory and antioxidant properties, which may help reduce inflammation-induced toxicity and oxidative stress. Forsythiasides have been extensively documented to protect against oxidative stress via activation of the Nrf2/HO-1 pathway, providing hepatoprotective effects and further supporting the observed high viability of treated cells in experiments. Flavonoids identified in our study, including cynaroside (luteolin-7-O-glucoside), cosmosioside (apigenin-7-O-glucoside), crisiliol, and cirsilineol, are well-known for possessing broad therapeutic windows and minimal cytotoxicity in numerous cellular models. For instance, luteolin glycosides like cynaroside have demonstrated significant anti-inflammatory, and antioxidant properties, often linked to hepatoprotective rather than hepatotoxic effects. Apigenin glycosides have also been documented to possess strong anti-inflammatory and antioxidant effects, with minimal toxicity observed at therapeutically relevant doses (Asmaey et al., 2024; Wang et al., 2024).

The triterpenoids isolated, 24-methylenecycloartanol and moretenyl acetate, represent another class of phytochemicals typically associated with low toxicity. Various triterpenoids and sterols are known for a wide therapeutic index and beneficial metabolic effects, including modulation of cholesterol metabolism and improvement in insulin sensitivity, with no significant reports of acute hepatotoxicity at therapeutic doses (Asmaey et al., 2024). While *in vitro* results strongly indicate the safety of these compounds at the doses tested, it remains critical to interpret these findings within the broader context of herbal safety literature. Indeed, some concerns have been raised historically about liver toxicity associated with the chronic or high-dose consumption of *T. polium* extracts. However, such hepatotoxicity has primarily been attributed to specific furano-diterpene derivatives (teucrin A), rather than the glycosylated flavonoids or other non-furanic diterpenoids that was isolated (Fiorentino et al., 2011; Stefkov et al., 2011). Importantly, the isolated compounds differ structurally and chemically from the known hepatotoxic constituents, suggesting a significantly reduced or negligible risk at therapeutic doses.

In this context, current paper findings align well with previous toxicological assessments indicating mild or negligible hepatotoxicity of *T. polium* fractions and isolated compounds at moderate doses. For example, Fiorentino et al. (2011) emphasized the generally low cytotoxicity of purified neo-clerodane diterpenoids towards HepG2 cells, supporting potential for safe therapeutic application (Fiorentino et al., 2011). Al-Asmari et al. (2014) likewise discovered that *T. polium* showed mild hepatotoxicity only at significantly high concentrations, likely due to the protective antioxidant constituents abundantly present in its extracts (Al-Asmari et al., 2014). These studies collectively underscore that while caution in dosing and prolonged use is always warranted, the isolated compounds from the current study are likely safe and potentially beneficial at therapeutic concentrations.

Overall, our findings substantiate the hypothesis that *T. polium* contains GU-enhancing molecules that are non-toxic to human-derived cells. These results position the plant as a credible source of lead molecules for further pharmacological development. Future studies should explore the molecular targets of these compounds, such as glucose transporter modulation or AMPK activation, to deepen mechanistic understanding and assess *in vivo* efficacy.

4. Conclusion

This study provides novel evidence of the antidiabetic potential of *T. polium* by identifying, for the first time, eight distinct bioactive compounds associated with enhanced GU in HepG2 cells. Utilizing a bioassay-guided fractionation approach, the ethyl acetate extract was systematically investigated, resulting in the isolation of structurally diverse constituents with pronounced glucose-lowering effects. Notably, these compounds demonstrated significant activity without compromising cell viability, supporting their potential as safe therapeutic candidates. The outcomes of this study advance comprehension of the therapeutic potential of *T. polium* and provide a foundation for subsequent research to exploring the *in vivo* efficacy, molecular mechanisms, and possible synergistic interactions of its active components in the context of diabetes management.

CRediT authorship contribution statement

Mahdi Yaghoobi: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Mahdi Moridi Farimani:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Haibo Hu:** Writing – review & editing, Software. **Razieh Yazdanparast:** Validation, Data curation, Conceptualization. **Walter Luyten:** Visualization, Validation, Supervision, Project administration.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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