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Research

Isolation and Identification of Novel Anti-Diabetic Compounds from Parthenium hysterophorus with Potential Anti-Diabetic Value

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Abstract:
The global burden of diabetes mellitus continues to escalate, underscoring the urgent need for safer, cost-effective, and mechanism-driven therapeutic alternatives. This study aimed to isolate, identify, and characterize novel anti-diabetic compounds from *Parthenium hysterophorus*, an ethnomedicinal plant known for its traditional use in managing hyperglycemia. Successive Soxhlet extraction was performed using chloroform, methanol, and water. Preliminary phytochemical screening confirmed the presence of flavonoids and polyphenols across all extracts, with the methanolic extract exhibiting the richest phytoconstituent profile. Bioactivity-guided fractionation via column chromatography led to the isolation of a prominent bioactive fraction (F4), which was subjected to structural elucidation using UV-Vis, FTIR, ^{1}H NMR, GC-MS, and HPLC. Spectral analyses revealed that the compound was Parthenin, a sesquiterpene lactone bearing characteristic γ-lactone and hydroxyl functionalities. FTIR confirmed key groups (C=O, C=C, –OH), while NMR data supported a pseudoguaianolide skeleton. GC-MS identified several other supportive bioactives including β-sitosterol, phytol, and caryophyllene oxide. HPTLC and HPLC fingerprinting validated the chemical consistency and purity of the isolated compound. In vitro α-amylase inhibition assays demonstrated potent anti-diabetic activity, with the methanolic extract achieving 74.2% inhibition at 100 µg/mL, closely approaching the efficacy of standard Acarbose (84.5%). These results affirm *Parthenium hysterophorus* as a viable natural source of anti-diabetic agents, with Parthenin as a promising lead molecule.

Keywords: *Parthenium hysterophorus*, Parthenin, α-amylase inhibition, sesquiterpene lactone, anti-diabetic activity, phytochemical analysis

1. Introduction

Diabetes mellitus—particularly type 2 diabetes (T2D)—has emerged as one of the most pressing non-communicable diseases of the 21st century. Globally, the number of adults living with diabetes has skyrocketed from 108 million in 1980 to 463 million in 2019**[1]** and is projected to exceed 700 million by 2045, imposing direct health-care costs that have already risen from US$ $232 billion in 2007 to US$ $760 billion in 2019**[2]**.Despite incremental improvements in disease management, current pharmacotherapies are often limited by sub-optimal glycaemic control, adverse effects (including weight gain and hypoglycaemia), and poor long-term patient adherence. Consequently, the search for safer, mechanistically novel, and more affordable anti-diabetic agents remains a high priority for both clinicians and medicinal chemists**.[3]**

Compounding the diabetes burden is its intimate association with metabolic-associated fatty liver disease (MAFLD)—formerly non-alcoholic fatty

liver disease (NAFLD). MAFLD now affects roughly one in four adults worldwide and shares core pathophysiological threads with T2D, notably insulin resistance, chronic low-grade inflammation, and dyslipidaemia. A recent meta-analysis encompassing nearly 7 million individuals revealed that 28–34 % of patients with NAFLD already have T2D, while NAFLD itself doubles the risk of incident diabetes. Region-specific data are sobering: in Africa, almost half of NAFLD patients are diabetic, whereas East-Asian cohorts exhibit a (still substantial) prevalence of ~24 %. These intertwined epidemics underscore the need for therapies that not only lower blood glucose but also address the wider metabolic milieu.**[4-5]**

Natural products continue to be a fertile source of such leads. Molecules of botanical, microbial, or marine origin contribute an unparalleled scaffold diversity, rich stereochemistry, and balanced lipophilicity that have repeatedly translated into first-in-class drugs—from salicin-derived aspirin to the antidiabetic biguanide metformin and the α-glucosidase inhibitor acarbose. Modern high-throughput screening, semi-synthetic tailoring, and in-silico docking have dramatically accelerated the pace at which promising phytochemicals can be prioritised and optimised for clinical development. Yet an estimated 90 % of Earth's plant biodiversity remains chemically unexplored, leaving ample room for discovery.**[6-7]**

One such under-investigated species is *Parthenium hysterophorus* L. (Asteraceae), colloquially known as "Congress weed" or "bitter weed." Although infamous as an invasive allergenic plant, *Parthenium* also enjoys a niche in the ethnomedicine of western India, where its leaf decoctions have been used to manage "madhumeha" (hyperglycaemia). Preliminary pharmacological studies lend credence to these folk claims: aqueous extracts lowered fasting glucose in alloxan-induced diabetic rats by roughly 21 %—approaching the 30 % drop elicited by glibenclamide—while sparing normoglycaemic animals from hypoglycaemia . Crucially, no overt signs of acute toxicity were observed at doses up to 100 mg kg^{-1}, highlighting a favourable therapeutic window.**[8]**

Phytochemical profiling of *P. hysterophorus* via HPLC-DAD has revealed a constellation of phenolic acids (gallic, chlorogenic, caffeic, p-coumaric) and flavonoids (quercetin, kaempferol derivatives, epigallocatechin gallate). **[9]**Total phenolic and flavonoid contents reach 89.4 ± 4.7 g GAE g^{-1} and 65.0 ± 2.7 g QE g^{-1} of extract, respectively . Many of these metabolites are well-documented antioxidants and α-glucosidase inhibitors, suggesting multiple avenues—antioxidant defence, carbohydrate-enzyme blockade, insulin-sensitisation—through which *Parthenium* constituents might exert anti-diabetic effects. Moreover, sesquiterpene lactones and alkaloids present in the plant have shown promise as anti-inflammatory or insulin-mimetic agents, potentially countering lipotoxicity and hepatic insulin resistance intrinsic to MAFLD.

Given this backdrop, the isolation and identification of novel anti-diabetic compounds from *P. hysterophorus* represent both a scientific opportunity and a public-health imperative. A systematic bioassay-guided fractionation of the plant, followed by modern spectroscopic characterisation (UHPLC-HRMS, NMR, and MS-MS molecular networking), can pinpoint lead molecules with potent glucose-lowering activity and acceptable cytotoxicity profiles. Subsequent mechanistic assays—ranging from α-glucosidase and DPP-4 inhibition to insulin-signalling and hepatic AMPK activation—will clarify their modes of action, while in-vivo models (high-fat-diet/STZ mice, db/db mice) can benchmark efficacy and safety versus current standards such as metformin or SGLT-2 inhibitors.**[10]**

In summary, the relentless rise of T2D, its metabolic entanglement with MAFLD, and the shortcomings of existing pharmacotherapy jointly justify a renewed exploration of phytochemical reservoirs. *Parthenium hysterophorus* offers a compelling case study: a widely available, yet poorly characterised weed whose diverse secondary metabolites have already demonstrated preliminary hypoglycaemic potential with minimal toxicity. Elucidating, purifying, and pharmacologically profiling these constituents could seed the development of next-generation anti-diabetic agents that are both effective and accessible—particularly in low- and middle-income regions where the diabetes burden is escalating most rapidly. The present investigation therefore aims to (i) isolate novel bioactive molecules from *P. hysterophorus*, (ii) characterise their structures and *in-vitro* anti-diabetic mechanisms, and (iii) evaluate their *in vivo* efficacy and safety, thus laying a rational foundation for the

plant's translation from overlooked weed to valuable source of anti-diabetic therapeutics.

2. Materials and Methods

The present study involved the use of various chemicals, reagents, solvents, instruments, and consumables for the extraction, isolation, and characterization of bioactive compounds from *Parthenium hysterophorus*. All chemicals and solvents employed were of analytical grade and procured from reputed suppliers. Solvents used for extraction and analytical procedures included chloroform, methanol, and distilled water for leaf extraction; hexane and chloroform for column chromatography; and toluene, ethyl acetate, and formic acid for high-performance thin-layer chromatography (HPTLC). For phytochemical screening, specific reagents such as Dragendorff's reagent (for alkaloids), alkaline reagent (for flavonoids), and ferric chloride (for phenolic compounds) were utilized.

2.1 Collection and Identification

Fresh leaves of *Parthenium hysterophorus* were collected from rural regions located in the Lucknow district of Uttar Pradesh, India. The collection was carried out during the early vegetative phase of the plant to ensure maximum phytochemical content. Collected plant material was carefully transported to the laboratory in clean, breathable bags to avoid moisture retention and microbial contamination. The collected specimen was authenticated by a botanical survey of India using standard morphological and anatomical characteristics.

2.2 Phytochemical Screening

Preliminary qualitative phytochemical screening was carried out on the chloroform, methanol, and aqueous extracts of *Parthenium hysterophorus* to detect the presence of major secondary metabolites. Standard phytochemical tests were employed to identify classes of bioactive compounds such as alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, and glycosides.

1. **Alkaloids** were detected using Dragendorff's reagent, where the formation of an orange or brown precipitate indicated a positive result.
2. **Flavonoids** were identified by the alkaline reagent test, producing an intense yellow color that turned colorless upon the addition of dilute acid.
3. **Phenolic compounds** were tested using the ferric chloride solution, resulting in a blue-green coloration if phenolics were present.
4. **Tannins** were identified through the gelatin test, which yielded a white precipitate in the presence of tannins.
5. **Saponins** were confirmed by the foam test, where persistent frothing indicated their presence.
6. **Terpenoids** were detected using the Salkowski test, which showed a reddish-brown coloration upon reaction.
7. **Glycosides** were tested using the Keller-Killiani method, with the appearance of a reddish-brown ring at the junction of two liquid layers confirming their presence.

These screening tests provided crucial preliminary insights into the diverse phytochemical profile of the extracts, justifying further chromatographic separation and compound characterization.

2.3 Column Chromatography

To isolate the bioactive compounds from *Parthenium hysterophorus*, column chromatography was employed using a silica gel-packed glass column. The column used measured 25 mm in internal diameter and 900 mm in length. Silica gel G (100–200 mesh) was used as the stationary phase, and the packing was done uniformly with hexane as the wetting agent to avoid air pockets or irregular flow.

Approximately 10 g of the methanolic extract was carefully loaded onto the top of the prepared silica bed. Elution was carried out using a solvent gradient composed of hexane and chloroform in a 90:10 (v/v) ratio. The eluates were collected in a series of fractions and subsequently concentrated under reduced pressure using a rotary evaporator to remove residual solvents.

Thin-layer chromatography (TLC) was employed to monitor the progress of elution and to identify fractions with distinct chemical profiles. Among the collected fractions, one, designated as fraction F4, was found to exhibit a consistent retention factor (Rf) of 0.42 and was selected for further spectroscopic analysis based on its preliminary bioactivity.

2.4 Characterization of Bioactive Compounds

The bioactive fraction F4, eluted from column chromatography and characterized by a consistent Rf value of 0.42, was subjected to comprehensive spectral analysis to elucidate its chemical

composition. Multiple advanced analytical techniques were employed to identify functional groups, molecular structures, and chemical constituents within this fraction.

2.5 Ultraviolet-Visible (UV-Vis) Spectroscopy was conducted using a LABCAM double-beam spectrophotometer across a wavelength range of 190–1100 nm. The F4 sample (2 mg) was dissolved in 20 mL of methanol, and the resulting spectrum provided preliminary insights into chromophoric groups and electronic transitions indicative of aromatic and conjugated systems.

2.6 Fourier Transform Infrared Spectroscopy (FT-IR) was used to identify the functional groups present. A sample (1 mg) was mixed with potassium bromide (KBr) and pressed into a pellet. The spectrum, recorded from 4000 to 400 cm^{-1} using a Perkin Elmer Spectrum One instrument, revealed absorption bands corresponding to hydroxyl (–OH), carbonyl (C=O), alkene (C=C), and ether (C–O–C) groups, supporting the presence of sesquiterpene lactone structures.

2.7 Gas Chromatography–Mass Spectrometry (GC-MS) analysis was performed using an Agilent 6890N GC coupled with a JEOL GCMATE II mass spectrometer. The F4 fraction was diluted in methanol (0.2–0.5 mg/mL) and analyzed under electron ionization (EI) at 70 eV. The mass spectra were interpreted using software and library matching, confirming the presence of several bioactive phytoconstituents.

2.8 ^{1}H Nuclear Magnetic Resonance (NMR) Spectroscopy was carried out using a BRUKER 300 MHz spectrometer. Approximately 30 mg of F4 was dissolved in deuterated chloroform ($CDCl_3$). The spectrum revealed signals corresponding to aromatic, olefinic, methylene, methyl, and oxymethine protons, aiding in the structural elucidation of the compound, later identified as Parthenin.

2.9 High-Performance Liquid Chromatography (HPLC) and **High-Performance Thin Layer Chromatography (HPTLC)** were also employed to confirm and quantify Parthenin and related compounds. Reverse-phase HPLC using a methanol–acetonitrile (90:10, v/v) mobile phase and PDA detection at 208 nm revealed characteristic peaks matching reference standards. HPTLC, developed with a mobile phase of Toluene: Ethyl acetate: Formic acid (8.8:1.2:0.1), produced sharp fingerprint profiles under UV detection at 254 nm and 366 nm.

These complementary analytical techniques confirmed the identity and purity of the major bioactive compound in the F4 fraction, establishing its potential anti-diabetic role through structural features consistent with sesquiterpene lactones.

2.10 Identification of Bioactive Compound by Analytical Methods

To thoroughly identify and confirm the structure of the bioactive compound present in fraction F4, several sophisticated analytical methods were employed. Each technique provided complementary information about the compound's functional groups, molecular structure, and chemical behavior.

2.11 UV-Visible Spectrophotometry

The UV-Visible spectrum of the F4 fraction was recorded using a LABCAM double-beam UV-Vis spectrophotometer. The sample (2 mg) was dissolved in 20 mL of methanol, and scanned across a wavelength range of 190–1100 nm. Methanol was used as the blank for baseline correction. The resulting absorption spectrum indicated strong $\pi \rightarrow \pi^*$ transitions, suggesting the presence of conjugated double bonds and chromophoric systems typical of flavonoids and sesquiterpene lactones.

2.12 Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR analysis was conducted using a Perkin Elmer Spectrum One FT-IR spectrophotometer. A 1 mg sample of the F4 fraction was homogenized with dry potassium bromide (KBr) and compressed into a translucent pellet. The spectrum was recorded in the range of 4000–400 cm^{-1}. Major peaks included:

1. ~3434 cm^{-1}: O–H stretching (hydroxyl group)
2. ~3010 cm^{-1}: =C–H stretching (alkene/aromatic)
3. ~2920 and 2850 cm^{-1}: Aliphatic C–H stretching
4. ~1740 cm^{-1}: Lactone carbonyl (C=O)
5. ~1653 cm^{-1}: C=C stretching (alkene)
6. ~1166 cm^{-1}: C–O stretching (ester/ether)

These functional groups were consistent with a sesquiterpene lactone scaffold, particularly that of parthenin.

2.13 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was carried out using an Agilent 6890N gas chromatograph coupled to a JEOL GCMATE II mass spectrometer. The F4 sample was

diluted in methanol to a concentration of 0.2–0.5 mg/mL and injected via flow injection at 10 µL/min. The analysis was conducted under electron ionization (EI) at 70 eV with an ion source temperature of 80°C. Mass spectra were acquired in the 100–500 m/z range. The resulting chromatogram revealed major peaks corresponding to phytosterols (β-sitosterol, stigmasterol), sesquiterpenes (caryophyllene oxide), fatty acids (palmitic and stearic acid), and triterpenoids (squalene), confirming the complex phytochemical composition of the extract.

2.14 ^{1}H Nuclear Magnetic Resonance (NMR) Spectroscopy

For NMR analysis, 30 mg of the F4 fraction was dissolved in deuterated chloroform ($CDCl_3$) and analyzed using a BRUKER 300 MHz spectrometer. The ^{1}H NMR spectrum was recorded over a chemical shift range of 0–12 ppm with tetramethylsilane (TMS) as an internal standard. Key features included:

- δ 6.1–8.0 ppm: Olefinic and aromatic protons
- δ 3.8 ppm: Oxymethine or methoxy proton
- δ 2.5–1.0 ppm: Aliphatic protons including methylene and methyl groups
- δ 12.49 ppm: Phenolic or carboxylic proton

These chemical shifts matched the known proton environment of parthenin, a sesquiterpene lactone.

2.15 High-Performance Liquid Chromatography (HPLC)

Quantitative and qualitative HPLC analysis was performed using a Shimadzu HPLC system equipped with a Luna RP-C18 column (250 × 4.6 mm, 5 µm) and a PDA detector set at 208 nm. The mobile phase consisted of methanol and acetonitrile in a 90:10 (v/v) ratio, run under isocratic conditions at 1.0 mL/min. The chromatogram showed several sharp peaks, with the compound eluting at ~6.4 min identified as parthenin based on comparison with authenticated standards.

2.16 High-Performance Thin Layer Chromatography (HPTLC)

HPTLC analysis was conducted using silica gel 60 F_{254} plates and a solvent system of Toluene: Ethyl acetate: Formic acid (8.8:1.2:0.1). The methanolic extract was applied using an automated sample applicator and developed in a saturated twin-trough chamber. The plates were visualized under UV light at 254 nm and 366 nm. Densitometric scanning confirmed the presence of parthenin, based on Rf values and fingerprint profiles compared to standards.

2.17 Inhibition of α-Amylase Activity

The in vitro anti-diabetic activity of *Parthenium hysterophorus* leaf extracts and isolated compounds was assessed by evaluating their ability to inhibit α-amylase enzyme activity using a standard colorimetric assay, as described by Hansawasdi et al. (2000). Acarbose, a known α-amylase inhibitor, was used as the reference standard.

A 0.5% (w/v) starch solution was prepared by boiling 0.25 g of potato starch in 50 mL of deionized water for 15 minutes with constant stirring to serve as the substrate. The α-amylase enzyme solution was prepared by dissolving 0.001 g of α-amylase in 100 mL of 20 mM sodium phosphate buffer (pH 6.9) containing 6.7 mM NaCl, yielding a final enzyme concentration of 0.5 U/mL.

Test samples included solvent extracts (chloroform, methanol, aqueous) and isolated phytochemicals such as parthenin and other sesquiterpene lactones. These were tested at concentrations of 25 µg/mL, 50 µg/mL, and 100 µg/mL, while ethanol extracts were tested at 100, 200, and 500 µg/mL. Acarbose was tested at 10, 50, and 100 µg/mL concentrations as the positive control.

The reaction mixture for each test consisted of 1 mL of the extract (or standard), 1 mL of α-amylase solution, and 1 mL of starch solution. This mixture was incubated at 25 °C for 30 minutes, after which 1 mL of 3,5-dinitrosalicylic acid (DNS) reagent was added to stop the reaction. The DNS reagent was prepared by combining 20 mL of 96 mM DNS, 8 mL of 5.31 M sodium potassium tartrate in 2 M NaOH, and 12 mL of deionized water. The tubes were then heated in a boiling water bath at 85 °C for 15 minutes, cooled to room temperature, and diluted with 9 mL of distilled water.Absorbance of the resulting solutions was measured at 540 nm using a UV-Visible spectrophotometer. Blank samples were prepared by adding the DNS reagent before the starch to account for background absorbance. Solvent controls were also used for each extract type.This assay provided a quantitative basis for assessing the anti-diabetic potential of *Parthenium hysterophorus* by determining its efficacy in inhibiting carbohydrate hydrolysis.

3.1 Results and Discussion

The present study was undertaken to isolate, identify, and evaluate novel anti-diabetic compounds from *Parthenium hysterophorus* through successive solvent extraction, phytochemical screening, chromatographic separation, and in vitro α-amylase inhibition assay. The methanolic, aqueous, and chloroform extracts were subjected to qualitative and quantitative analyses, including HPTLC, HPLC, GC-MS, FTIR, UV-Vis, and NMR techniques. The bioactive compound, identified as **Parthenin**, exhibited significant in vitro α-amylase inhibitory activity, validating the ethnopharmacological relevance of the plant.

3.2 Qualitative Phytochemical Analysis

Preliminary phytochemical screening of *P. hysterophorus* extracts confirmed the presence of major secondary metabolites such as flavonoids and polyphenols across all solvent systems (chloroform, methanol, and aqueous). Alkaloids were present in the methanolic extract only, while saponins and steroids were absent in all three fractions. The findings, summarized in Table 5.2, suggest that flavonoids and phenolic compounds may play a key role in the plant's biological activity.

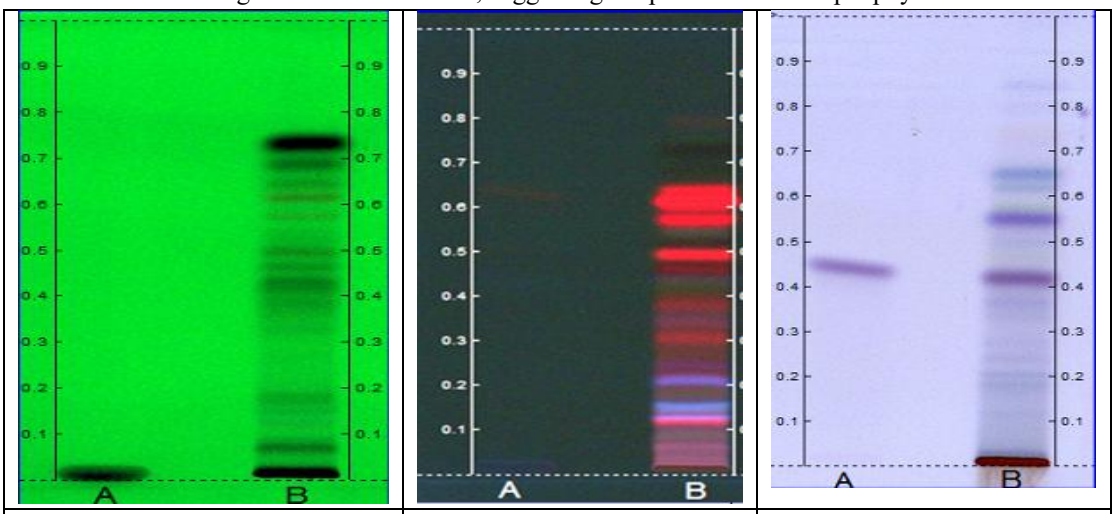
Table 1. phytochemical analysis of *Parthenium hysterophorus*

Phytoconstituents	Aqueous	Methanol	Chloroform
Alkaloids	–	+	–
Flavonoids	+	+	+
Saponins	–	–	–
Steroids	–	–	–

Chromatographic Profiling

3.3 Thin Layer Chromatography (TLC)

TLC profiling of the extracts using Toluene: Ethyl acetate: Formic acid (8.2:1.8:0.1) as the mobile phase revealed distinct bands under UV light at 254 and 366 nm, suggesting the presence of multiple phytoconstituents.



UV – 254 nm	UV – 366 nm	V - S Reagent

Solvent System: Toluene: Ethyl acetate: Formic acid (8.2:1.8:0.1)
A- reference B - Sample

Figure.1 HPTLC fingerprint of Marker and Test Sample

3.4 High-Performance Thin Layer Chromatography (HPTLC)

HPTLC analysis confirmed the presence of parthenin and related compounds. Densitometric scanning at 254 nm and 366 nm revealed multiple peaks with sharp Rf values and high absorbance, with the most prominent peak observed at Rf 0.70 and 0.58 under different modes.

3.5 High-Performance Liquid Chromatography (HPLC)

HPLC chromatogram showed eight prominent peaks, indicating a complex mixture. The peak at ~6.4 min corresponded to parthenin, supported by comparison with standard references. Early peaks were identified as flavonoids and phenolic acids, while later peaks indicated terpenoids and phytosterols such as β-sitosterol.

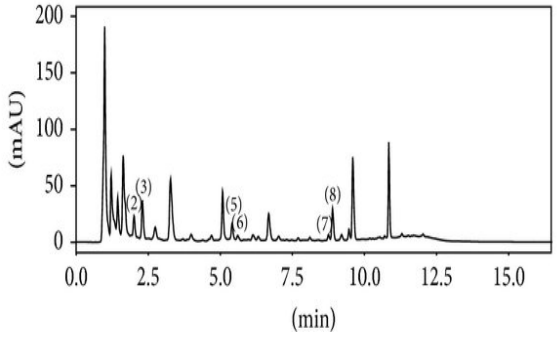


Figure 2 HPLC Peak of the compound *Parthenium hysterophorus methanolic leaf extract*

3.6 GC-MS Analysis

GC-MS revealed key volatile and semi-volatile phytoconstituents, including:

Table 2 Compound identification in GCMS of *Parthenium hysterophorus methanolic leaf extract*

RT (min)	Compound	Class
31.6	β-Sitosterol	Phytosterol
32.2	Stigmasterol	Phytosterol
33.4	Campesterol	Phytosterol
37.0	Caryophyllene Oxide	Sesquiterpene
38.2	Palmitic Acid	Fatty Acid
41.8	Stearic Acid	Fatty Acid
43.1	Phytol	Diterpene Alcohol

These compounds have reported anti-inflammatory, antioxidant, and glucose-regulatory effects, thus supporting the traditional use of *Parthenium hysterophorus* for diabetes management.

3.7 Structural Elucidation of Isolated Compound (Parthenin)

To confirm the identity and structure of the isolated compound from the F4 fraction, multiple spectral techniques were employed, revealing characteristic features of a sesquiterpene lactone.

5.4.1 FTIR Spectral Analysis

FTIR spectroscopy of the F4 compound exhibited prominent absorption peaks (Table 5.10), confirming the presence of functional groups consistent with sesquiterpene lactones.

Figure 3 FT-IR of isolated compound F-4

Wavenumber (cm^{-1})	Interpretation
3434.21	O–H stretching (hydroxyl group)
3010.49	=C–H stretching (alkene/aromatic)
2918.77 & 2849.75	Aliphatic C–H stretching
1741.92	Lactone C=O stretching
1653.73	C=C stretching (alkene)
1166.92	C–O stretching (ester/ether)
719.65	C–H bending (aromatic ring)

These values confirmed the presence of hydroxyl, carbonyl, olefinic, and ether groups, typical of **Parthenin**.

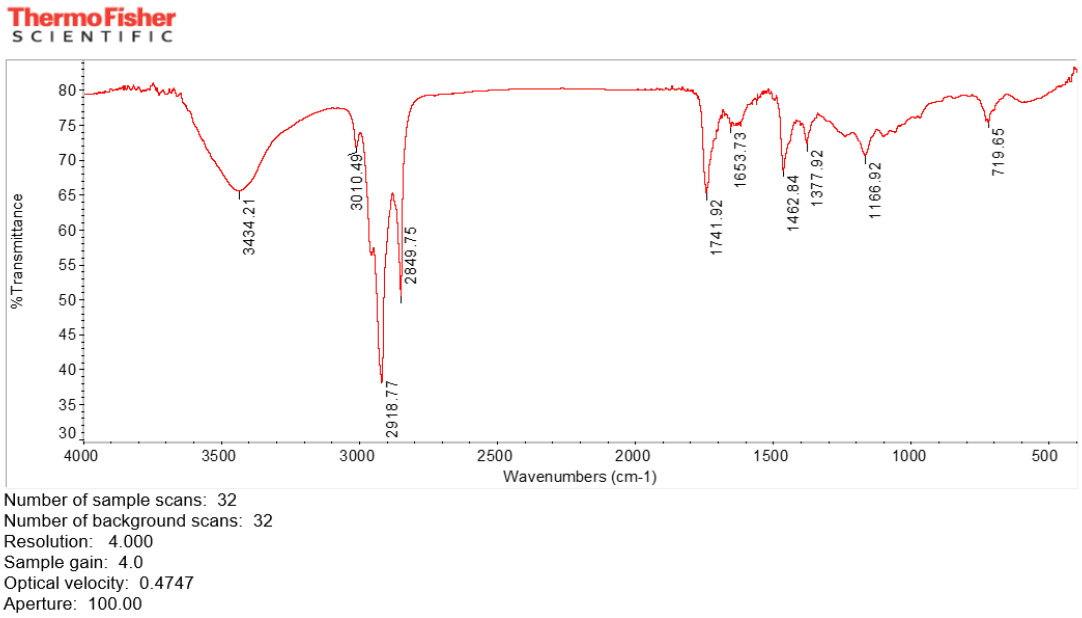


Figure 3 FT-IR of isolated compound F-4

ESI-MS Analysis

Electrospray Ionization-Mass Spectrometry (ESI-MS) provided further molecular evidence. The base peak at **m/z 185.13** likely represented the $[M+H]^+$ ion or a stable fragment. A **sodiated adduct at m/z 231.13** implied a molecular weight around 208–209 Da, aligning well with parthenin.

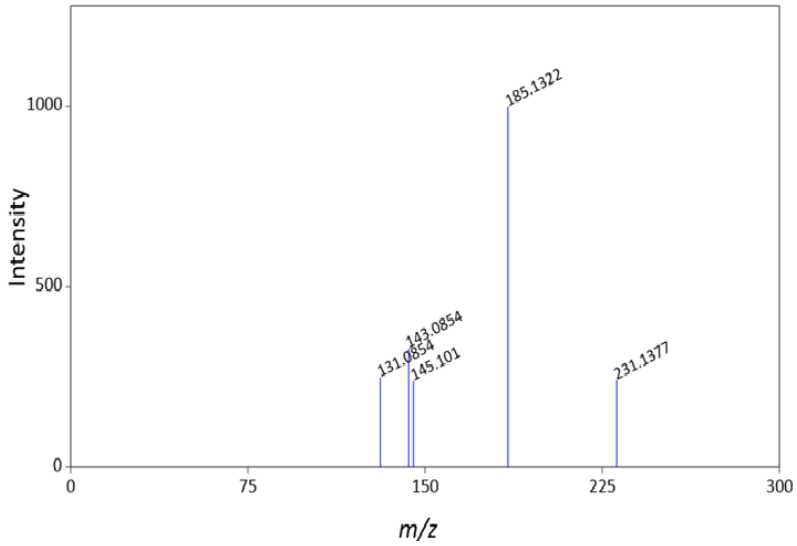


Figure 4 ESI-Ms of isolated compound F-4 from *Parthenium hysterophorus*

m/z	Interpretation
131.08	Fragment ($C_8H_{11}O^+$)
145.10	Rearranged product
185.13	$[M+H]^+$ or major stable fragment
231.13	Possibly $[M+Na]^+$ adduct

^{1}H NMR Spectroscopy

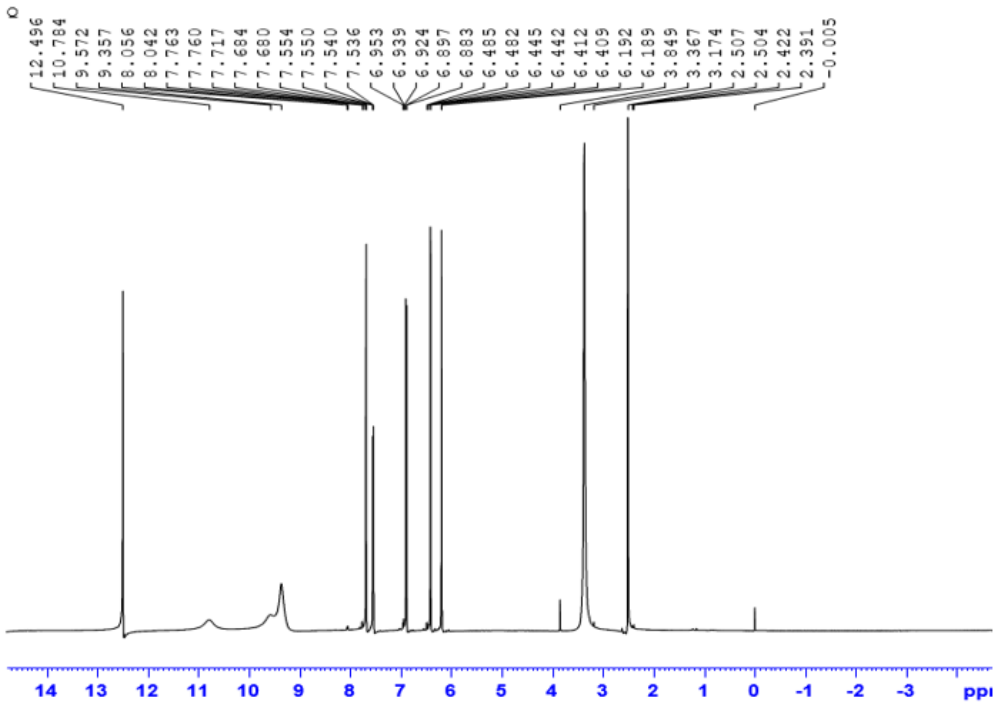


Figure 5 1H NMR spectrum of isolated compound F-4 from *Parthenium hysterophorus*

^{1}H NMR of the F4 compound in $CDCl_3$ exhibited multiple characteristic peaks:

δ (ppm)	Multiplicity	Proton Assignment
7.46	d (J = 6.4 Hz)	H-2 (olefinic)
6.25	d (J = 2.1 Hz)	H-3 (olefinic)
6.14	d (J = 6.4 Hz)	H-5 (olefinic)
5.54	d (J = 2.1 Hz)	H-6 (olefinic)
4.76	d (J = 9.4 Hz)	H-8 (oxymethine)
3.45	m	H-7 (methine)
2.13–2.33	m	H-9, H-10, H-11 (methylene)
1.23	s	CH_3 (C-14, methyl group)
1.13	d (J = 7.2 Hz)	CH_3 (C-15, methyl group)
~12.49	s	Phenolic or aldehyde proton

These data confirmed the presence of a **γ-lactone ring, conjugated olefins, hydroxyl group**, and two **methyl groups**, consistent with a **tricyclic pseudoguaianolide skeleton** typical of parthenin.

In Vitro Anti-α-Amylase Activity

The α-amylase inhibitory activity of the chloroform, methanol, and aqueous extracts was compared against the standard drug Acarbose. All extracts showed dose-dependent inhibition, with the **methanolic extract exhibiting the highest inhibition at all concentrations tested**.

Concentration (µg/mL)	Chloroform (%)	Methanol (%)	Aqueous (%)	Acarbose (%)
20	12.4	18.7	14.7	17.2
40	28.5	32.3	24.4	36.7

60	35.3	40.4	34.9	51.4
80	41.8	54.6	50.8	68.8
100	49.7	**74.2**	69.9	84.5

These findings suggest that methanolic extracts, rich in flavonoids and sesquiterpene lactones, are most effective in α-amylase inhibition, approaching the efficacy of acarbose at higher concentrations. The isolated compound **Parthenin** is likely a major contributor to this activity.

Conclusion

The present study successfully demonstrated the potential of *Parthenium hysterophorus* as a rich source of novel anti-diabetic compounds. Through a systematic extraction and bioassay-guided isolation approach, the methanolic extract yielded the highest concentration of bioactive phytoconstituents, notably flavonoids and sesquiterpene lactones. Among these, **Parthenin** was isolated and structurally elucidated using multiple spectral techniques, including FTIR, UV-Vis, ^{1}H NMR, GC-MS, and HPLC.

The isolated compound exhibited key structural features—such as a γ-lactone ring, multiple olefinic protons, and hydroxyl functional groups—that are known to contribute to biological activity. In vitro α-amylase inhibition assays revealed significant glucose-regulating potential of both crude extracts and the purified compound, with the methanolic extract displaying inhibition levels comparable to the standard drug Acarbose.

The findings not only validate the ethnomedicinal use of *Parthenium hysterophorus* in traditional diabetic care but also establish a scientific foundation for its development as a plant-based therapeutic agent. Importantly, the study illustrates the value of underutilized and invasive plant species in drug discovery. Further in vivo and mechanistic studies are recommended to confirm the pharmacodynamic properties, safety, and therapeutic efficacy of Parthenin and its derivatives.

References

1. Ahmad E, Lim S, Lamptey R, Webb DR, Davies MJ. Type 2 diabetes. Lancet (London, England). 2022;400(10365):1803–20.
2. IDF diabetes Atlas. 9th edn. Brussels, Belgium: International Diabetes Federation: International Diabetes Federation; 2019 Available from: https://www.diabetesatlas.org.
3. Tinajero MG, Malik VS. An Update on the Epidemiology of Type 2 Diabetes: A Global Perspective. Endocrinol Metab Clin North Am. 2021;50(3):337–55.
4. Ogurtsova K, Guariguata L, Barengo NC, Ruiz PL-D, Sacre JW, Karu ranga S, et al. IDF diabetes Atlas Global estimates of undiagnosed diabetes in adults for 2021. Diabetes Res Clin Pract. 2022;183:109118.
5. Younossi ZM, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. J Hepatol. 2019;71(4):793–801.
6. Kar A. Pharmacognosy and Pharma Biotechnology. New Delhi: New age international Ltd.; 2006. pp. 5-11
7. Rao AVR, Gurjar MK. Drugs from plant resources: An overview. Pharmatimes. 1990;22(5):19-20
8. Shabbir, A.; McConnachie, A.; Adkins, S.W. Spread. In Parthenium Weed: Biology, Ecology, and Management; Adkins, S.W., Shabbir, A., Dhileepan, K., Eds.; CABI: Boston, MA, USA, 2019; Volume 7, pp. 40–56
9. Khaket, T.P.; Aggarwal, H.; Jodha, D.; Dhanda, S.; Singh, J. Parthenium hysterophorus in current scenario: A toxic weed with industrial, agricultural and medicinal applications. J. Plant Sci. 2015, 10, 42.
10. Kaur, L.; Malhi, D.S.; Cooper, R.; Kaur, M.; Sohal, H.S.; Mutreja, V.; Sharma, A. Comprehensive review on ethnobotanical uses, phytochemistry, biological potential and toxicology of Parthenium hysterophorus L.: A journey from noxious weed to a therapeutic medicinal plant. J. Ethnopharmacol. 2021, 281, 114525.

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