

LC-MS QTOF ANALYSIS OF VALERIANA WALLICHII DC. AND INTEGRATED IN SILICO AND IN VITRO ANALYSIS FOR POTENTIAL ANTIDIABETIC AND ANTI-GLYCATION ACTIVITIES

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Abstract

Traditional medicinal plant *Valeriana wallichii* is comprised of diverse medicinal phytochemicals with a broad range of bioactivities. This investigation comprehensively evaluates antidiabetic and antiglycation activities of *V. wallichii*. An integrated analytical-computational and *In vitro* approach was used, comprising LC–MS Qtof analysis, *In silico* ADME profiling and docking analysis and antioxidant, antidiabetic and AGEs inhibitory assays. Phytochemical profiling identified multiple compounds, like phenolics, flavonoids, phenolics and terpenoids. Significant activity antioxidant potential was shown by ethyl acetate part (H₂O₂ scavenging: 81.2%, DPPH IC₅₀ 0.091 mg/mL), α -glucosidase inhibition (IC₅₀ 0.13 mg/mL) and antiglycation effects, including decreased fructosamine levels (~0.48 mM/mL), higher carbonyl trapping (~23 μ M/mg), and inhibition of β -amyloid formation. Further, a marked protection against groups was also noticed in the case of the ethyl acetate fraction. Favorable drug likeness and ADMET profiles were noticed during *In silico* analysis with valerianic acid and Valeriandoid F showing strong binding affinities (up to -7.3 kJ·mol⁻¹) against key protein targets including 3CJJ, 3TOP and 4F5S. These interactions were stabilized by hydrogen bonding and hydrophobic bonds. It was thus evident that *V. wallichii*, especially its semi-polar fraction, can modulate oxidative stress, carbonyl formation, and protein glycation pathways related to diabetic complications. Further *In vivo* validation is required to confirm its clinical applicability.

Key words: Advanced glycation, molecular docking, fructosamine, carbonyl entrapment, bovine serum albumin

Introduction

A common metabolic disorder, i.e., diabetes mellitus (DM), is mainly linked to an increase in blood glucose levels occurring because of impairment in insulin secretion, insulin or both (Jadon *et al.*, 2024). This long-continued and persistent hyperglycemia generally initiates numerous interconnected pathogenic pathways, including oxidative stress, boosted polyol flux, protein kinase C activation and non-enzymatic glycation of nucleic acids, proteins and lipid (Hussain, 2025). The last pathway is generally called Advanced glycation end products formation (AGEs) and is a main mechanism pertaining to the commencement and progression of diabetic complications (Yubero-Serrano & Pérez-Martínez, 2020; Khalid *et al.*, 2022).

The major pathway for AGEs formation is called the Maillard reaction, starting with the creation of Schiff base and proceeding through Amadori rearrangement (Twarda-Clapa *et al.*, 2022). Several reactive dicarboxylic intermediates, especially the MGO (methylglyoxal glyoxal) and 3-deoxyglucosone, assist this process and further augment carbonyl stress during the underlying condition of diabetes (Li *et al.*, 2018). As of their production, a concurrent tissue damage may occur through 2 key pathways. In the first

instance, a direct modification of protein structure occurs through enhanced cross-linking activity. This results in a marked increase in vascular elasticity, enzymatic activity and modification of architecture in the extracellular matrix (Zgutka *et al.*, 2023). Secondly, a stable interaction of the reactive moieties occurs with RAGEs (receptor for advanced glycation end products) (Yan *et al.*, 1997) thereby activating a complex cascade of intracellular signaling like transforming growth factor- β (TGF- β)-associated responses, extracellular signal-regulated kinase and c-Jun N-terminal kinase, and nuclear factor kappa B (NF- κ B). All these mechanisms facilitate inflammation, promotion of cellular dysfunctions and increase in oxidative stress (Shanmugam *et al.*, 2003; Parwani & Mandal, 2023). All these mechanisms are generally involved in facilitating the AGEs contribution towards retinal damages, endothelial dysfunctions, arterial stiffnesses, renal injuries, neuropathological consequences and accelerated CVS (cardiovascular disease) in diabetics patients (Yubero-Serrano & Pérez-Martínez, 2020).

Since conventional antidiabetics are currently unable to completely eradicate or lessen the glycation-driven molecular injuries, an exceedingly increased need to use medicinal plants against diabetes AGE formation exists. (Naganarasimha *et al.*, 2025). Several investigations have

previously proved that plant-derived terpenoids, alkaloids, phenolics, flavonoids and several related secondary metabolites can inhibit AGE formation. This activity is mainly due to high antioxidant activities, reactive carbonyl trapping, metal chelation and interference with protein cross-linking (Velichkova *et al.*, 2021). This pharmacological outline styles ethnomedicinal and traditional plants as key striking candidates for multitarget interventions in diabetes and related complications.

Valeriana wallichii DC. is an important Himalayan medicinal species used in traditional systems of medicine for insomnia, anxiety, epilepsy, psychosomatic disorders, pain, and inflammatory conditions (Tyagi *et al.*, 2022). Phytochemical studies indicate that the species contains structurally diverse constituents, including valerenic acid-related compounds, valeric acid, valepotriates such as dihydrovaltrate, sesquiterpenoids, flavonoids including hesperidin and 6-methylapigenin, alkaloids, lignans, and volatile oil components (Mathela *et al.*, 2005; Glaser *et al.*, 2015). It has been extensively reported in earlier investigations that several important compounds classes including flavonoids, terpenoids, alkaloids and tannins are commonly found in *V. wallichii*. (Jugran *et al.*, 2019; Li *et al.*, 2022). These compound classes interfere with carbohydrate digesting enzymes, lower oxidative stress and decrease dicarbonyl-mediated glycation, thus finding usefulness in management strategies for DM (Velichkova *et al.*, 2021).

Nonetheless, the Anti AGEs/diabetic potential of *V. wallichii* remains less explored despite traditional and ethnopharmacological importance. Consequently, this study was planned to appraise the AGEs inhibitory and antidiabetic activities of *V. wallichii*, with emphasis on its likelihood to constrain carbohydrate metabolizing enzymes (α glucosidase) and suppress glycation-linked oxidative stress.

Materials and Methods

Plant material and extraction: The rhizomes of *V. wallichii* were procured from a local herbal store and authenticated. The plant material was then washed thoroughly, dried and milled into a powdered form. The powdered material was macerated using 100% methanol for 72–96 hours with intermittent shaking. Filtration of the extract was accomplished by using Whatman's filter paper. This was followed by concentrating the extract under vacuum pressure using a rotary evaporator to attain the concentrated methanolic extract. The crude extract was then subjected to solvent–solvent fractionation to obtain fractions of varying polarity. All extracts were dried and stored at 4°C for further analysis.

Phytochemistry

Preliminary analysis: During initial screening, the presence of major phytoconstituents like alkaloids, tannins, saponins and flavonoids etc. were checked by using standard procedures (Hodoşan *et al.*, 2025). Whereas for quantitative screening, the *Folin Ciocalteu* method (total Phenolic Content (TPC) and Aluminum chloride based methods (total flavonoid contents (TFC) was used (Luță *et al.*, 2023).

LC–MS analysis: The LC–MS/QTOF analysis was accomplished by using a Triple Quadrupole Mass

Spectrometer (TSQ Quantis (ThermoElectron Scientific, USA) that is equipped with a heated electro spray ionization (H-ESI) source. General analysis conditions were as; Flow rate was 10 μ L/min, Sheath gas flow rate 15 units, Auxiliary gas flow rate 3 units, Capillary voltage 4.0 kv, Capillary Temperature, 275°C and mass scan range was 30–1500 m/z. The Fragmentation (MS/MS) was performed with collision energy ranging from 10–40 V. For analysis and interpretation, the Xcalibur software was used.

Antioxidant assays: Radical Scavenging Assay (DPPH). A commonly employed radical scavenging assay (DPPH assay) was used for determination of antioxidant potential. Briefly mixing 60 μ L of DPPH solution (methanolic, 0.1 mM) with 60 μ L of plant extract at varying concentrations (0.93–120 μ g/mL) was performed. The combination was nurtured in the dark area for 35 minutes, and by using a UV spectrophotometer, readings were taken ($\lambda=517$ nm). Trolox was used as a reference standard (Amin *et al.*, 2024).

Ferric reducing antioxidant power (FRAP assay): A previously set procedure was used during FRAP assay [21]. Initially a working FRAP reagent (TPTZ, ferric chloride, and acetate buffer) was prepared and an aliquot part of 30 μ L of extract was mixed with the FRAP reagent. The blend was later place for 35 min at room temperature (25°C). Finally, by using a UV spectrophotometer, readings were taken ($\lambda=560$ nm) (Amin *et al.*, 2024).

H₂O₂ antioxidant assay: H₂O₂ assay was performed by standard procedure (Bhatti *et al.*, 2015). Briefly, the test sample (450 μ L) or positive control solution were reacted with H₂O₂ solution (550 μ L, 2 mM). The mixture was gently mixed followed by placing undisturbed for 10 min. Finally, by using a UV spectrophotometer, readings were taken ($\lambda=230$ nm). Percentage inhibition was calculated as;

$$\% \text{ inhibition} = \left(1 - \frac{A_a}{A_b} \right) \times 100$$

where A_a represents the sample absorbance, and A_b represents the control absorbance. Trolox was used as positive control.

Phosphomolybdate assay: The phosphomolybdenum assay was performed by a modified method (Diwan *et al.*, 2012). In brief, an aliquot part of test sample (0.1 mL; in MeOH) was placed carefully in a vial (3mL capacity) already having 1 mL of reagent (0.6 M H₂SO₄, 28 mM NaHPO₄·7H₂O, and 4 mM (NH₄)₂ MoO₄, trailed by incubation at 95°C for 90 min (water bath). Afterwards, test samples were shaken slightly and permitted to cool at room temperature. Finally by using a UV spectrophotometer, readings were taken ($\lambda=765$ nm). Following formula was designated to calculate the percent inhibition.

$$\% \text{ Inhibition} = (1 - A_a / A_b) \times 100$$

where A_a represents the sample absorbance, and A_b represents the control absorbance.

Antidiabetic assay

Enzyme inhibition assay: Enzyme inhibition assay was performed by mixing 100 μ L of extract and α -glucosidase enzyme solution (0.57 U/mL, 100 μ L) and incubating at 37°C for 10 minutes. Subsequently, substrate solution (200 μ L) was added and placed undisturbed for an additional 20 minutes. The reaction was terminated using 100 μ L of 1 M sodium carbonate, and absorbance was recorded (λ = 405 nm). IC₅₀ values were calculated (Naz Qaisrani *et al.*, 2025).

Antiglycation assays

BSA glucose assay: The oxidative model (BSA-MGO) was employed by using our previously employed protocol (Naz Qaisrani *et al.*, 2025). In brief, the EO (30 μ L) with BSA (135 μ L) and MGO (135 μ L) was incubated for 24 hrs at 60°C. Afterwards, the fluorescent intensity was recorded, and % inhibition was calculated as below;

$$\% \text{ Inhibition} = (1 - (\text{fla} / \text{flb})) \times 100$$

where fla is the fluorescence intensity of the sample, and flb indicates the fluorescence intensity of the control.

BSA MGO assay: The non-oxidative model will encompass BSA-Glucose treatment for one week to 21 days at 37°C. In this case, fluorescent intensity will be considered as a measure of successful glycation (Amin *et al.*, 2024). Inhibition levels were determined as follows;

$$\% \text{ Inhibition} = (1 - (\text{fla} / \text{flb})) \times 100$$

where fla is the fluorescence intensity of the sample, and flb indicates the fluorescence intensity of the control. IC₅₀ values for the tested samples were calculated from linear regression of the scatter plots.

Fructosamine estimation: The AGEs product “fructosamine” was estimated using an already-developed protocol (Elrherabi *et al.*, 2024). Briefly stating, the NBT solution (0.75mM, in carbonate buffer (0.1 M, pH 10.35). Moreover, the glycated samples (40 μ L) were mixed with this NBT solution (0.8 mL) at 37°C for 30 min. Afterwards, the readings of treated samples was recorded using a UV spectrophotometer (λ =530nm). Fructosamine concentration was calculated using the standard 1-deoxy-1-moeopholino-fructose curve ($Y=0.00X+0.014$, $R^2=982$) and was expressed in μ M/mg of protein.

Inhibition of β -Amyloid formation assay: The Congo red dye-binding method was employed to detect β -amyloid formation in BSA (Witter *et al.*, 2018). Briefly, the glycated sample (50 μ L) was mixed with Congo red dye (50 μ L; 100 μ M) and kept at 25°C (20 minutes). Afterwards, absorption was measured at 530 nm using a spectrophotometer.

Free carbonyl contents: In this assay, the AGEs with free carbonyl groups (glycated samples) were assessed using a previously defined protocol (Heckmann *et al.*, 2024). Briefly, the DNPH (2,4-dinitrophenylhydrazine) (500 μ L; 10 mM; in 2.5 M HCl) was mixed with the glycated sample (500 μ L) and incubated for one hour at RT (room temperature). Afterwards, precipitation was performed using TCA (1.0 mL, 20%). The precipitate was then washed several times with an ethanol: ethyl acetate mixture

(1:1 v/v, 1 mL), and the pellet was further dissolved in urea solution (1 mL, 6 M). Finally, the absorbance was recorded at 365 nm using a spectrophotometer.

Thiol estimation: The Protein-thiol contents in glycated samples were estimated using a slightly modified Ellman’s assay (Meeprom *et al.*, 2013). Briefly, an aliquot (10 μ L) of glycated samples was incubated with 90 μ L of DTNB (5 mM 5,5'-dithiobis (2 2-nitrobenzoic acid) in 100 mM phosphate buffer (pH 7.4) at 25°C (15 minutes). Afterwards, the samples' absorbance was recorded at 412 nm using a microplate reader. The final concentration of free thiol was estimated from the L-cysteine standard curve (0.031–4.0 mM).

In silico analysis

Docking: The compound target interaction was accomplished by Molecular docking studies using AutoDock Vina using previously set protocol (Trott & Olson, 2010). The key target proteins including 3CJJ (Devi Alaparathi *et al.*, 2016), 3TOP (Singh *et al.*, 2018), and 4F5S (Soliman *et al.*, 2022) were retrieved PDB (Protein Data Bank) followed by removal of co-associated ligands and water molecules using Discovery Studio (2025). Further proteins and ligand preparation was accomplished in Atudock vina followed by molecular docking on active pockets (CaSTp tool). Binding interactions were visualized using PyMOL and Discovery Studio 2.0. Blind docking was used during investigation and redocking was performed by using SwissDock (Bugnon *et al.*, 2024).

PASS prediction and ADMET analysis: Biological activity prediction was performed using online PASS tool(ways2drugs). The ADMET analysis was accomplished by ADMET labs 3.0, CASTp and molsoft online tools (Amanullah *et al.*, 2025).

Statistical analysis

During this investigation, all experiments were repeated thrice and the values were expressed as $\pm$ SD (standard deviation). The significance of data (statistical) was strengthened by the use of one-way ANOVA ($p<0.05$). This was trailed by Tukey's post hoc analysis ($p<0.05$).

Results

LCMS-MS²: The LC-MS/QTOF analysis of *V. wallichii* rhizome extract was accomplished by using already set protocol (experimental section). As series of compounds were noticed both in positive (+ve) and negative (-ve) ionization modes depending on nature of compounds and ionization pattern. In case of -ve ionization mode, identified compounds included Daphnetin, 7,8-dihydroxycoumarin (RT 1.73 min), dihydroxy-Valerate (RT 1.23 min), Kaempferol-3-o-rutinoside (RT 6.22 min), Norvaline (RT 0.86 min), 4-hydroxybenzoate (RT 1.55 min), volvatrate C (RT 5.37 min) and Hesperidin (RT 6.64 min) (Fig. 1, Table 1). Most prominent compounds from +ve ionization mode included longiflorone (RT 8.96 min), Valernal (RT 9.17 min), Valarenic acid (RT 9.60 min), Orientin (RT 11.04 min) and Valerioandoid F (RT 11.22 min) (Table 2). The fragmentation patterns, MS and MS² spectra of all major compounds with both +ve and -ve ionization modes is presented in the supplementary information.

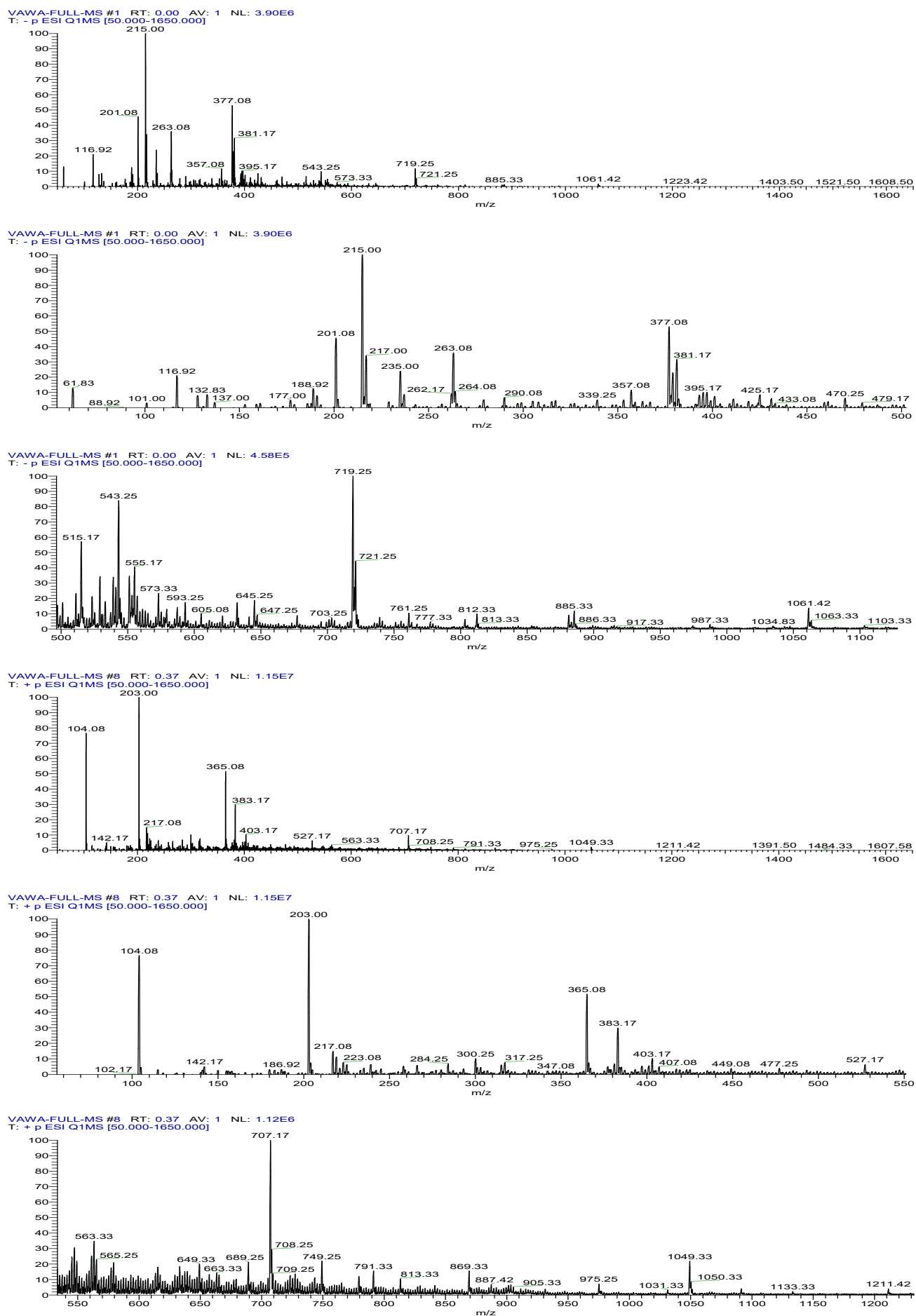


Table 1. LCMS-MS² analysis ((-) ESI-MS/MS (m/z) of *Valerian wallichii* rhizome extract.

S.No	RT	Molecular formula	Observed mass	Exact mass	Ion type	Fragments ((+) ESI-MS/MS (m/z)	Compound	References
1.	0.86	C ₁₅ H ₁₇ NO ₂	116.92	117.15	M ⁻¹	73.00, 70.92, 69.01, 59.00	Norvaline	Mass Bank of North America
2.	1.05	-	127.67	128.2	M ⁻¹	127.67, 83.67, 81.24	Unknown	
3.	1.23	C ₉ H ₁₀ O ₄	133.17	134.21	M ⁻¹	132.33 114.75, 88.58, 75.0, 72.50, 71.00, 58.67	dihydroxy-Valerate	Mass Bank of North America
4.	1.55	C ₇ H ₅ O ₃	137.01	138.17	M ⁻¹	137.01, 107.92, 93.08, 81.00, 65.08	4-hydroxybenzoate	Mass Bank of North America
5.	1.62	-	161.33	162.4	M ⁻¹	161.33, 129.17, 114.8, 89.00, 75.00, 71.00, 57.17	Unknown	
6.	1.73	C ₉ H ₆ O ₄	177.00	178	M ⁻¹	177.00, 132.92, 115.8, 105.17, 100.08, 88.83, 84.83, 76.75	Daphnetin, 7,8-dihydroxycoumarin	Doi. 10.3390/molecules20045740
7.	3.47	C ₁₈ H ₃₂ O ₂	279.08	280.04	M ⁻¹	279.08, 250.92, 203.17, 188.75, 118.83, 89.00, 59.08	Linoleic acid	Mass Bank of North America
8.	4.55	C ₇ H ₅ O ₃	377.17	378.12	M ⁻¹	341.33, 179.17, 161.08, 119.08, 89.08, 70.67	Unknown	
9.	4.87		395.17	396.02	M ⁻¹	395.17, 363.2, 214.25, 179.18, 117.08, 101.17	Unknown	
10.	5.01		425.17	426.12	M ⁻¹	393.25, 361.17, 316.2, 274.15, 231.1, 161.17, 111.08	Unknown	
11.	5.37	C ₂₅ H ₄₀ O ₁₁	515.2	516.13	M ⁻¹	515.2 478.12, 400.5, 353.12, 303.25, 262.83, 191.2, 179.08, 134.25, 116.92	Volvatrate C	DOI: 10.1002/jssc.201601406
12.	5.82	C ₇ H ₅ O ₃	555.25	556.04	M ⁻¹	555.25, 523.25, 437.17, 393.25, 361.17, 271.17, 218.17, 173.17, 160.58, 128.17	Unknown	
13.	6.22	C ₂₇ H ₃₀ O ₁₅	593.33	594.14	M ⁻¹	593.33, 561.08, 529.17, 414.17, 339.17, 294.33, 251.08	Kaempferol-3-o-rutinoside	Doi:10.1016/j.phyplu.2021.100025
14.	6.49		631.58	632.18	M ⁻¹	631.58, 599.83, 503.25, 290.00, 272.08, 200.17, 128.25	Unknown	
15.	6.64		645.00	646.21	M ⁻¹	609.08, 301.00, 260.67, 271.02, 178.9	Hesperidin	Mass Bank of North America
16.	6.82		722.13	721.33	M ⁻¹	541.2, 379.17, 201.1	Unknown	

Table 2. LCMS-MS² analysis ((+) ESI-MS/MS (m/z) of *Valerian wallichii* rhizome extract.

S.No	RT	Molecular formula	Observed mass	Exact mass	Ion type	Fragments ((+) ESI-MS/MS (m/z)	Compound	References
1.	8.96	C ₁₀ H ₁₆ O	217.08	216.04	M+1	173.07, 133.25, 125.17	Longiflorone	10.1016/j.phyplu.2021.100025
2.	9.17	C ₁₅ H ₂₂ O	219.17	218.12	M+1	203.13, 161.34, 121.27, 81.0680, 69.0696	Valerenal	10.1016/j.phyplu.2021.100025
3.	9.60	C ₁₅ H ₂₂ O ₂	258.23	234.33	M+23	127.33, 140.30, 141.30, 201.46,	Valerenic acid	10.1016/j.prmcm.2025.100674
4.	11.04	C ₃₁ H ₅₀ O ₁₁	447.25	446.07	M+1	327.21, 285.24, 101.12, 56.12	Orientin	10.1016/j.phyplu.2021.100025
5.	10.59	C ₁₉ H ₂₄ O ₈	403.12	380.15	M+23	303.21, 285.12, :241.11	Desacetylisovaltrate	10.1002/jssc.201601406
6.	11.04	C ₂₂ H ₃₂ O ₈	447.19	424.12	M+23	261.12;161.14(8.78), 155.13,149.13,	Didrovaltrate	10.1002/jssc.201601406
7.	11.22	C ₂₃ H ₃₄ O ₉	477.21	454.13	M+23	279.31, 261.41, 177.3, 149.21	Valeriandoid F	10.1002/jssc.201601406

Table 3. Drug Likeness of Key Compounds from *V. wallichii*.

S. No	Major compounds	Smiles	Drug likeness score	MW <500Da	Log p<5	H-bond donors (S)	H-bonds acceptors <10	M.Ref**
1.	Valerenal	<chem>C[C@@H]1CC[C@H](C2=C(C(C[C@H]12)C)/C=C(\C)/C=O</chem>	-0.55	217.17	4.20	0	1	69.24
2.	Valenianic acid	<chem>C[C@@H]1CC[C@H](C2=C(C(C[C@H]12)C)/C=C(\C)/C(=O)O</chem>	-0.16	234.16	3.72	1	2	70.81
3.	Valeriandoid F	<chem>COC[C@@]1([C@H]([C@@H]2OC(C(C(C)O)=O)[H])C(C(C(C)O)=O)O)C(C(C(C)O)=O)O</chem>	-0.28	454.22	2.33	1	9	114.72

** Molar refractivity

Lipinski properties: All compounds satisfied Lipinski drug-likeness criteria, with molecular weights ranging from 217.17 to 454.22 Da and LogP values below 5. **Valerenal** exhibited the highest lipophilicity (LogP 4.20) and minimal hydrogen-bonding capacity (0 donors, 1 acceptor). **Valerianic acid** showed balanced physicochemical properties, with moderate lipophilicity (LogP 3.72), one hydrogen-bond donor, and two acceptors. **Valeriandoid F** displayed the highest molecular weight (454.22 Da) and hydrogen-bond acceptor count (9), indicating increased structural complexity. Drug-likeness scores were negative for all compounds, with valerianic acid showing the least unfavorable value (-0.16), followed by Valeriandoid F (-0.28) and Valerenal (-0.55). Also, non-odorous compounds were found to be toxic (Table 3).

Table 4. Toxicity profiling of analysis of key compounds from *V. wallichii*.

Compounds	Hepatotoxicity	Mutagenicity	Cytotoxicity
Valerenal	Inactive	Inactive	Inactive
Valerianic acid	Slight	Inactive	Inactive
Valeriandoid F	Inactive	Inactive	Inactive

ADMET analysis: The major compounds of *V. wallichii*, including valerenal, valerianic acid and valeriandoid F, were screened for ADMET analysis using ADMET lab 3.0 as explained earlier. It was noticed that all analysed compounds possess auspicious preliminary pharmacokinetic profile. Further, a higher GI (gastrointestinal) absorption was recorded alongside acquiescence with main bioavailability parameters (Tables 4-5; Figs. 2-4). The PSA (polar surface area) of valerianic acid and Valerenal was low with a moderate MV (molecular volume) level, that explains a good skin penetration and membrane permeability. On the other hand, due to comparably higher MW and elevated polarity, a reduced passive diffusion for valeriandoid F was predicted. In case of Boiled egg analysis for BBB (Blood brain barrier) valuation, moderate permeability for valerenal and valerianic acid was recorded, with higher levels in case of valeriandoid F, explaining key impact on CNS. Among all analyzed compounds, no P-glycoprotein substrate activity was noticed

in case of all compounds. Similarly, the Cytochrome P450 profiling presented multiple CYP isoform inhibition by valeriandoid F compared to other analysed compounds. In the case of toxicity analysis, a slight AMES toxicity was recorded for valeriandoid F and valerenal mild hepatotoxicity signals in all compounds (Tables 4-6, Figs. 2-4).

PASS analysis: PASS prediction indicated that Valeriandoid F exhibited the highest probability of biological activity, particularly for antineoplastic, immunosuppressive, hepatoprotective, and enzyme inhibitory functions (Pa up to 0.821) (Table 7). Valerenal showed moderate predicted activities related to neurological and metabolic functions, including dementia treatment and insulin promotion. Valeric acid demonstrated lower but relevant probabilities for antioxidant and antidiabetic activities, including free radical scavenging and alpha-glucosidase inhibition.

Molecular docking analysis: Molecular docking analysis of valerenal, valerianic acid and valeriandoid F specified differential binding affinities (ΔG) of the compounds across targets (3CJJ, 3TOP, 4F5S). In case of protein 4F5S, highest binding energy (ΔG -7.3 kJ·mol⁻¹) was recorded in case of valerianic acid and 3CJJ (ΔG -7.0 kJ·mol⁻¹) that resulted in stable hydrogen bonding interactions. In case of 3TOP on the other hand the valeriandoid F displayed significant binding with 3TOP (ΔG -7.2 kJ·mol⁻¹), accompanied by multiple interaction residues (H bonding, hydrophobic interactions). Valerenal displayed comparatively weaker binding affinities (-5.2 to -6.9 kJ·mol⁻¹) against analysed targets and scarcer hydrogen bond interactions was seen (Table 8, Figs. 5-7).

Phytochemical analysis: A detailed quantitative phytochemical profiling of *V. wallichii* indicated a diverse array of secondary metabolites including alkaloids, flavonoids, phenolics, tannins and terpenoids (Tables 9-10). Quantitative analysis demonstrated that the ethyl acetate fraction contained the highest total phenolic content (38.4 ± 1.1 mg GAE/g) and total flavonoid content (20.3 ± 1.6 mg QE/g) followed by the crude methanolic extract.

Table 5. Bioavailability properties of major components from *V. wallichii*.

Sample	MolPSA (Å ²)	Mol Vol (Å ³)	pKa<0	BBB	Stereocenters
Valerenal	14.88	280.27	0. /16.12	4.42	3
Valerianic acid	29.13	289.67	0. /4.89	4.31	3
Valeriandoid F	94.47	493.12	0. /14.27	16.8	4

BBB The Blood-Brain Barrier (BBB) Score: 6-High,0-Low

Table 6. ADMET profiling of major components from *V. wallichii*.

Sample	Valerenal	Valerianic acid	Valeriandoid F
GI absorbtion	High	High	High
BBB permeant	No	Yes	No
BBB Score	4.42	4.31	16.8
Pgp substrate	No	No	No
CYP1A2 inhibitor	No	No	Yes
CYP2C19 inhibitor	Yes	No	Yes
CYP2C9 inhibitor	Yes	Yes	Yes
CYP2D6 inhibitor	No	No	Yes
CYP3A4 inhibitor	No	No	Yes
Log K _p (skin permeation)cm/s	-5.37	-5.43	-7.03
AMES Toxicity	Yes (slight)	No	Yes (Slight)
Hepatotoxicity	Yes (slight)	Yes (Slight)	Yes (Slight)

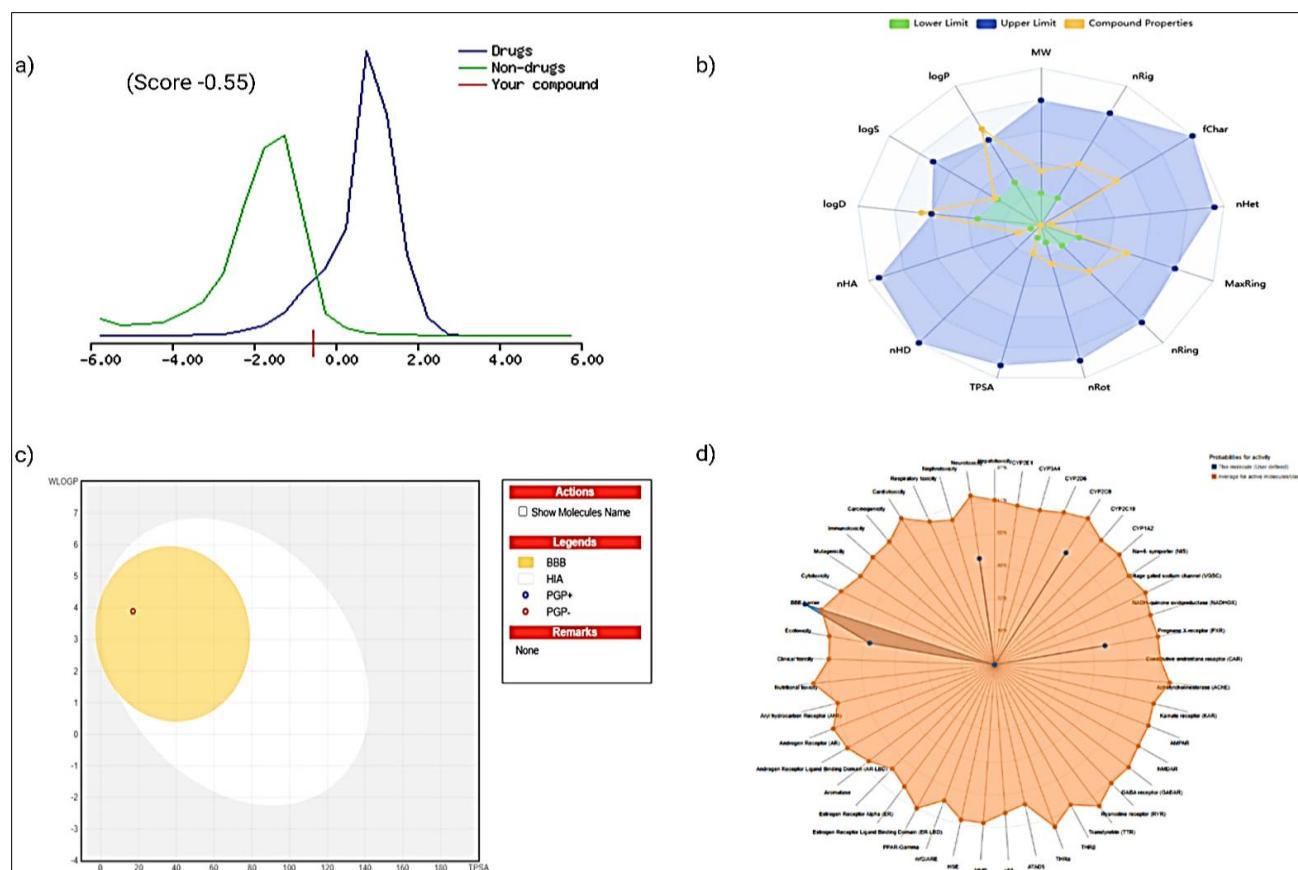


Fig. 2. *In silico* ADMET analysis of Valerenal, including drug likeness a) Bioavailability radad b), Boiled egg analysis c) and Toxicity profiling d).

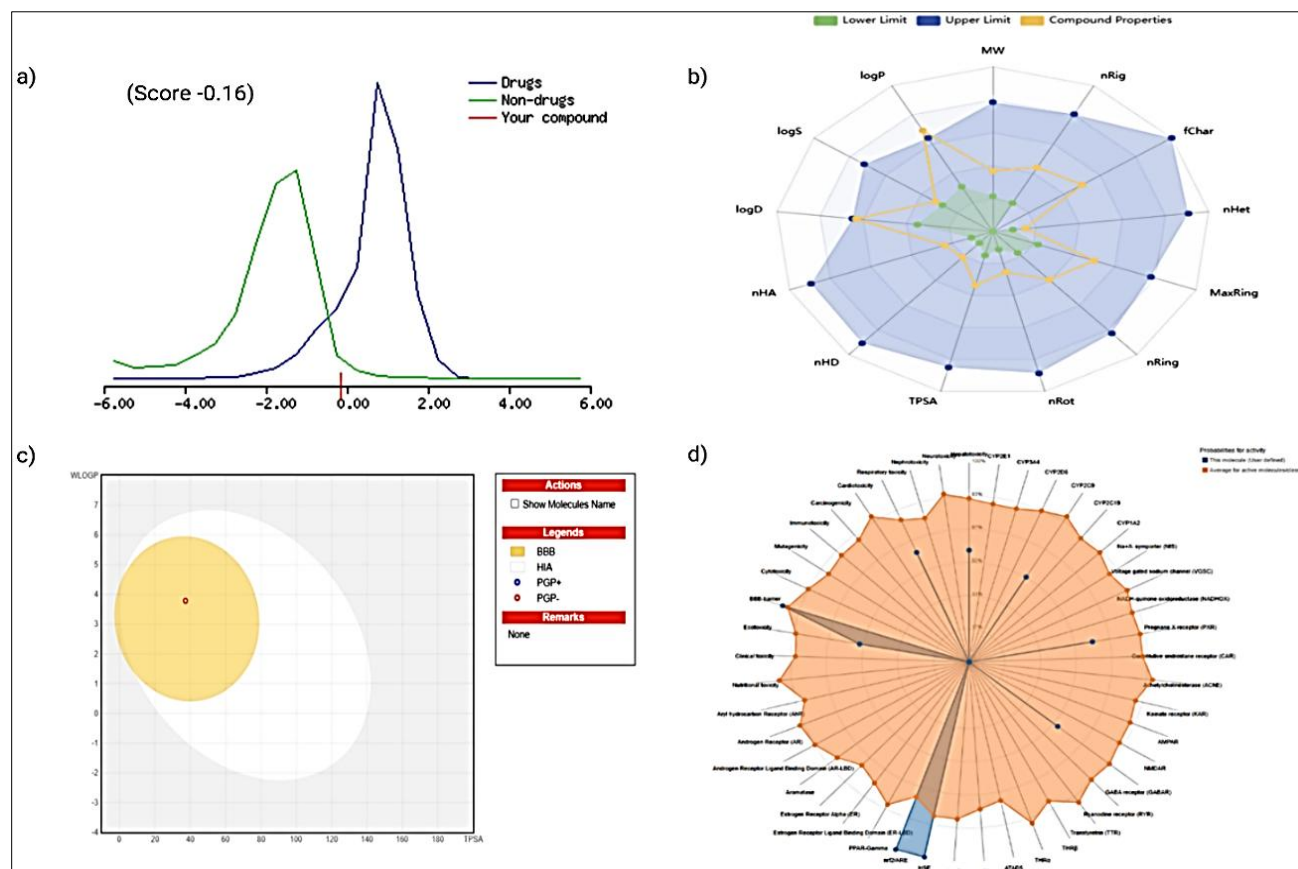


Fig. 3. *In silico* ADMET analysis of Valerenic acid, including drug likeness a) Bioavailability radad b), Boiled egg analysis c) and Toxicity profiling d).

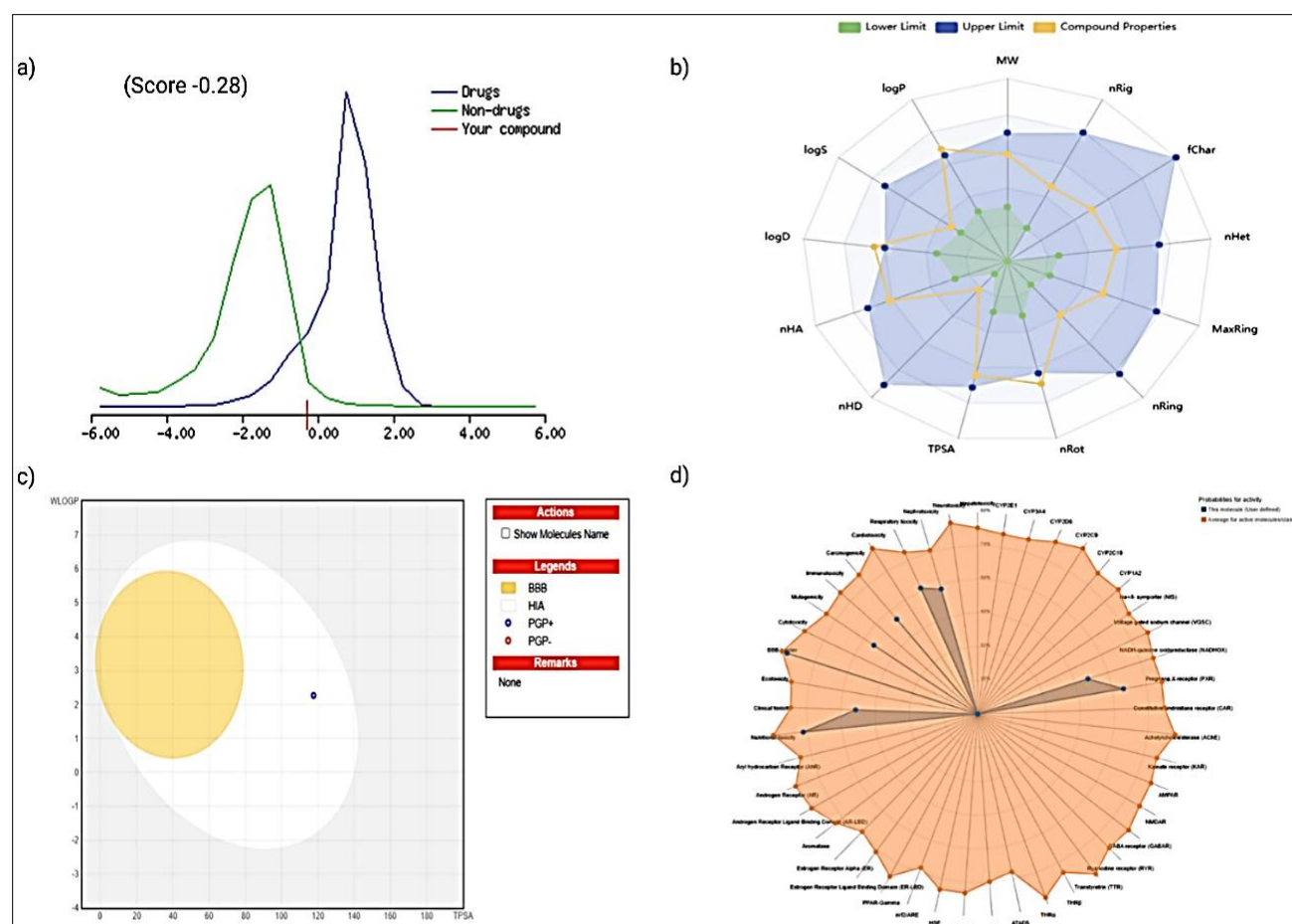


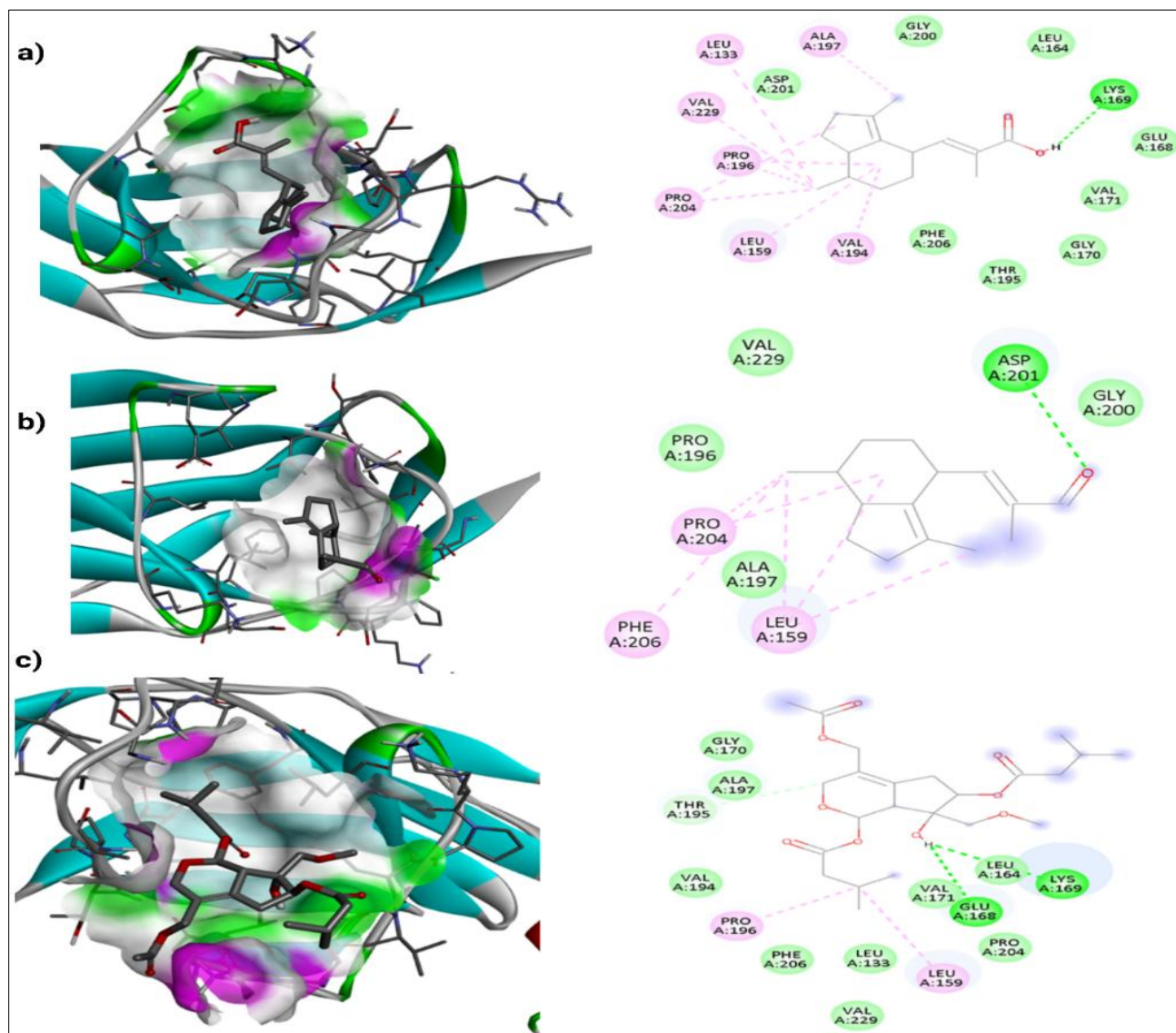
Fig 4. *In silico* ADMET analysis of valerendoid F, including drug likeness a) Bioavailability radar b), Boiled egg analysis c) and Toxicity profiling d).

Table 7. PASS prediction analysis of major compounds from *V. wallichii*.

Plant	Major compound	Pa	Pi	Activity
Valeriana wallichii	Valeriandoid F	0.821	0.009	Antineoplastic
		0.690	0.039	CYP2H substrate
		0.688	0.012	Cholesterol antagonist
		0.654	0.058	CDP-glycerol glycerophosphotransferase inhibitor
		0.623	0.026	Immunosuppressant
		0.595	0.013	Hepatoprotectant
		0.572	0.010	Antineoplastic (lung cancer)
		0.588	0.053	Phosphatase inhibitor
		0.492	0.019	Caspase 8 stimulant
		0.472	0.014	Hepatic disorders treatment
	Valerenal	0.446	0.030	Dementia treatment
		0.312	0.242	Membrane permeability inhibitor
		0.336	0.103	Insulin promoter
		0.234	0.104	Lipid peroxidase inhibitor
		0.133	0.069	Nitric oxide antagonist
	Valeric acid	0.288	0.243	Diabetic neuropathy treatment
		0.315	0.027	Free radical scavenger
		0.288	0.008	Diabetic nephropathy treatment
		0.284	0.005	Antidiabetic (type 1)
		0.222	0.045	Antioxidant
		0.150	0.010	Alpha-glucosidase inhibitor

Table 8. Docking score and interaction analysis of major components in *Valeriana wallichii*.

Ligand	Binding free energy ΔG (kJ mol ⁻¹)	Pose	No of H bonds	H Bond interaction residues	Other interaction residues
3CJJ					
Valeric acid	-7.0	1	1	Lys169	Glue 168, Val171, Gly170, Thr195, Phe206, Val194, Leu159, Pro 204, Val229, Asp201, Leu133, Ala197, Gly200
Valerenal	-5.2	2	1	Asp201	Leu159, Ala197, Phe206, Pro204, pro196, Val229, Gly200
Valeriandoid F	-5.4	1	2	Lys169, Glu168	Val229, Leu133, Phe206, pro196, Val194, the195, Ala197, Gly170, Leu164, Val171, Pro204, Leu159,
3TOP					
Valerianic acid	-5.7	1	2	Ser1292, Met1283	Arg1377, Val1363, Ile1587, Asp1357, Tyr1251, Gln1286, Asp1281, Ile1280, Glu1284, Arg1285
Valerenal	-5.8	1	0	0	Lys1737, Thr1614, Tyr1686, Thr1611, Tyr1685, Thr1607, Gln1610, Pro1722, Glu1721, Tyr1798, Trp1719
Valeriandoid F	-7.2	1	1	Thr1586, Trp1369	Phe1427, Asp1157, Lys1460, Asp1526, Tyr1251, Arg1510, Met1421, Ile1280, Asp1279, His1584, Ile1315, Trp1418, Thr1589, Gly1588, Gln1561
4F5S					
Valerianic acid	-7.3	1	1	Arg185	Tyr137, Tyr160, Phe133, Lys136, Glu125, Pro117, Leu115, Leu122, Glu140, Ile141
Valerenal	-6.9	1	1	Arg185	Ile181, Tyr137, Glu140, Lys136, Leu115, Leu122, Pro117, Tyr160
Valeriandoid F	-6.1	1	1	Arg194, Arg217, Arg435, Thr190, Tyr451	Glu152, Arg256, Arg198, Tyr149, Ser 191, His287, Lys187, Asp450, Ala290, Glu291, Tyr156

**Fig. 5. Interaction(2D) and docking analysis of *V. wallichii* compounds, including Valerianic acid, a) Valerine, b) and Valeriandoid F, c) and 2D interaction analysis with 3CJJ protein.**

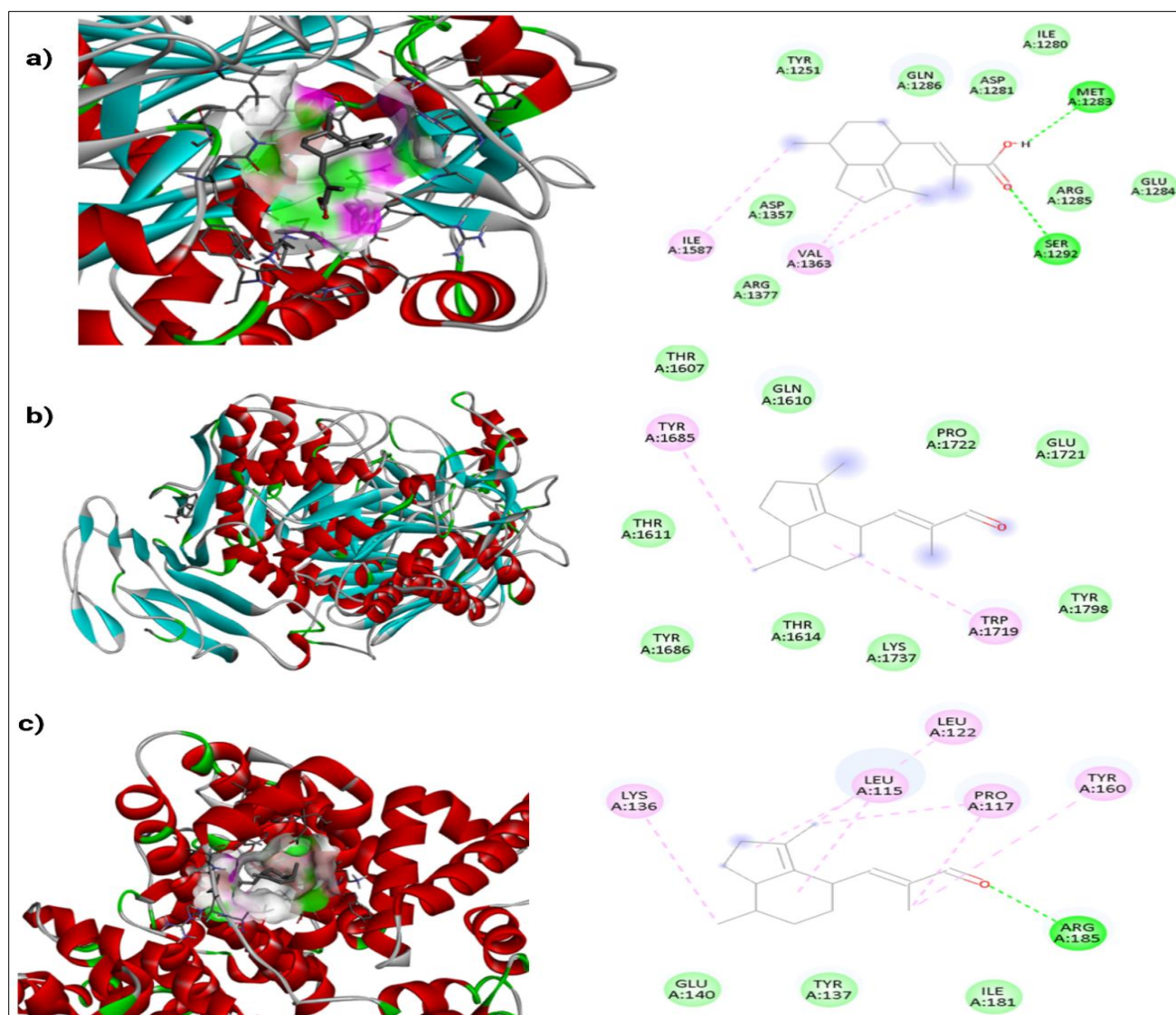


Fig. 6. Interaction(2D) and docking analysis of *V. wallichii* compounds including Valerinic acid a) valeranine b) and Valeriandoid F c) and 2D interaction analysis with 3TOP protein.

Table 9. Phytochemical analysis of *V. wallichii*.

S. No.	Sample	Flavonoids	Alkaloids	Saponins	Terpenoids	Tannins
1.	Extract-1	+++	+	+	++	++
2.	Extract-2	+++	++	++	++	+++
3.	Extract-3	+	-	++	++	+++
4.	n-hexane extract	-	-	-	-	-
5.	Std	4.5*	75±1.1*	0.35	0.056	

Extract-1 (ethanolic extract); extract-2 (ethyl acetate extract) and extracts-3 (Aqueous extract) (+++ = highly present, ++ = moderately present, + = Present, - = Absent)

Antioxidant activity: The Antioxidant assays demonstrated extract-dependent activity of *Valeriana wallichii*. The ethyl acetate extract exhibited the strongest antioxidant potential, with the lowest DPPH IC₅₀ (0.091 mg/mL), highest H₂O₂ scavenging activity (81.2%), and low phosphomolybdenum EC₅₀ (0.31 mg/mL) (Table 11). The aqueous extract showed moderate activity across assays, while the ethanolic extract displayed comparatively weaker DPPH scavenging (IC₅₀: 1.1 mg/mL) and FRAP activity (IC₅₀: 1.4 mg/mL). The n-hexane extract showed no detectable activity. The standard (Trolox) demonstrated strong reference antioxidant capacity across assays.

Antidiabetic activity: The ethyl acetate extract showed the strongest α -glucosidase inhibitory activity (IC₅₀ 0.13 mg/mL), approaching the standard acarbose (IC₅₀ 0.181 mg/mL). In antiglycation assays, the aqueous extract demonstrated the highest inhibition in the BSA–MGO (oxidative) model (IC₅₀ 0.32 mg/mL), while the ethanolic extract showed the strongest activity in the BSA–Glc (non-oxidative) model (IC₅₀ 0.64 mg/mL) (Table 12). The ethyl acetate extract exhibited comparatively lower antiglycation activity in the BSA–Glc system. The standard (rutin) showed superior inhibition across both glycation models.

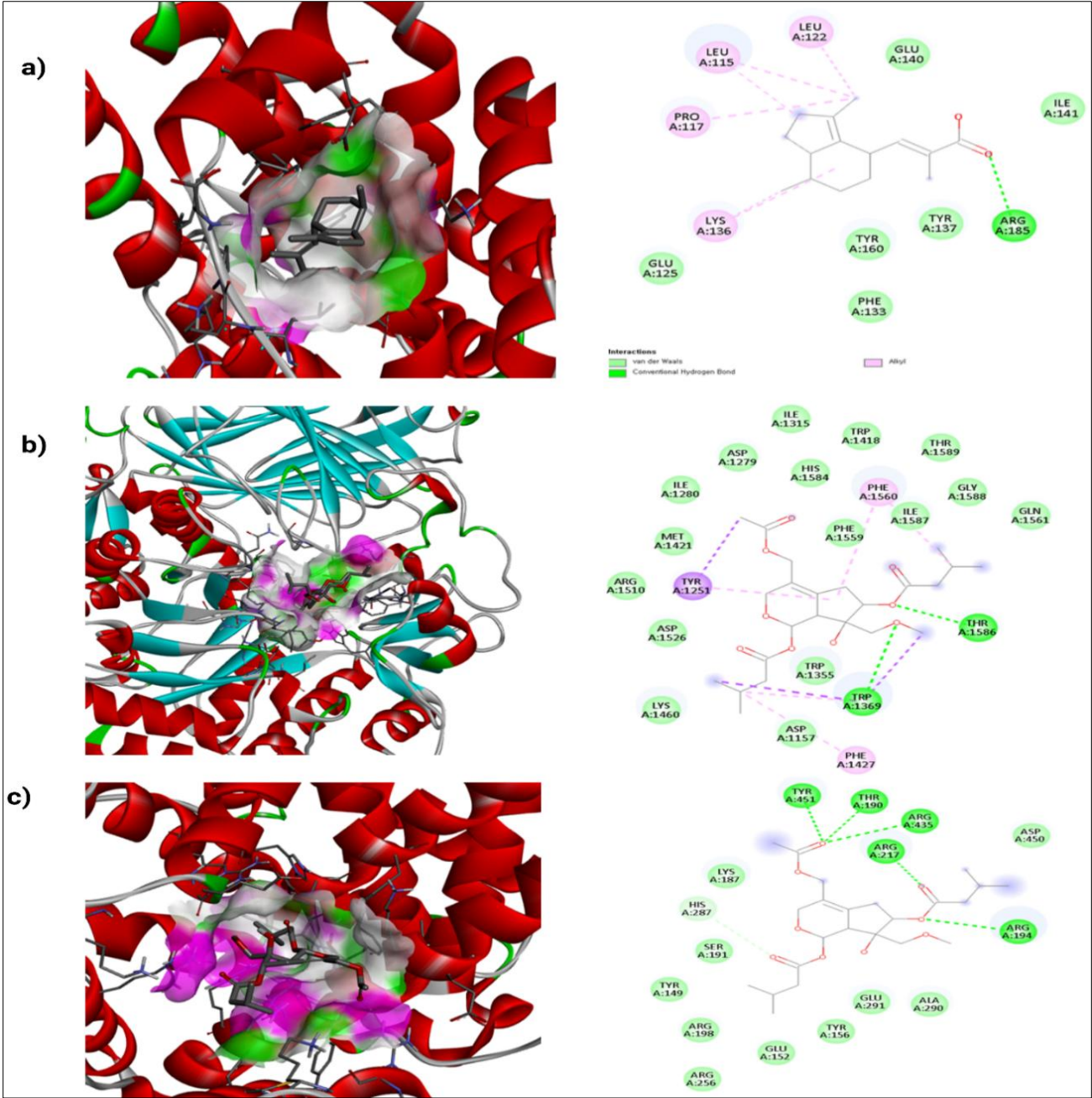


Fig. 7. Interaction (2D) and docking analysis of *V. wallichii* compounds including Valerinic acid a) a) Valerinine b) and Valerianoid F c) and 2D interaction analysis with 4F5S protein.

Table 10. Quantitative analysis of *Valleriana wallichii*.

S. No.	Sample	Total phenolics (GAE mg/g)	Total flavonoids** (mg QE/g DW)
1.	Extract-1	25.1 ± 1.2	14.2 ± 0.5
2.	Extract-2	38.4 ± 1.1	20.3 ± 1.6
3.	Extract-3	22.4 ± 1.2	-
4.	n-hexane extract	-	-

Extract-1(ethanolic extract); extract-2 (ethyl acetate extract) and extrcats-3 (Aqueous extract); GAE= Gallic acid, QE =quercetin

AGEs mechanistic insights: During β -amyloid assay, the ethyl acetate extract showed the strongest protective effect and was classified as protective, indicating excellent antiglycation-related activity (Table 13). The ethanolic and aqueous extracts showed moderate protection, suggesting partial inhibition of protein structural changes associated

with glycation. Free carbonyl entrapment varied across samples, in comparison with CG (control) showing the highest activity in EA (~23 μ M/mg) followed by EO (~29 μ M/mg) and AE (~31 μ M/mg) showing entrapment of free carbonyl groups. Fructosamine levels varied across samples, with CG (control) showing the highest concentration (~0.61 mM/mL), while EA exhibited the lowest level (~0.48 mM/mL), followed by EO (~0.52 mM/mL) and AE (~0.56 mM/mL). Lower fructosamine levels indicate stronger inhibition of early glycation products. L-cysteine levels varied across samples, with CG (control) showing the highest concentration (~0.58 mmol L-cysteine/mg protein), while EA exhibited the lowest level (~0.26), followed by EO (~0.31) and AE (~0.42). Lower L-cysteine depletion indicates stronger protection against protein oxidation and glycation (Fig. 8).

Table 11. Oxidative stress lowering potential of *V. wallichii* extracts in different antioxidant models.

S. No.	Sample	DPPH (IC ₅₀ mg/mL)	H ₂ O ₂ (% inhibition)	Phosphomolybde-Num (EC ₅₀ mg/mL)	FRAP IC ₅₀ mg/mL
1.	Extract-1	1.1	56.0 ± 1.4	0.83	1.4
2.	Extract-2	0.091	81.2 ± 1.6	0.31	0.56
3.	Extract-3	0.65	52.1 ± 1.4	0.63	0.48
4.	Extract-4	-	-	-	-
5.	Std	4.5*	75 ± 1.1*	0.35	0.056

Extract-1 (ethanolic extract); extract-2 (ethyl acetate extract) and extracts-3 (Aqueous extract); extract-4(n-hexane); *Trolox

Table 12. The antidiabetic and anti-AGEs potential of *V. wallichii* extracts.

Sample	α -glucosidase ³	BSA-MGO ³	BSA-Glc ³
Extract-1	0.96	1.4	0.64
Extract-2	0.13	0.87	1.6
Extract-3	0.63	0.32	1.2
Standard	0.18 ¹	0.075 ¹	0.083 ¹

Extract-1 (ethanolic extract); extract-2 (ethyl acetate extract) and extracts-3 (Aqueous extract); ¹Acarbose, ²rutin; (IC₅₀ mg/mL)

Table 13. Investigation on using various extracts from *V. wallichii* regarding its protective role against β -amyloid formation.

Sample	Final mg/mL	Remarks*
Extract-1	0.2	Moderate
Extract-2	0.2	Protective
Extract-3	0.2	Moderate

Extract-1 (ethanolic extract); extract-2 (ethyl acetate extract) and extracts-3 (Aqueous extract)

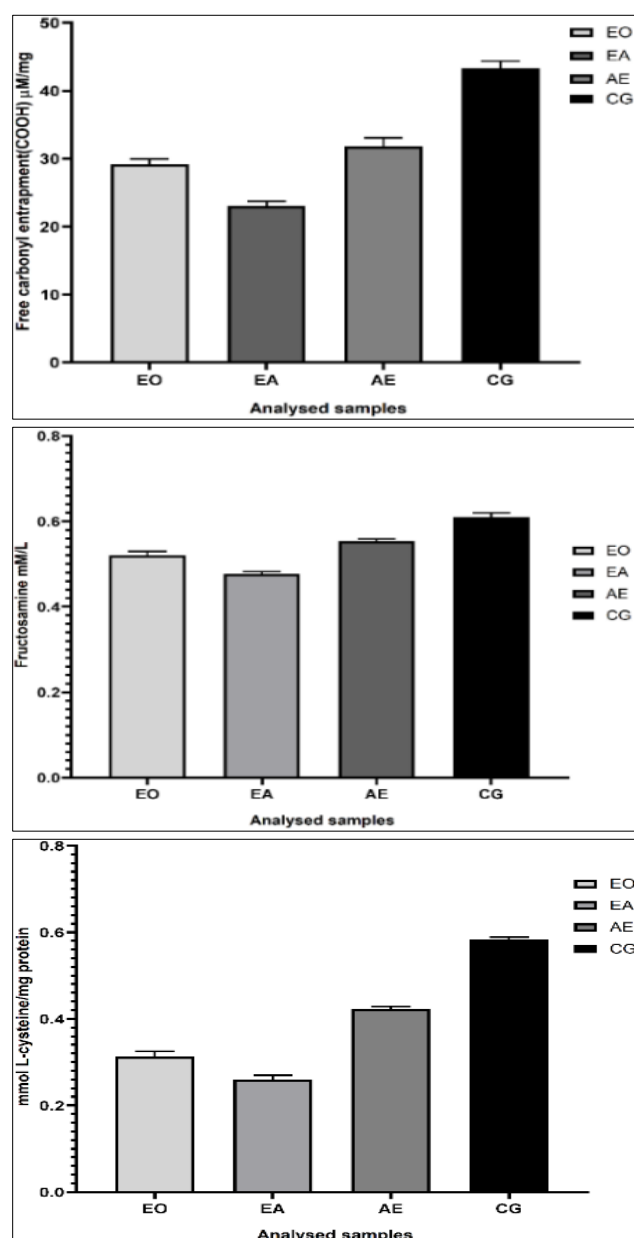


Fig. 8. The AGEs inhibition by various *V. wallichii* extracts through a) Carbonylic entrapment b) fructosamine levels and c) thiol groups estimation.

Discussion

The detailed LCMS analysis of *V. wallichii* revealed a wide array of compounds that is consistent with earlier findings (Jamal *et al.*, 2023; Balikai *et al.*, 2025). The evaluated compounds showed acceptable preliminary drug-likeness, as all remained within Lipinski thresholds for molecular weight, lipophilicity, and hydrogen-bonding parameters. Valerianic acid displayed the most balanced profile, suggesting comparatively favorable permeability and physicochemical suitability. It has been established already that lipophilic molecules possess significant membrane diffusion as noticed in case of valeranal, however solubility could be a limiting factor in this case. On the other hand, in case of valerianoid F although Lipinski's rule of 5 is followed, yet its higher molecular weight and H-bond acceptor count may result in lowering passive permeability (Lipinski *et al.*, 2001; Veber *et al.*, 2002).

The detailed ADMET analysis of *V. wallichii* compounds showed a balanced pharmacokinetic and safety profile. This observation favors oral absorption, permeability with least toxicity signals (Pires *et al.*, 2015). It was further noticed that all compounds possess pertinent skin permeation profiles, which additionally propose that low MW molecules with less polar nature is suitable for transdermal or topical formulations (Gupta *et al.*, 2019). These outcomes are consistent with previous *In silico* investigations that are related with pharmacokinetics, toxicity profiles of molecules in early stage drug discovery (Daina *et al.*, 2017). In PASS (prediction of activity spectra of substances) analysis, highest prediction scores ($pa > pi$) was recorded in case of Valerianoid F showing interaction with numerous therapeutic targets. It is important to note here that despite robustness of this PASS prediction there always exists a great need to experimentally validate the outcomes (Lagunin *et al.*, 2000; Filimonov *et al.*, 2014).

During docking studies, it was evident that Valerianoid F and Valerianic acid possess sturdier and comparably stable binding interaction than valeranal. This is most likely to be due to higher number and favorable hydrogen bonding and wider hydrophobic interactions (Vinod *et al.*, 2023). The broad and variable binding affinities of these molecules across different protein targets propose target-specific interaction profiles and this mainly

pertains to structural complexities (Arif *et al.*, 2021). These observations are consistent with recognized principles of molecular docking, where binding affinity is governed by hydrogen bonding, hydrophobic interactions and conformational functional groups (Morris *et al.*, 2009).

The compounds distribution in different fraction was relied on chemical nature of these compounds and polarity of used solvents (Wakeel *et al.*, 2019). On the other hand, no promising compounds were observed in *n*-hexane fraction. A comparably high concentration of phenolic compounds as observed in the ethyl acetate fraction reveal greater efficiency in extracting semi-polar bioactive compounds (Tsaturyan *et al.*, 2025). This is significant since flavonoids and phenolic compounds are best known for their ability to act as free radical scavengers, metal chelators, and inhibitors of oxidative processes, making implications in Diabetes and AGEs.

The antioxidant experiments included three diversified mechanism and it was evident ethyl acetate and aqueous extracts possess significant inhibitions. It was thus hypothesized that key antioxidant components of *V. wallichii* mainly reside in semi-polar to polar solvent fractions. This observation is consistent with the reported evidence of the influence of solvent polarity on the effectiveness of phytochemical extraction process (Dai and Mumper, 2010). Since these fractions are rich in flavonoids, terpenoids and organic acids, it is evident that the molecules can either donate hydrogen atoms or electrons to neutralize free radicals (ROS) (Prior *et al.*, 2005). Contrary to this, no inhibition was noticed in case of *n*-hexane fraction (non-polar components), indicating a lesser involvement of non-polar constituents in lowering oxidative stress (Choi *et al.*, 2018). Thus among all analyzed extracts, ethyl acetate fraction possesses higher potential in mitigating oxidative stress, that is very important in management of AGEs and DM (Sies *et al.*, 2017).

Overall, the results indicate that different solvent extracts selectively target distinct antiglycation pathways, reflecting variation in phytochemical composition. The strong α -glucosidase inhibition by the ethyl acetate extract suggests enrichment of semi-polar bioactive compounds capable of modulating carbohydrate digestion and postprandial glucose levels (Tadera *et al.*, 2006). In contrast, the aqueous extract showed higher efficacy in the oxidative BSA-MGO model, indicating the presence of polar antioxidant constituents that may inhibit reactive carbonyl species and oxidative glycation pathways (Wu & Yen, 2005). The activity observed in the BSA-Glc model suggests potential interference with early-stage glycation reactions. These findings support the role of plant-derived phenolics and related compounds in inhibiting advanced glycation end product (AGE) formation through both antioxidant and carbonyl-trapping mechanisms, which are relevant to diabetes-associated complications (Yamagishi *et al.*, 2012).

It is evident from these findings that a significant protection against β -sheet aggregation is provided by ethyl acetate extract. It is most likely be due to presence of flavonoids, phenolics and terpenoids (semi-polar bioactive), that are generally associated with antioxidant and AGEs inhibition properties (Dai and Mumper, 2010). The pragmatic inhibition of β -amyloid formation is consistent with the potential role of AGEs in promotion of protein aggregation and misfolding (Singh *et al.*, 2001). These

findings therefore strongly favor the potential of *V. wallichii* extracts in lowering glycation related damages to protein structures that are related to diabetes and neurodegenerative conditions (Ott *et al.*, 2014). Likewise, the carbonyl entrapment assay outcomes also suggest that all analysed compounds were able to trap carbonylic moieties, that is one of key mechanisms in reducing the formation of AGE. A decreased carbonyl burden is considered important with pharmacological perspective in diabetics since the reactive carbonyl species mainly contribute toward modification of proteins, thereby lead to advancement of metabolic disorders (like diabetes) (Singh *et al.*, 2001). These findings support the role of plant-derived phytochemicals in mitigating carbonyl stress and oxidative damage (Ott *et al.*, 2014). Fructosamine represents early glycation intermediates (Amadori products), and its reduction indicates suppression of initial glucose-protein interactions, which are critical in the progression toward advanced glycation end products (AGEs). This mechanism is well established in the pathophysiology of diabetes and its complications (Brownlee, 2005). The enhanced activity of EA may be attributed to semi-polar phytochemicals that interfere with glycation and oxidative pathways. Such inhibition of early glycation markers has been shown to reduce downstream AGE formation and associated metabolic damage (Thornalley, 2003). Overall, comparably elevated L-cysteine levels were noticed in EA, EO, and AE compared to control (CG₋). It was thus evident that EA possess significant protective effects against thiol oxidation. Preservation of L-cysteine reflects reduced oxidative and carbonyl stress, which are key drivers of protein modification and advanced glycation end product (AGE) formation. Maintenance of thiol groups is critical for protein stability and cellular redox balance, particularly in metabolic disorders such as diabetes (Townsend *et al.*, 2003). These findings support the role of plant-derived bioactive compounds in protecting thiol integrity and limiting oxidative damage (Schafer & Buettner, 2001).

Conclusions

V. wallichii exhibits notable antidiabetic and antiglycation activity, with the ethyl acetate extract showing the highest efficacy. The observed effects are associated with inhibition of oxidative stress, carbonyl formation, and protein glycation. *In silico* results further support its pharmacological potential. These findings indicate that *V. wallichii* may serve as a promising source of bioactive compounds for managing glycation-related diabetic complications, pending further *In vivo* validation.

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Authors' contributions: A.S.K., S.A., and S.C. performed the experimental work, data analysis, and writing and editing of the final draft. M.Z. and A.H.S. contributed to computational analysis, interpretation of results and writing the draft. A.A. and A.H.S. conceptualized the study, supervised the research, and finalized the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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