

Integrated in silico and LC-MS Profiling of *Achillea millefolium* Against Olanzapine-Induced Metabolic Dysregulation

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Highlights

- *Achillea millefolium* phytochemicals engage 179 OLZ-linked targets in silico
- Apigenin shows the strongest DRD2 binding (−9.5 kcal/mol); quercetin targets HMGCR (−8.5 kcal/mol)
 - MD simulations confirm partial complex stability over 100 ns
- LC-MS validates apigenin, quercetin, luteolin, and resveratrol in hydroalcoholic extract
- AM is a credible multi-target candidate for antipsychotic metabolic complications

ABSTRACT

Background and Purpose:

Olanzapine (OLZ), a widely prescribed second-generation antipsychotic, is strongly associated with metabolic adverse effects including significant weight gain, dyslipidaemia, and insulin resistance. These effects arise through multifactorial mechanisms involving dopaminergic, serotonergic, histaminergic, and muscarinic receptor antagonism, resulting in olanzapine-induced obesity (OIO) and metabolic syndrome. *Achillea millefolium* (AM), a medicinal herb rich in diverse phytoconstituents including apigenin, quercetin, resveratrol, luteolin, linalool, and terpenoids, exhibits documented anti-inflammatory, antioxidant, and metabolic-regulatory activities. This study evaluated AM phytochemicals against OLZ-induced metabolic dysregulation using integrated in silico and in vitro approaches.

Experimental Approach:

A total of 59 AM phytochemicals were screened for drug-likeness and ADMET profiles; 44 met selection criteria. Network pharmacology was employed to identify OLZ-modulated targets and enriched KEGG pathways. Molecular docking was performed against six key targets (DRD2, HTR2C, GSK3B, CHRM1, CHRM2, HMGCR), followed by 100 ns molecular dynamics (MD) simulation of the top complexes. In vitro analysis included total phenolic content (TPC), total flavonoid content (TFC), and LC-MS characterisation of the AM hydroalcoholic extract.

Key Results:

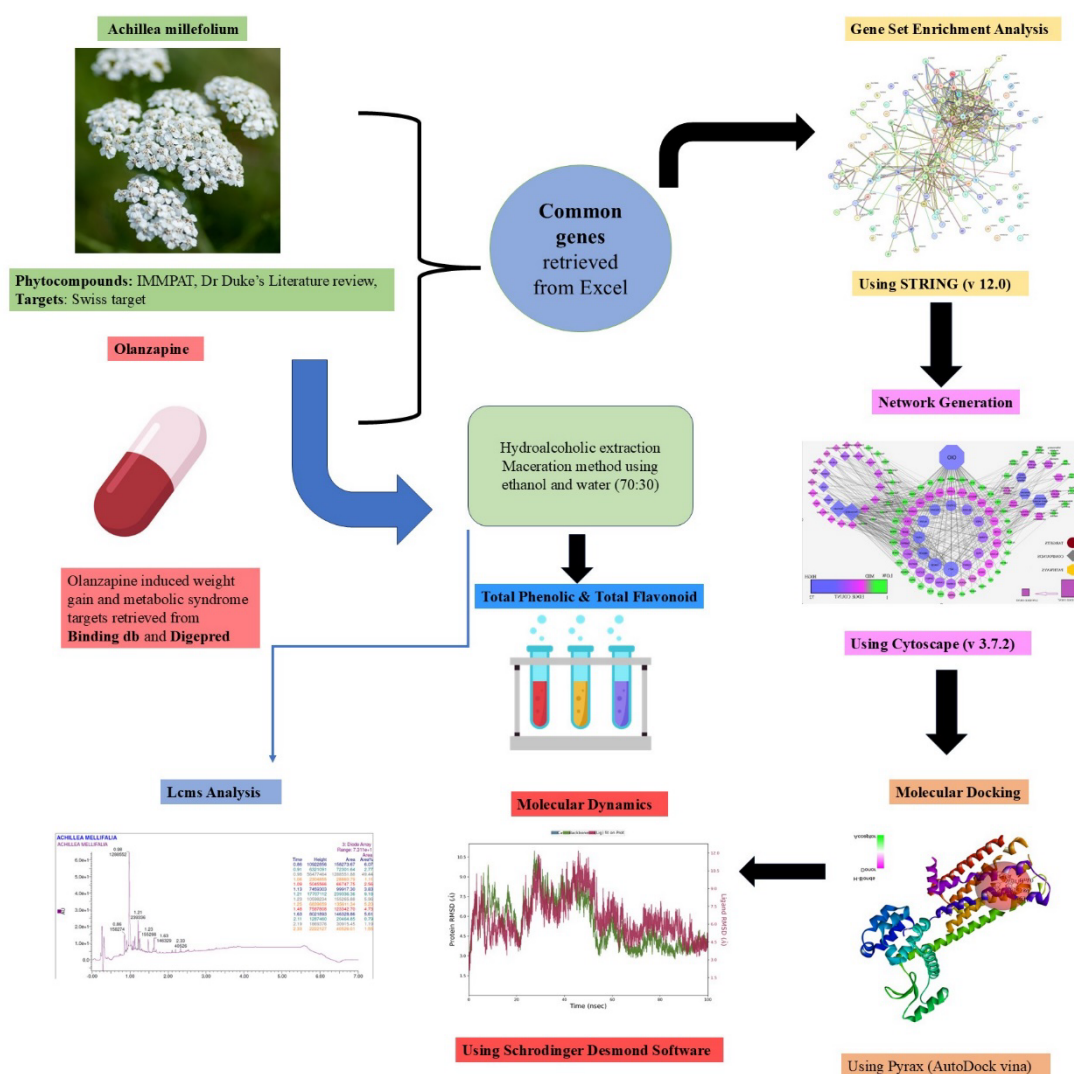
Network pharmacology identified 179 OLZ-modulated targets; KEGG enrichment highlighted dopaminergic synapse, insulin resistance, AMPK, and PPAR signalling pathways. Apigenin showed the highest binding affinity at DRD2 (−9.5 kcal/mol) and quercetin at HMGCR (−8.5 kcal/mol, 10 active-site contacts). MD simulations confirmed partial binding stability with preserved radius of gyration. TPC was 26.07 ± 1.876 mg GAE/g and TFC was 72.78 ± 5.697 mg QE/g. LC-MS identified apigenin, quercetin, luteolin, and resveratrol in the extract.

Conclusion and Implications:

These findings establish AM as a promising multi-target candidate for adjunct management of antipsychotic-induced metabolic complications, warranting further in vivo and pharmacokinetic validation.

Keywords: *Achillea millefolium*; olanzapine-induced metabolic syndrome; network pharmacology; molecular docking; molecular dynamics

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1. Introduction

Olanzapine (OLZ) is a widely used second-generation antipsychotic (SGA) prescribed for the treatment of schizophrenia, bipolar disorder, and as an adjunct therapy for major depressive disorder [1]. OLZ exerts its antipsychotic action primarily through antagonism of dopamine (D2) receptors in the mesolimbic pathway, which leads to a reduction in positive symptoms such as hallucinations and delusions [2]. Additionally, it blocks 5-HT_{2A} receptors in the mesocortical pathway, leading to increased dopaminergic transmission that helps alleviate negative and cognitive symptoms [3]. Furthermore, OLZ has a lower affinity for D2 receptors in the nigrostriatal pathway, which contributes to a reduced incidence of extrapyramidal side effects compared to typical antipsychotic agents (2-5). Despite its therapeutic benefits, OLZ is strongly associated with metabolic adverse effects, including significant weight gain, dyslipidaemia, impaired glucose homeostasis, and an increased risk of cardiovascular complications [7,8].

Weight gain, in particular, is one of the most concerning adverse effects, not only because of its impact on physical health but also due to its broader clinical effects. The development of obesity increases the risk of other conditions such as type-2 diabetes, hypertension, cardiovascular disease, some forms of cancer, and sexual dysfunction. These complications can negatively affect a patient's quality of life, psychological well-being and also lead to reduced adherence or complete discontinuation of therapy, increasing the risk of symptom relapse and hospitalisation [9].

Therefore, it is essential to understand and minimise OLZ-induced weight gain in order to enhance patient safety and long-term therapeutic results.

The mechanisms underlying OLZ-induced weight gain and metabolic dysregulation are not completely understood; however, multiple pathways have been implicated. OLZ antagonizes several central receptors, including M3, 5-HT_{2C}, D2, and H1 receptors, leading to increased appetite, reduced satiety, and impaired glucose and lipid metabolism [10,11]. Genetic variations in serotonin receptor genes may further increase susceptibility to these metabolic effects. Clinically, these alterations manifest as hyperphagia, reduced physical activity, insulin resistance, dyslipidaemia, and obesity [12,13]. Additionally, SGAs disrupt hypothalamic appetite regulation by upregulating orexigenic neuropeptides such as neuropeptide Y (NPY) and agouti-related peptide (AgRP), while downregulating anorexigenic neuropeptides including pro-opiomelanocortin (POMC) and cocaine-and amphetamine-regulated transcript (CART) within the arcuate nucleus (Arc) of the hypothalamus [14–16]. OLZ treatment also increases ghrelin receptor (GHS-R1a) expression and circulating ghrelin levels, contributing to hyperphagia and weight gain [17]. Furthermore, OLZ-induced obesity and dyslipidaemia are associated with elevated pro-inflammatory cytokines, decreased adiponectin levels, and hyperleptinemia [12].

Achillea millefolium (AM), commonly known as Yarrow, is a medicinal plant rich in flavonoids, polyphenols, terpenoids, and essential oils, including apigenin, luteolin, and dicaffeoylquinic acids [18–20]. These phytoconstituents exhibit anti-inflammatory, antioxidant, and metabolic regulatory activities through modulation of inflammatory cytokines, NF- κ B, COX/LOX pathways, ROS scavenging, and insulin-related signalling [21–24]. Despite its pharmacological potential, the role of AM in olanzapine-induced obesity and metabolic dysregulation remains underexplored. Therefore, this study employed an integrated in-silico approach involving network pharmacology, molecular docking, and molecular dynamics simulation, along with LC-MS validation, to investigate the therapeutic potential of AM-derived phytoconstituents against antipsychotic-induced metabolic complications.

2. Materials & Methods

2.1 Computational pharmacology

2.1.1 Mining of bioactive constituents and their information

The phytochemicals of AM were compiled through published literature [18,25] and phytoconstituent databases, including Dr. Duke's Phytochemical and Ethnobotanical Database (<https://phytochem.nal.usda.gov/phytochem/search/list>) (accessed on 10-09-2025), IMPPAT (<https://cb.imsc.res.in/imppat/>) (accessed on 10-09-2025), and the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) (accessed on 11-09-2025) [26,27]. These databases were used to obtain detailed chemical profiles of the compounds, such as molecular formula, molecular weight, and their canonical SMILES representations.

2.1.2 Drug-Likeness Evaluation and ADMET Analysis

To assess the pharmacokinetic activity of the identified compounds, drug-likeness properties were predicted using the Molsoft online platform (<http://molsoft.com/mprop/>) (accessed on 14-09-2025), and ADMET characteristics were evaluated using the pkcsim web server (<https://biosig.lab.uq.edu.au/pkcsim/>) (accessed on 15-09-2025) [28]. These analyses were carried out to screen phytochemicals with favourable absorption, distribution, metabolism, excretion, and toxicity profiles.

2.1.3 Prediction of side-effects

To evaluate the possibility of any toxic or adverse effects, the ADVERPred web server (<http://www.way2drug.com/adverpred/>) was used (accessed on 17-09-2025) [29]. This tool estimates the probability of key side effects, such as arrhythmia, myocardial infarction, cardiac failure, nephrotoxicity, and hepatotoxicity, by comparing the structure of the selected compounds with those of known drugs. The results are expressed as Pa, representing the probability that a compound may cause a particular side effect, and Pi, indicating the probability that the compound is unlikely to cause that effect.

2.1.4 Identification of OLZ-regulated/binding targets

The chemical structure of OLZ was obtained in SMILES format from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) (accessed on 20-09-2025) [26]. Potential molecular targets of OLZ were predicted using DIGEP-Pred (<https://way2drug.com/digep-pred/>) (accessed on 23-09-2025) with a pharmacological activity score cutoff of ≥ 0.5 . Additionally, Binding DB (<https://www.bindingdb.org/>) (accessed

on 25-09-2025) was used to identify experimentally reported binding interactions, applying a minimum binding probability of ≥ 0.7 . [30]

2.1.5 Functional enrichment analysis

The protein sets derived from the phytocompounds and OLZ (Gene IDs) were submitted to STRING v12.0 (<https://string-db.org/>) (accessed on 1-10-2025) to evaluate protein-protein interaction (PPI) networks [31]. KEGG (<https://www.genome.jp/kegg/>) (accessed on 5-10-2025) was used to identify pathways relevant to metabolic and obesity-related conditions [32]. The resulting interaction and pathway data were visualised and integrated using Cytoscape v3.7.2, and network topology was assessed using the “Edge Count” parameter to determine the most interconnected nodes within the network [33].

2.1.6 Molecular docking

Based on NP analysis, six target proteins, DRD2, HTR2C, GSK3B, CHRM1, CHRM2, and HMGCR, were prioritised for molecular docking using AutoDock4.0 (Morris et al. 2009) on the PyRx 0.8 platform (<https://pyrx.sourceforge.io/>) to identify probable binding affinity. The selected phytocompounds and their respective standard reference drugs were used as ligand molecules (Table 1). The two-dimensional structural coordinates of the phytocompounds were retrieved in SDF format from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>) [26] and subsequently converted to PDB format using BIOVIA Discovery Studio Visualizer 2019 [33].

The three-dimensional X-ray crystallographic structures of the target proteins were retrieved from the RCSB Protein Data Bank (<https://www.rcsb.org>) (accessed 13-10-2025) and saved in PDB format [34]. Active site prediction and binding pocket characterisation for each target protein were performed using the PrankWeb server (<https://prankweb.cz>) [35]. A docking grid box was created for each protein and centred on the projected active site, with box dimensions set to include all important binding residues. The lowest-energy binding positions were selected using the computed binding affinity scores (kcal/mol) after standard docking procedures were performed consistently to all protein-ligand pairs [28].

Following docking, protein-ligand interaction profiles including hydrogen bonds, hydrophobic contacts, and π -interactions were visualised and analysed using BIOVIA Discovery Studio Visualizer 2019 [33].

2.1.7 Molecular dynamics studies

To determine the stability of the complex formed, a 100 ns MD simulation of the protein/ligand complex (systems) was performed using the software package Desmond. The systems were put in a cubic box of side length $10 \text{ \AA} \times 10 \text{ \AA} \times 10 \text{ \AA}$, solved using the Simple Point Charge model of water under the periodic boundary condition. The systems were neutralised by adding a counterion solution containing Na^+ and Cl^- . The SHAKE algorithm was used to restrict water to its geometry and to maintain the bond lengths and bond angles of the heavy atoms. Furthermore, long-range interactions (beyond $9.0 \text{ \AA}$) designed for Coulomb forces were treated through the Particle Mesh Ewald approach. The Lennard-Jones forces were computed with a cutoff of $10 \text{ \AA}$. The systems were then minimised and equilibrated according to their respective Desmond default protocols, followed by the production run of the 100 ns trajectory at constant temperature and pressure (300 K, 1.01325 bar) using the Nosé-Hoover chain thermostat and the Martyna-Tobias-Klein barostat with relaxation times of 1.0 ps and 2.0 ps, respectively. Coulomb forces for short-range interactions were computed over $9.0 \text{ \AA}$. At the end of the production run, RMSD, RMSF, and radius of gyration (rGyr) were calculated for all the residues, along with monitoring the protein/ligand interactions throughout the entire 100 ns simulation to assess the stability of the intermolecular contacts.[28]

Table 1. Target proteins, corresponding PDB IDs, and selected ligands used for molecular docking and MD studies [36].

Protein Name	PDB ID (Chains)	Ligand
Dopamine receptor (DRD2)	6CM4 ,7JVR	Apigenin, Quercetin, Resveratrol
Muscarinic acetylcholine receptor M1 (CHRM1)	6ZG4	Quercetin, Resveratrol
Muscarinic acetylcholine receptor M2 (CHRM2)	5YC8	Quercetin, Resveratrol
5-hydroxytryptamine receptor 2C (5HT2C)	6BQH,6BQG	Apigenin

3-hydroxy-3-methyl-glutaryl-coenzymeA reductase (HMGCR)	3CDA,1HWL	Quercetin, Resveratrol
Glycogen Synthase Kinase-3 Beta (GSK3B)	6GN1,4JIR	Apigenin, Quercetin, Resveratrol

2.2 In-Vitro Screening Methods

2.2.1 Plant Collection and Plant Extraction

AM (whole dried plant) was procured from Nanda Medicinal Plants Traders, College Road, near Haripur, Soil, Himachal Pradesh 175136, India (GPS coordinates: 32°09'09.9"N 77°10'39.1"E). The plant name was verified using The Plant List (<http://www.theplantlist.org>; accessed 15 August 2025). A voucher specimen has been deposited in the Herbarium, Department of Pharmacognosy, KLE College of Pharmacy, Belagavi (Voucher No. KLE/PHCOG/2025/001). The supplier provided a certificate of identity confirming the botanical source, and batch characterisation data (including TPC and TFC) were obtained as quality control parameters (see Sections 2.2.2 and 2.2.3).

The whole dried plant material was ground into a fine powder. Hydroalcoholic extraction was carried out using the maceration technique, where the powdered plant material was soaked in a solvent mixture of 70% ethanol and 30% distilled water in a tightly sealed container for 7 days, with occasional shaking to ensure thorough mixing. After the maceration period, the mixture was filtered to separate the plant residue from the extract. The filtrate was then evaporated using a water bath at 50°C to remove the solvent and obtain a concentrated dry extract. The dried extract was then stored in an airtight container for further use [19,20].

2.2.2 Determination of Total Flavonoid Content (TFC)

The TFC of AM was determined by the aluminium chloride colourimetric method using quercetin as the reference standard. Briefly, 1 mL of extract solution (1 mg/mL) was diluted with distilled water, followed by the sequential addition of 5% sodium nitrite and 10% aluminium chloride at 5-min intervals. The reaction mixture was then treated with 1.0 M sodium hydroxide, and the final volume was adjusted with distilled water. Extract samples (100, 80, 60, 40, and 20 µg/mL) and quercetin standard solutions (3.125-50 µg/mL) were prepared similarly. Absorbance was measured at 510 nm using a UV-visible spectrophotometer. TFC was calculated from the quercetin calibration curve and expressed as milligrams of quercetin equivalents (QE) per gram of extract [37,38].

2.2.3 Determination of Total Phenolic Content (TPC)

The TPC of AM extract was determined using the Folin-Ciocalteu method, with gallic acid as the calibration standard. Briefly, 1 mL of plant extract solution (1 mg/mL) was mixed with 9 mL of distilled water in a 25 mL volumetric flask, followed by the addition of 2.5 mL of Folin-Ciocalteu reagent. After 5 min, 10 mL of 7.5% sodium carbonate solution was added, and the final volume was adjusted with distilled water. The reaction mixture was incubated at room temperature for 90 min. Gallic acid standard solutions (100-200 µg/mL) were prepared using the same procedure. Absorbance of both sample and standard solutions was measured at 760 nm using a UV-visible spectrophotometer. TPC was calculated from the gallic acid calibration curve and expressed as milligrams of gallic acid equivalents per gram of extract [37,39,40].

2.2.4 LC-MS-Based Phytochemical Characterisation

Phytochemical profiling of the extract was carried out using an LC-MS system equipped with an Inertsil ODS-3 reverse-phase column. Chromatographic separation was achieved through gradient elution using two mobile phases: 0.1% formic acid in water and acetonitrile, at a flow rate of 1 mL/min. Mass spectrometric detection was performed using a Waters Micromass ZQ instrument operating in electrospray ionisation (ESI) mode, with a scan range of 100-1000 m/z. This scanning range was chosen to ensure comprehensive coverage of both low and medium-molecular-weight phytochemicals characteristic of the plant material under investigation. All acquired data were processed and interpreted using MassLynx software, enabling effective compound separation, identification, and quantification [41].

3. Results

3.1 Computational pharmacology

3.1.1 Retrieving phytochemicals and their intended protein targets

A total of 59 phytocompounds were identified in AM (Supplementary Table S1), with resveratrol and quercetin showing the strongest protein-regulatory potential. Most of the other constituents predominantly targeted AKT1, GSK3B, HMGCR, and DRD2 (Supplementary Table S2).

3.1.2 Retrieving of OLZ-modulated protein targets

Olanzapine induced obesity (OIO) is closely associated with metabolic disturbances such as insulin resistance, dyslipidaemia, hypertension, and weight gain. To understand the molecular basis of these effects, a total of 179 potential protein targets related to OIO were identified. (Supplementary Table S3).

3.1.3 Calculation of druglike property and ADMET profile

Out of the 59 phytocompounds screened, 49 fulfilled the predefined drug-likeness criteria. Compounds with intestinal absorption above 70% were selected to ensure adequate oral bioavailability following administration. For toxicity evaluation, compounds were further filtered using an established probability threshold of ≤ 0.6 , excluding molecules with higher predicted toxicity. Based on the combined drug-likeness, intestinal absorption, and toxicity criteria, a final panel of 44 phytocompounds was selected as suitable candidates for further pharmacological and mechanistic investigations (Supplementary Table S4).

3.1.4 Functional enrichment analysis and key pathways

The network analysis revealed a densely connected set of proteins involved in the metabolic abnormalities associated with OLZ treatment. Central metabolic regulators such as INSR, AKT1, GSK3B, PIK3CG, and IGF1R formed the core of the network, while receptors including DRD2, CHRM1, CHRM2, and several HTR family members established strong links to central appetite-regulating circuits (Fig 1). KEGG pathway enrichment highlighted several neurologically linked pathways, most notably the dopaminergic synapse and serotonergic synapse pathways, both of which play important roles in appetite control, reward-driven feeding, and energy balance and are significantly disrupted by OLZ. Alongside these CNS pathways, several metabolic and obesity-related pathways were also enriched, including insulin resistance, AMPK signalling, adipocytokine signalling, PPAR signalling, non-alcoholic fatty liver disease, insulin secretion, and type II diabetes mellitus. These pathways were collectively regulated by key nodes, including AKT1, GSK3B, INSR, DRD2, HMGCR, and LDLR, indicating coordinated effects on neurotransmission, glucose regulation, and lipid metabolism. Together, these findings suggest that AM phytocompounds may beneficially influence both CNS signalling and peripheral metabolic pathways central to OIO and metabolic dysregulation.

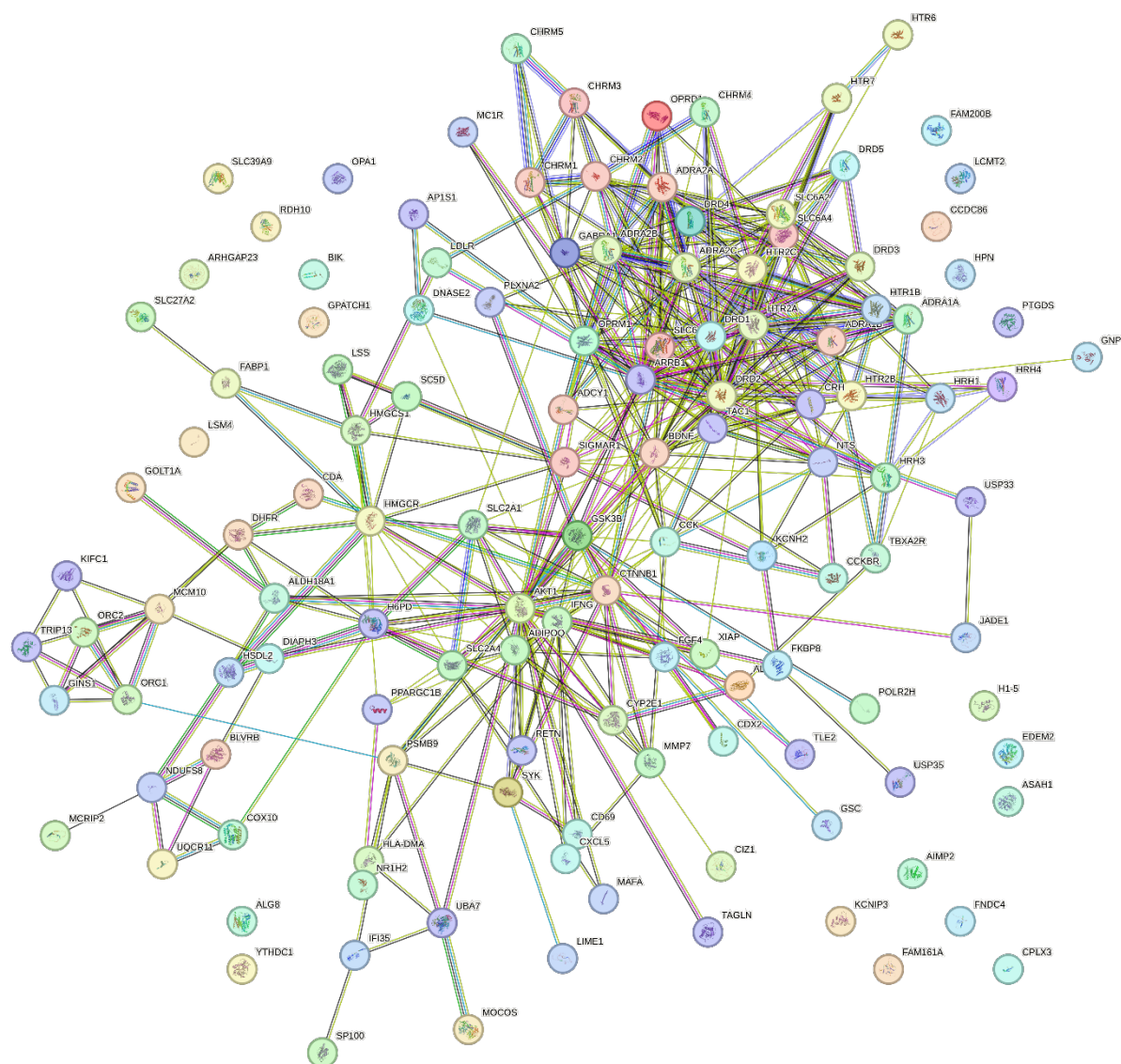


Fig 1: Protein interaction network showing molecular targets of selected phytoconstituents.

3.1.5 Network Generation and Analysis

Finally, the compound-protein, protein-pathway, and integrated compound-protein-pathway networks were constructed and visualised using Cytoscape v3.7.2 (Fig 2). In this network, Resveratrol, Quercetin, Apigenin, Linalool, Inulin, and Borneol emerged as the most enriched compounds, exhibiting the highest edge counts and interacting with multiple targets simultaneously. These phytochemicals showed strong connectivity with key proteins implicated in OLZ-induced metabolic dysfunction, including AKT1, GSK3B, HMGCR, LDLR, ADIPOQ, SLC2A4, DRD2, CHRM1, and CHRM2. Collectively, these high-degree interactions highlight the central involvement of both metabolic regulators and neurotransmitter-linked genes, particularly those associated with dopaminergic and serotonergic pathways in the development of OIO. The multi-target engagement demonstrated by these compounds suggests their potential to modulate several interconnected mechanisms underlying glucose imbalance, lipid dysregulation, and excessive weight gain.

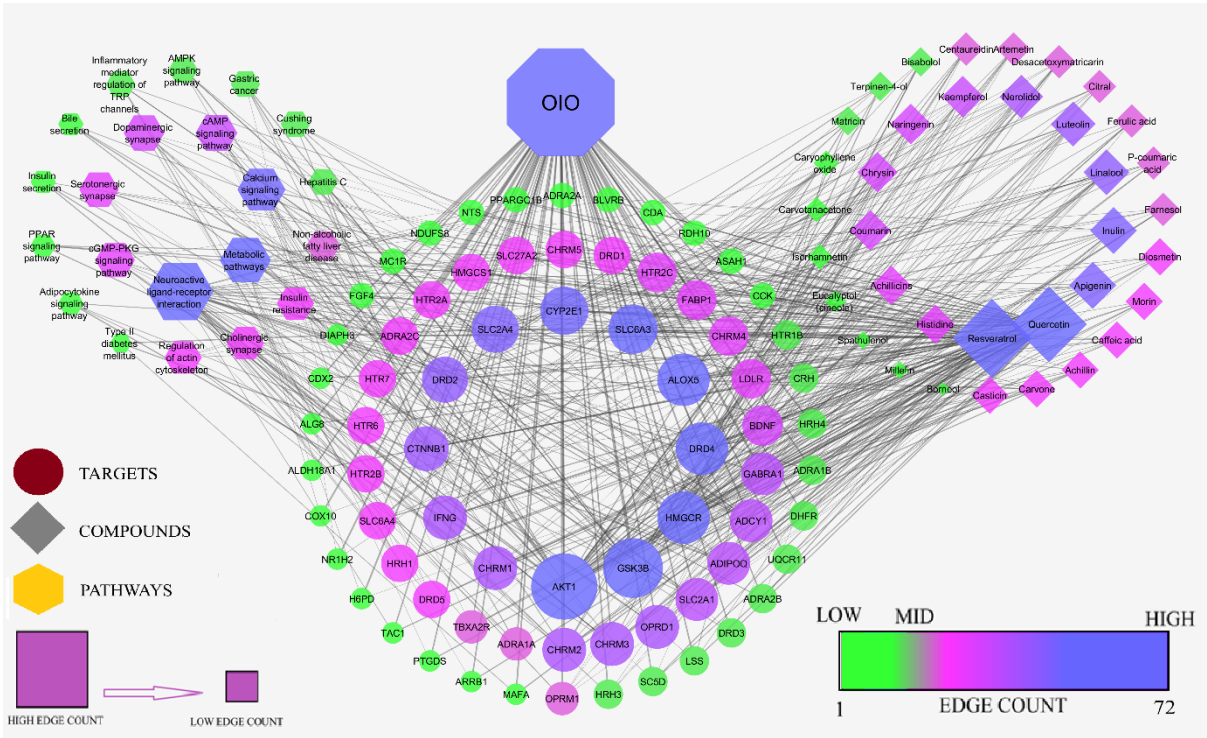


Fig 2: Network representation of AM phytochemicals with their probable protein molecules involved in OIO.

Table 2: Significantly enriched KEGG pathways of phytochemical-regulated genes

KEGG ID	Pathway name	Gene count	FDR	Gene set enrichment analysis
hsa04080	Neuroactive ligand-receptor interaction	33	3.93E-26	DRD4, OPRD1, CHRM3, NTS, HRH4, HTR2B, HTR2C, CRH, ADRA2A, HTR6, DRD2, CHRM1, ADRA1B, TAC1, HTR7, HRH3, HTR1B, ADRA1A, CHRM5, DRD3, DRD1, CCK, HRH1, ADRA2C, GABRA1, TBXA2R, OPRM1, CHRM2, CHRM4, HTR2A, MC1R, ADRA2B
hsa04020	Calcium signalling pathway	16	7.06E-11	CHRM3, HTR2B, HTR2C, HTR6, ADCY1, DRD5, CHRM1, ADRA1B, HTR7, ADRA1A, CHRM5, DRD1, HRH1, TBXA2R, CHRM2, HTR2A
hsa04728	Dopaminergic synapse	9	2.77E-05	DRD4, SLC6A3, DRD5, GSK3B, DRD2, DRD3, DRD1, ARRB1, AKT1
hsa04726	Serotonergic synapse	8	7.64E-05	HTR2B, SLC6A4, HTR2C, HTR6, HTR7, HTR1B, ALOX5, HTR2A
hsa04024	cAMP signalling pathway	10	0.00011	HTR6, ADCY1, DRD5, CHRM1, DRD2, HTR1B, DRD1, CHRM2, BDNF, AKT1
hsa04931	Insulin resistance	7	0.00051	SLC27A2, PPARGC1B, SLC2A4, GSK3B, SLC2A1, AKT1, NR1H2
hsa04725	Cholinergic synapse	7	0.00052	CHRM3, ADCY1, CHRM1, CHRM5, CHRM2, CHRM4, AKT1

hsa04022	cGMP-PKG signalling pathway	8	0.00069	OPRD1, ADRA2A, ADCY1, ADRA1B, ADRA1A, ADRA2C, AKT1, ADRA2B
hsa04750	Inflammatory mediator regulation of TRP channels	5	0.0111	HTR2B, HTR2C, ADCY1, HRH1, HTR2A
hsa04932	Non-alcoholic fatty liver disease	6	0.0116	NDUFS8, GSK3B, ADIPOQ, CYP2E1, AKT1, UQCR11
hsa04810	Regulation of actin cytoskeleton	7	0.012	FGF4, CHRM3, CHRM1, CHRM5, DIAPH3, CHRM2, CHRM4
hsa04920	Adipocytokine signalling pathway	4	0.0262	SLC2A4, ADIPOQ, SLC2A1, AKT1
hsa04152	AMPK signalling pathway	5	0.0268	HMGCR, SLC2A4, ADRA1A, ADIPOQ, AKT1
hsa03320	PPAR signalling pathway	4	0.0312	SLC27A2, FABP, HMGCS1, ADIPOQ
hsa04911	Insulin secretion	4	0.0404	CHRM3, ADCY1, CCK, SLC2A1
hsa05010	Alzheimer disease	8	0.0411	CHRM3, CHRM1, NDUFS8, GSK3B, CHRM5, AKT1, UQCR11, CTNNB1
hsa01100	Metabolic pathways	19	0.0416	RDH10, COX10, BLVRB, SC5D, HMGCR, ADCY1, ALG8, NDUFS8, HMGCS1, ALDH18A1, PTGDS, ALOX5, CDA, ASAH1, LSS, DHFR, CYP2E1, UQCR1, H6PD
hsa04976	Bile secretion	4	0.043	HMGCR, ADCY1, SLC2A1, LDLR
hsa05226	Gastric cancer	5	0.043	FGF4, GSK3B, CDX2, AKT1, CTNNB1
hsa04930	Type II diabetes mellitus	3	0.0451	SLC2A4, MAFA, ADIPOQ
hsa04934	Cushing syndrome	5	0.0451	CRH, ADCY1, GSK3B, LDLR, CTNNB1
hsa05160	Hepatitis C	5	0.0453	IFNG, GSK3B, AKT1, LDLR, CTNNB1

3.1.6 Molecular Docking

Molecular docking was performed against six key targets (Table 1) using Apigenin, Quercetin, and Resveratrol as lead phytochemicals. Apigenin recorded the highest binding affinity overall at DRD2-6CM4 (-9.5 kcal/mol, 7 active site interactions), while Quercetin outperformed at DRD2-7JVR (-9.1 kcal/mol, 7 active site interactions). At CHRM1-6ZG4, Quercetin scored -8.3 kcal/mol and Resveratrol -7.5 kcal/mol with 7 active site contacts. At CHRM2-5YC8, Quercetin and Resveratrol recorded -8.3 and -6.9 kcal/mol, respectively, both engaging TRP422. Apigenin was the sole compound docked at HTR2C, recording -6.8 kcal/mol (6BQH) and -7.0 kcal/mol (6BQG), though with no active site contacts at either structure. At HMGCR-3CDA, Quercetin demonstrated the strongest

active site engagement overall (-8.5 kcal/mol, 11 interactions, 10 active site contacts), forming extensive hydrogen bonds with ASP690, LYS692, ASN686, SER684, ARG590, GLY560, SER565, CYS561, and ALA751. At GSK3B-6GN1, Apigenin recorded -8.3 kcal/mol with 8 active site interactions, followed by Quercetin (-8.2 kcal/mol) and Resveratrol (-7.4 kcal/mol). Overall, Apigenin and Quercetin consistently demonstrated superior binding affinities and active site engagement across all targets compared to Resveratrol. Complete binding scores and interaction details are summarised in Table 3.

Table 3: Binding affinity and molecular interactions of phytochemicals with selected target proteins.

Protein Target	Complex	B.E kcal/mol	HBI	NHBI	No of Interactions	No of Interactions with active site
6CM4	Apigenin	-9.5	SER193, CYS118, TYR416	PHE389, PHE390, TRP386, ASP114	7	7
	Quercetin	-8.1	LEU94	THR412, TRP413, GLU95	4	4
	Resveratrol	-6.3	-	ALA1074, PHE1104	2	0
7JVR	Apigenin	-7.7	VAL320, PHE278, PHE234	ARG150, LEU192, HIS62	6	6
	Quercetin	-9.1	ASP246, CYS148, SER147, MET101	THR274, LYS210	7	7
	Resveratrol	-7.7	ASN230, ASP290	-	2	2
6ZG4	Quercetin	-8.3	TYR404, SER388	LEU183	3	3
	Resveratrol	-7.5	ASP393, SER388, TYR404	PRO186, TRP400, THR189, LEU183	8	7
5YC8	Quercetin	-8.3	PHE181	TRP422	2	2
	Resveratrol	-6.9	TYR80	TYR426, TRP422	3	3
6BQH	Apigenin	-6.8	GLY362	SER380, PHE379	3	0
6BQG	Apigenin	-7	ILE63	PRO365, LEU366, VAL359, MET66	5	0
1HWL	Quercetin	-8.5	GLU700, ILE699, ILE638, TRP698	ALA585, LYS606, GLU610, HIS635, THR636	10	0

	Resveratrol	-7.3	-	ASN529, ALA564, TYR479	3	3
3CDA	Quercetin	-8.5	ASP690, LYS692, ASN686, SER684, ARG590, GLY560, SER565, CYS561, ALA751	GLU559, LEU853	11	10
	Resveratrol	-7	-	GLU610, ALA585	2	0
6GN1	Apigenin	-8.3	GLU137, VAL135, ASP200	ILE62, VAL70, LYS85, LEU132, CYS199	8	8
	Quercetin	-8.2	GLU137, LYS85	ILE62, VAL70, CYS199,	5	5
	Resveratrol	-7.4	VAL135, ILE62	VAL70, LYS85	4	4
4JIR	Apigenin	-8	ASN64	VAL70, LEU132, ALA83, CYS199, VAL135	6	0
	Quercetin	-7.7	TYR221, TYR221, ARG220	-	3	0
	Resveratrol	-6.6	ASP133, ASN64	CYS199, ALA83, LEU132, VAL70, SER261	7	2

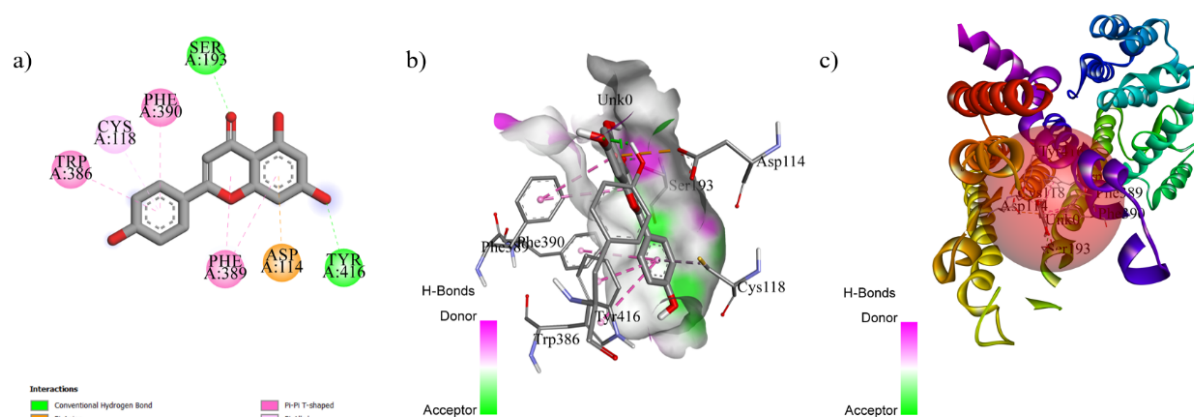


Fig 3: Molecular docking interactions of apigenin with DRD2 (6CM4). **(a)** 2D ligand interaction diagram illustrating the types and patterns of interactions within the binding site. **(b)** 3D binding pose of apigenin within

the catalytic pocket. **(c)** Ribbon representation showing apigenin (pink sphere) docked within the catalytic binding pocket of DRD2.

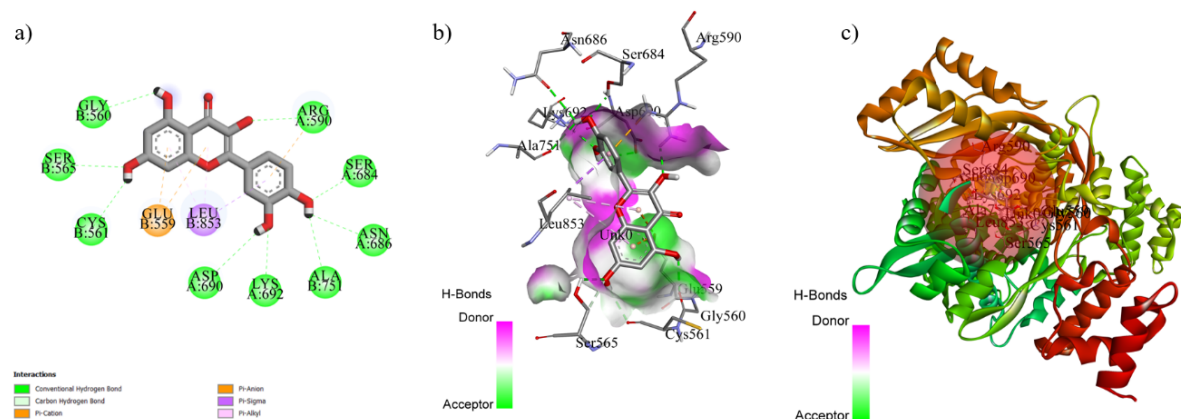


Fig 4: Molecular docking interactions of quercetin with HMGCR (3CDA). **(a)** 2D ligand interaction diagram illustrating the types and patterns of interactions within the binding site. **(b)** 3D binding pose of quercetin within the catalytic pocket. **(c)** Ribbon representation showing quercetin (pink sphere) docked within the catalytic binding pocket of HMGCR.

3.1.7 Molecular dynamics

3.1.7.1 Stability of apigenin with human dopamine D2 receptor (DRD2, PDB ID: 6CM4)

A 100 ns molecular dynamics simulation was performed to evaluate the stability of the apigenin-DRD2 (6CM4) complex. The protein backbone RMSD increased from ~ 2 Å to ~ 18 Å during 25-30 ns and later fluctuated between 8-12 Å, indicating incomplete conformational equilibration. In contrast, ligand RMSD stabilised at ~ 2 -3 Å after 60 ns, suggesting relatively stable ligand binding despite protein fluctuations. The radius of gyration remained stable (3.84-3.96 Å), confirming preservation of overall protein compactness. RMSF analysis revealed high flexibility in loop regions (residues ~ 90 -110, 150-220, and 250-280), which likely contributed to the elevated backbone RMSD, whereas ligand atoms showed minimal fluctuations (< 0.5 Å). Interaction analysis demonstrated persistent contacts with ASP114, TRP386, PHE197, PHE389, VAL115, and CYS118, maintaining ~ 5 -6 interactions throughout the simulation. Overall, although ligand binding remained relatively stable, the non-converging protein RMSD suggests that longer simulations or enhanced sampling approaches are required for complete stability assessment.

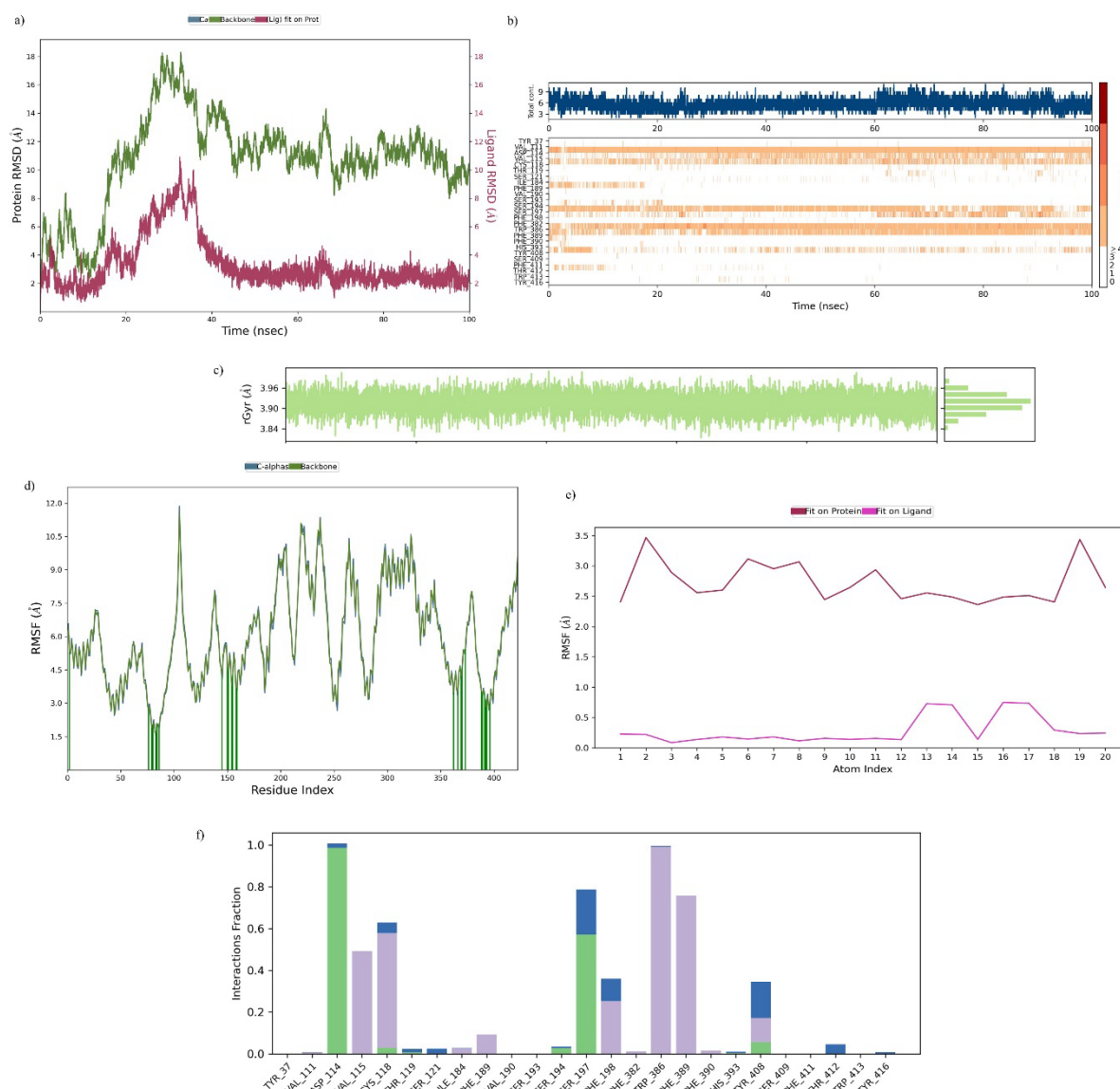
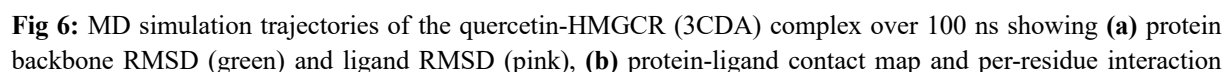


Fig 5: MD simulation trajectories of the apigenin-DRD2 (6CM4) complex over 100 ns showing **(a)** protein backbone RMSD (green) and ligand RMSD (pink), **(b)** protein-ligand contact map and per-residue interaction frequency, **(c)** radius of gyration (rGyr), **(d)** per-residue protein RMSF, **(e)** per-atom ligand RMSF fitted on protein and ligand, and **(f)** residue-level interaction fraction across the trajectory.

3.1.7.2 Stability of quercetin with human HMG-CoA reductase (HMGCR, PDB ID: 3CDA)

A 100 ns molecular dynamics simulation was conducted to evaluate the stability of the quercetin-HMGCR (3CDA) complex. The protein backbone RMSD initially increased to ~10-11 Å during the first 40 ns and later converged to ~3-5 Å after 60 ns, indicating progressive equilibration of the system. Ligand RMSD showed early fluctuations (6-12 Å) due to ligand repositioning but stabilised to ~3-5 Å in the latter half of the simulation, suggesting partial convergence of the binding pose. The radius of gyration remained stable (3.84-4.00 Å), confirming maintenance of overall protein compactness. RMSF analysis revealed higher flexibility in the N-terminal and loop regions, whereas the catalytic domain remained comparatively stable. Ligand per-atom RMSF indicated good internal rigidity, with most atoms showing fluctuations below 1 Å when aligned to the ligand. Protein-ligand interaction analysis demonstrated stable contacts (10-15 throughout the trajectory), with key interactions involving ILE802, ASN658, ASP690, GLN66, and MET659. Overall, the results suggest stable and meaningful engagement of



frequency, **(c)** radius of gyration (rGyr), **(d)** per-residue protein RMSF, **(e)** per-atom ligand RMSF fitted on protein and ligand, and **(f)** residue-level interaction fraction across the trajectory.

3.2 In-Vitro Experimental Findings

3.2.1 Total Phenolic Content (TPC)

The total phenolic content of the extract was estimated using the Folin-Ciocalteu method and expressed as gallic acid equivalents (GAE). The obtained results are shown in Table 4.

Table 4. Total phenolic content of samples (mg GAE/g of extract)

Sample	Total phenolic content (mg GAE/g)	Gallic acid standard calibration equation	R ²
AM hydroalcoholic extract	26.07 ± 1.876	y = 0.0264x + 0.1586	0.9924

3.2.2 Total Flavonoid Content (TFC)

The total flavonoid content was determined by the aluminium chloride colourimetric assay and expressed as quercetin equivalents (QE). The obtained results are shown in Table 5.

Table 5: Total flavonoid content of the samples expressed as quercetin equivalents (QE)

Sample	Total flavonoid content (mg QE/g of extract)	Quercetin standard calibration equation	R ²
AM hydroalcoholic extract	72.78 ± 5.697	y = 0.0096x + 0.006	0.9908

3.2.3 LC-MS-Based Phytochemical Characterisation of AM Hydroalcoholic Extract

LC-MS analysis of the AM hydroalcoholic extract revealed fourteen resolved peaks in the DAD chromatogram, with the major peak at RT 0.98 min showing the highest abundance (49.44%). Total Ion Chromatograms in both ES⁺ and ES[−] modes confirmed the presence of diverse ionisable phytoconstituents. Ten representative compounds were identified, including flavonoids such as resveratrol, apigenin, quercetin, kaempferol, luteolin, and casticin, along with histidine and the terpenoids citral, nerolidol, and linalool. Multiple retention times observed for certain compounds suggested possible isomeric or co-eluting forms. Overall, the LC-MS findings validated the phytochemicals predicted in the in-silico study and support further pharmacological evaluation of the extract.

Table 6: LC-MS characterisation of ten selected bioactive compounds identified in the AM hydroalcoholic extract, with corresponding molecular weights and retention times.

Compounds	Molecular Weight	Retention Time
Resveratrol	228.24	2.127
Apigenin	270.24	1.632
Quercetin	302.23	0.868
Kaempferol	286.24	2.127, 1.493
Luteolin	286.24	1.493
Casticin	374.34	1.328
Histidine	155.15	1.326, 1.632
Citral	152.24	2.353
Nerolidol	222.37	2.127

Linalool	154.25	1.328,1.632

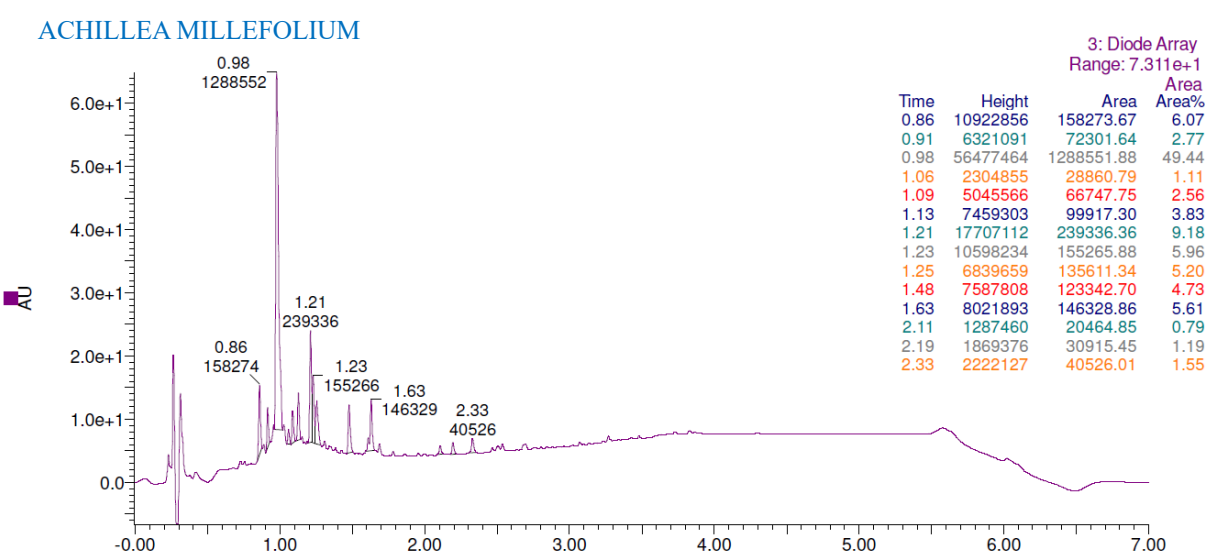


Fig 7: LC-MS chromatogram of the AM hydroalcoholic extract showing the DAD profile with fourteen resolved peaks (RT: 0.86-2.33 min), with the predominant peak at RT 0.98 min (area: 1,288,551.88; 49.44%) indicating the most abundant compound, supported by TIC profiles in both ES+ and ES- modes confirming the presence of diverse phytochemical constituents.

4. Discussion

The present study used a combination of *in-silico* and in vitro methods to assess how *Achillea millefolium* (AM) can help counter the metabolic side effects caused by OLZ. The metabolic disturbances associated with antipsychotic therapy remain a significant clinical limitation, particularly because they arise through various mechanisms involving appetite dysregulation, insulin resistance, oxidative stress, and altered lipid homeostasis [7,8]. Therefore, agents capable of modulating multiple pathological pathways simultaneously may hold particular therapeutic relevance. An *in-silico* investigation was conducted to evaluate the pharmacological relevance of phytoconstituents identified from AM in the context of OIO and metabolic dysregulation. A total of 59 phytocompounds were initially retrieved from available phytochemical databases and literature sources, out of which 44 met the criteria for drug-likeness, intestinal absorption (>70%), and acceptable toxicity profiles (probability threshold ≤ 0.6), establishing a refined panel of candidates for further pharmacological investigation. NP analysis showed a highly interconnected target landscape where AM phytocompounds, mainly resveratrol, quercetin, and apigenin, engaged key metabolic hubs (AKT1, GSK3B, INSR, PIK3CG, IGF1R, HMGCR) and neurotransmitter-related receptors (DRD2, CHRM1, CHRM2, HTR2C). KEGG pathway enrichment highlighted the dopaminergic and serotonergic synapse pathways as the most significantly enriched pathways, along with pathways related to insulin resistance, AMPK signalling, PPAR signalling, adipocytokine signalling, non-alcoholic fatty liver disease, and type 2 diabetes mellitus. This engagement across multiple pathways resembles the findings of Beerwala et al. (2025), who used a similar pharmacology approach to investigate *Garcinia indica* Choisy phytocompounds for OLZ-induced cardiometabolic issues, identifying AMPK, insulin signalling, and PPAR- γ pathways as central pathways [27]. The overlap in metabolic pathway targets across these two different plants supports the idea that polyphenol-rich herbal extracts can address the complex pathological effects of OLZ-induced metabolic syndrome more effectively than single-target treatments [42].

Apigenin showed the strongest binding affinity for DRD2 (6CM4; -9.5 kcal/mol), forming three hydrogen bonds with SER193, CYS118, and TYR416, and also exhibited significant binding at HTR2C (6BQH; - 6.8

kcal/mol). These interactions are important, as OLZ blocks DRD2 and 5-HT_{2C} receptors, which leads to increased appetite and dysregulation. The DRD2 binding profile of apigenin supports findings by Wasim M et al. (2024), who used AutoDock to show apigenin's high affinity for dopamine, serotonergic, cholinergic, and insulin receptors, suggesting it as a multi-target neuroprotective flavonoid [43]. Likewise, Amin et al. (2022) validated apigenin's interaction with 5HT_{1A}/5HT_{2A} receptors through 200 ns MD simulations and in vivo rodent models, showing antidepressant and anxiolytic effects [44]. Collectively, these findings suggest that apigenin may partially counteract OLZ's appetite-dysregulating effects through serotonergic and dopaminergic modulation.

Quercetin had strong binding with HMGCR at both 1HWL and 3CDA (-8.5 kcal/mol), forming 10-11 hydrogen bonds with important catalytic residues Asp690, Lys692, Ser684, Ile699, and Trp698, which are very similar to those reported by Riyad et al. (2023) in their study on quercetin-HMGCR inhibition in hypercholesterolemic rabbits, where quercetin improved lipid profiles and reduced HMGCR activity [45]. Palmado et al. (2024) further confirmed quercetin's lipid-normalising effect in a rat dyslipidaemia model, showing changes in HMG-CoA and ABCA1 transporter expression like those seen with simvastatin [46]. A 2024 systematic review and meta-analysis of 20 randomised controlled trials showed that quercetin supplementation significantly lowered fasting blood glucose (WMD: -1.03 mg/dL) and systolic blood pressure, highlighting its clinical relevance beyond computational evidence [47]. Quercetin also showed significant binding at GSK3 β (-8.2 kcal/mol), supporting its role in addressing insulin resistance related to OLZ-induced glucose dysregulation. Resveratrol demonstrated significant multi-target interactions with GSK3 β (-7.4 kcal/mol), CHRM1 (-7.5 kcal/mol), CHRM2 (-6.9 kcal/mol), and HMGCR (-7.3 kcal/mol). The hyperactivation of GSK3 β is a known effect of OLZ therapy that hampers glycogen synthase activity and increases insulin resistance [13]. Koushki et al. (2024) showed that resveratrol promotes glycogen synthesis through Akt phosphorylation-mediated GSK3 β inhibition in diabetic models [48], while Pan et al. (2025) indicated that resveratrol improves insulin resistance in skeletal muscle through the DDIT4/mTOR/GSK3 β pathway [49]. The binding of quercetin and resveratrol to muscarinic receptors is also noteworthy as OLZ's M3 receptor blockade reduces pancreatic insulin secretion. The cholinergic synapse pathway was significantly enriched in KEGG pathways (FDR = 0.00052), reflecting the broader role of muscarinic receptors in OLZ's metabolic effects.

The 100 ns MD simulations offered insight into the binding dynamics of the two prioritised complexes. For the apigenin-DRD2 system, the protein backbone RMSD did not converge within the simulation period, peaking at ~18 Å before partially declining to 8-12 Å after 60 ns. This behaviour is consistent with the known conformational plasticity of class A GPCRs simulated without a lipid bilayer, where extracellular loops undergo large fluctuations that inflate backbone RMSD without necessarily destabilising the ligand-binding site [50]. In support of this interpretation, the radius of gyration remained stable (3.84-3.96 Å), confirming the preservation of global protein architecture, and the ligand RMSD converged to ~2-3 Å after 60 ns, with persistent interactions at ASP114 and TRP386 (interaction fractions ~1.0) maintained throughout the trajectory. These data suggest that apigenin is retained within the orthosteric pocket despite protein backbone mobility, though extended simulations in a membrane-embedded environment are required before firm conclusions can be drawn about DRD2 complex stability. In contrast, the quercetin-HMGCR complex showed substantially better convergence, with protein backbone RMSD declining from ~10-11 Å to 3-5 Å after 60 ns and the ligand RMSD stabilising to ~3-5 Å in the latter half of the simulation. Dominant contacts at ILE802 (~1.55 interaction fraction), ASN658 (~1.05), ASP690, and GLN66 were sustained throughout the trajectory, with 10-15 stable contacts maintained overall. The rGyr remained stable (3.84-4.00 Å), confirming preservation of the HMGCR catalytic domain structure. Taken together, the quercetin-HMGCR complex provides stronger MD evidence of binding stability, while the apigenin-DRD2 system demonstrates promising but preliminary ligand-level stability that warrants further investigation. These observations are consistent with Khanal et al. (2020), who validated the translational relevance of both DRD2 and HMGCR as targets in OLZ-induced metabolic dysregulation using zebrafish models [51].

To experimentally confirm the presence of the phytochemicals identified in the *in-silico* study, in vitro phytochemical analyses were performed. The TFC measured 72.78 ± 5.697 mg QE/g, and the TPC was 26.07 ± 1.876 mg GAE/g. These Figs match those reported by Hayat et al.(2025) for the same species, confirming the extract's pharmacological potential [52].LC-MS analysis confirmed the presence of key bioactive flavonoids such as apigenin, quercetin, luteolin, and Casticin, alongside the amino acid Histidine and terpenoids Citral, Nerolidol, and Linalool, etc [18,53]. These findings confirm the presence of key phytochemical compounds identified in the

computational study, thereby validating the *in-silico* predictions and strengthening the pharmacological basis for further investigation of the AM hydroalcoholic extract.

Overall, this study combines experimental and computational data to demonstrate the significant potential of AM phytochemicals, especially apigenin, quercetin, and resveratrol, in mitigating the metabolic adverse effects of OLZ. The computational study identified these compounds as capable of acting on multiple disease-related targets, and the *in vitro* results confirmed that these same compounds are present in the AM extract. Even though these results are encouraging, further animal-based studies and pharmacokinetic investigations will be essential before any clinical application can be considered.

The study has some key limitations. All findings come from computational methods and need *in vivo* validation in established OIO models, such as female Sprague-Dawley rats, with measurements of body weight, glycaemic indices, and lipid profiles. The bioavailability of quercetin and apigenin after oral administration varies, and specific formulation strategies may be needed to achieve therapeutic plasma levels. Potential pharmacokinetic interactions between AM phytochemicals and OLZ, especially regarding CYP450-mediated metabolism, must also be examined before considering any clinical co-administration. Despite these limitations, the combined computational findings, experimental phytochemical data, and existing published evidence establish AM, particularly apigenin and quercetin, as credible candidates for further development as adjunct treatments to reduce OLZ-induced metabolic syndrome.

5. Conclusion

This study provides both computational and experimental evidence that supports AM phytochemicals, particularly apigenin and quercetin, as promising candidates for reducing OLZ-induced metabolic dysfunction. Network pharmacology showed that these compounds engage with multiple targets, including key metabolic regulators (AKT1, GSK3B, HMGCR, INSR) and neurotransmitter receptors (DRD2, HTR2C, CHRM1, CHRM2). These interactions affect pathways related to dopamine, serotonin, insulin resistance, and lipid metabolism. Molecular docking revealed that apigenin binds most strongly at DRD2 (-9.5 kcal/mol), while quercetin shows a strong binding at HMGCR (-8.5 kcal/mol). MD simulations confirmed partial binding stability over 100 ns. LC-MS analysis confirmed the presence of these bioactive compounds in the AM hydroalcoholic extract. This was supported by significant total flavonoid content (72.78 ± 5.697 mg QE/g) and total phenolic content (26.07 ± 1.876 mg GAE/g). Together, these results confirm AM as a promising therapeutic candidate, suggesting the need for further *in vivo* validation and pharmacokinetic studies before clinical application.

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The authors report there are no competing interests to declare.

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Data Availability Statement

The data supporting the findings of this study are available within the article and its supplementary materials. Additional data are available from the corresponding author upon reasonable request.

Ethical Statement

This study did not involve human participants or animal experiments; therefore, no ethical approval was required.

Declaration of Generative AI and AI-Assisted Technologies in the Manuscript Preparation Process

During the preparation of this work the authors used ChatGPT (OpenAI) and Claude (Anthropic) in order to perform grammar correction and language refinement. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Author Contributions

Iqra Mujawar: Conceptualisation; Data Curation; Methodology; Investigation; Formal Analysis; Writing Original Draft.

Sanjay Ugare: Conceptualisation; Supervision; Writing-Review and Editing; Project Administration.

Vishal Patil: Methodology; Software; Validation; Writing-Review and Editing.

Shamanand Mallapur: Resources; Writing-Review and Editing; Validation.

Sneha Hajare: Data Curation; Writing-Review and Editing.

Supplementary Material:

Table S1: Selected Active Phytocompounds of *Achillea Millefolium*; Table S2: List of Phytocompounds with their Targets; Table S3: Olanzapine modulated protein targets; Table S4: Drug-likeness and ADMET profiles of 44 selected phytocompounds.

List of Abbreviations

OLZ: Olanzapine; AM: *Achillea millefolium*; BE: Binding energy; HBI: Hydrogen-bond interactions; NHBI: Non-hydrogen-bond interactions; NP: Network Pharmacology; PPI: Protein-Protein Interaction; MD: Molecular dynamics; RMSD: Root-mean-square deviation; RMSF: Root-mean-square fluctuation; rGyr: Radius of gyration; OIO: Olanzapine-induced obesity; KEGG: Kyoto Encyclopedia of Genes and Genomes; BBB: Blood-brain barrier; Pa: Probability of activity; Pi: Probability of inactivity; SGA: Second-generation antipsychotic; TPC: Total phenolic content; TFC: Total flavonoid content; GAE: Gallic acid equivalents; QE: Quercetin equivalents; ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity; ESI: Electrospray ionisation.

7. Reference

- [1] Callaghan JT, Bergstrom RF, Ptak LR, et al. Olanzapine: Pharmacokinetic and Pharmacodynamic Profile. *Clinical Pharmacokinetics* 1999;37:177–93. <https://doi.org/10.2165/00003088-199937030-00001>.
- [2] Fulton B, Goa KL. Olanzapine: A Review of its Pharmacological Properties and Therapeutic Efficacy in the Management of Schizophrenia and Related Psychoses. *Drugs* 1997;53:281–98. <https://doi.org/10.2165/00003495-199753020-00007>.
- [3] Demirci Ö, Adar İ, Erbaş O. An Overview of Antipsychotics: Mechanisms of Action. *JEBMS* 2023;4:062–70. <https://doi.org/10.5606/jebms.2023.1047>.
- [4] Di Pasqua LG, Cagna M, Berardo C, et al. Detailed Molecular Mechanisms Involved in Drug-Induced Non-Alcoholic Fatty Liver Disease and Non-Alcoholic Steatohepatitis: An Update. *Biomedicines* 2022;10:194. <https://doi.org/10.3390/biomedicines10010194>.
- [5] Zhu Z, Gu Y, Zeng C, et al. Olanzapine-induced lipid disturbances: A potential mechanism through the gut microbiota-brain axis. *Front Pharmacol* 2022;13:897926. <https://doi.org/10.3389/fphar.2022.897926>.
- [6] Tripathi KD. *Essentials of Medical Pharmacology*. Jaypee Brothers Medical Publishers; 2018.
- [7] Hirsch L, Yang J, Bresee L, et al. Second-Generation Antipsychotics and Metabolic Side Effects: A Systematic Review of Population-Based Studies. *Drug Saf* 2017;40:771–81. <https://doi.org/10.1007/s40264-017-0543-0>.
- [8] ÜÇÖK A, GAEBEL W. Side effects of atypical antipsychotics: a brief overview. *World Psychiatry* 2008;7:58–62. <https://doi.org/10.1002/j.2051-5545.2008.tb00154.x>.
- [9] Fitzgerald I, O'Dwyer S, Brooks M, et al. Worth the Weight? Olanzapine Prescribing in Schizophrenia. A Review of Weight Gain and Other Cardiometabolic Side Effects of Olanzapine. *Front Psychiatry* 2021;12. <https://doi.org/10.3389/fpsy.2021.730769>.
- [10] Razavi BM, Lookian F, Hosseinzadeh H. Protective effects of green tea on olanzapine-induced-metabolic syndrome in rats. *Biomedicine & Pharmacotherapy* 2017;92:726–31. <https://doi.org/10.1016/j.biopha.2017.05.113>.
- [11] Kirk SL, Glazebrook J, Grayson B, et al. Olanzapine-induced weight gain in the rat: role of 5-HT_{2C} and histamine H₁ receptors. *Psychopharmacology* 2009;207:119–25. <https://doi.org/10.1007/s00213-009-1639-8>.
- [12] Evers SS, Calcagnoli F, van Dijk G, et al. Olanzapine causes hypothermia, inactivity, a deranged feeding pattern and weight gain in female Wistar rats. *Pharmacology Biochemistry and Behavior* 2010;97:163–9. <https://doi.org/10.1016/j.pbb.2010.05.029>.
- [13] Weston-Green K, Huang X-F, Deng C. Olanzapine treatment and metabolic dysfunction: a dose response study in female Sprague Dawley rats. *Behavioural Brain Research* 2011;217:337–46. <https://doi.org/10.1016/j.bbr.2010.10.039>.
- [14] Singh R, Bansal Y, Sodhi RK, et al. Berberine attenuated olanzapine-induced metabolic alterations in mice: Targeting transient receptor potential vanilloid type 1 and 3 channels. *Life Sciences* 2020;247:117442. <https://doi.org/10.1016/j.lfs.2020.117442>.

- [15] Rojczyk E, Pałasz A, Wiaderkiewicz R. Effect of short and long-term treatment with antipsychotics on orexigenic/anorexigenic neuropeptides expression in the rat hypothalamus. *Neuropeptides* 2015;51:31–42. <https://doi.org/10.1016/j.npep.2015.04.001>.
- [16] Fernø J, Varela L, Skrede S, et al. Olanzapine-Induced Hyperphagia and Weight Gain Associate with Orexigenic Hypothalamic Neuropeptide Signaling without Concomitant AMPK Phosphorylation. *PLOS ONE* 2011;6:e20571. <https://doi.org/10.1371/journal.pone.0020571>.
- [17] Zhang Q, Deng C, Huang X-F. The role of ghrelin signalling in second-generation antipsychotic-induced weight gain. *Psychoneuroendocrinology* 2013;38:2423–38. <https://doi.org/10.1016/j.psychneuen.2013.07.010>.
- [18] Far BF, Behzad G, Khalili H. *Achillea millefolium*: Mechanism of action, pharmacokinetic, clinical drug-drug interactions and tolerability. *Heliyon* 2023;9. <https://doi.org/10.1016/j.heliyon.2023.e22841>.
- [19] Zolghadri Y, Fazeli M, Kooshki M, et al. *Achillea Millefolium* L. Hydro- Alcoholic Extract Protects Pancreatic Cells by Down Regulating IL- 1 β and iNOS Gene Expression in Diabetic Rats. *Int J Mol Cell Med* 2014;3:255–62.
- [20] Nematy M, Mazidi M, Jafari A, et al. The effect of hydro-alcoholic extract of *Achillea millefolium* on appetite hormone in rats. *Avicenna J Phytomed* 2017;7:10–5.
- [21] Mushtaq Z, Sadeer NB, Hussain M, et al. Therapeutical properties of apigenin: a review on the experimental evidence and basic mechanisms. *International Journal of Food Properties* 2023;26:1914–39. <https://doi.org/10.1080/10942912.2023.2236329>.
- [22] Caporali S, De Stefano A, Calabrese C, et al. Anti-Inflammatory and Active Biological Properties of the Plant-Derived Bioactive Compounds Luteolin and Luteolin 7-Glucoside. *Nutrients* 2022;14:1155. <https://doi.org/10.3390/nu14061155>.
- [23] Yahfoufi N, Alsadi N, Jambi M, et al. The Immunomodulatory and Anti-Inflammatory Role of Polyphenols. *Nutrients* 2018;10:1618. <https://doi.org/10.3390/nu10111618>.
- [24] Öztürk H, Kolak U, Meric C. Antioxidant, Anticholinesterase and Antibacterial Activities of *Jurinea consanguinea* DC. *Rec Nat Prod* 2011.
- [25] İlhami Gülçin, Mshvildadze V, Gepdiremen A, et al. The antioxidant activity of a triterpenoid glycoside isolated from the berries of *Hedera colchica* : 3- O - (β - D -glucopyranosyl)-hederagenin. *Phytotherapy Research* 2006;20:130–4. <https://doi.org/10.1002/ptr.1821>.
- [26] Ugare SR, Khatib NA, Beerwalaa FA, et al. The Effectiveness of Hydroalcoholic Extract and Fraction of *Cucumis melo* on Amelioration of High Fat Diet Induced hepatotoxicity: A Comprehensive Approach Integrating Computational and Preclinical Validation. *J Young Pharm* 2024;16:469–79. <https://doi.org/10.5530/jyp.2024.16.61>.
- [27] Beerwala FA, Kolambkar SV, Karikazi Adilmehadi, et al. Harnessing the therapeutic potential of *Garcinia indica* choisy constituents against olanzapine-induced cardiac and metabolic disorders. In *Silico Research in Biomedicine* 2025;1:100010. <https://doi.org/10.1016/j.ins.2025.100010>.
- [28] Patil VS, Harish DR, Vetrivel U, et al. Hepatitis C Virus NS3/4A Inhibition and Host Immunomodulation by Tannins from *Terminalia chebula*: A Structural Perspective. *Molecules* 2022;27:1076. <https://doi.org/10.3390/molecules27031076>.
- [29] Khanal P, Patil VS, Bhandare VV, et al. Systems and in vitro pharmacology profiling of diosgenin against breast cancer. *Front Pharmacol* 2023;13. <https://doi.org/10.3389/fphar.2022.1052849>.
- [30] Patil VS, Khatib NA. Triterpene saponins from *Barringtonia acutangula* (L.) Gaertn as a potent inhibitor of 11 β -HSD1 for type 2 diabetes mellitus, obesity, and metabolic syndrome. *Clin Phytosci* 2020;6:61. <https://doi.org/10.1186/s40816-020-00210-y>.
- [31] Ugare SR, Khatib NA, Patil VS, et al. Computational and experimental pharmacology reveals hepatoprotective effect of *Cucurbita pepo* in isoniazid-induced liver cirrhosis. *J Appl Pharm Sci* 2023. <https://doi.org/10.7324/JAPS.2024.148502>.
- [32] Venkataramanan S, V Kamat R, Ugare S. Molecular Mechanism of Nishamalaki as Rasayana in Diabetes Mellitus. *IJAM* 2024;15:375–82. <https://doi.org/10.47552/ijam.v15i2.4552>.
- [33] Japti VP, Patil MB, Unger BS, et al. Network pharmacology-based toxicity, molecular docking, and molecular dynamics analysis of phytoconstituents from roots of *Nerium indicum* L. *Pharmacological Research - Modern Chinese Medicine* 2025;16:100640. <https://doi.org/10.1016/j.prmcm.2025.100640>.

- [34] K S S, Unger BS, Kodihally AKV, et al. Inula Racemosa Hook f. Modulates Metabolic Rate and Lipid Accumulation: An in Silico and in Vivo Study in High-Fat Diet Fed Zebrafish. *ChemistrySelect* 2025;10:e03359. <https://doi.org/10.1002/slct.202503359>.
- [35] Jakubec D, Skoda P, Krivak R, et al. PrankWeb 3: accelerated ligand-binding site predictions for experimental and modelled protein structures. *Nucleic Acids Res* 2022;50:W593–7. <https://doi.org/10.1093/nar/gkac389>.
- [36] Olanzapine manipulates neuroactive signals and may onset metabolic disturbances: *Journal of Biomolecular Structure and Dynamics*: Vol 42, No 13 n.d. <https://www.tandfonline.com/doi/abs/10.1080/07391102.2023.2235617> (accessed March 26, 2026).
- [37] Hayat S, Miana GA, Kanwal M, et al. Determination of Total Flavonoid Content and Phenolic Content, Antioxidant Assay, and Antiepileptic Activity of *Achillea millefolium* Extract. *Natural Product Communications* 2025;20:1934578X251319221. <https://doi.org/10.1177/1934578X251319221>.
- [38] Limenew A, Tadesse M. Phytochemical screening and peroxide value determination of methanolic extract of four traditional medicinal plants from Debre Tabor Town, Ethiopia. *J Med Plants Res* 2018;12:203–8. <https://doi.org/10.5897/JMPR2018.6579>.
- [39] Martins GR, Monteiro AF, Do Amaral FRL, et al. A validated Folin-Ciocalteu method for total phenolics quantification of condensed tannin-rich açai (*Euterpe oleracea* Mart.) seeds extract. *J Food Sci Technol* 2021;58:4693–702. <https://doi.org/10.1007/s13197-020-04959-5>.
- [40] Nikolaeva TN, Lapshin PV, Zagorskina NV. Method for Determining the Total Content of Phenolic Compounds in Plant Extracts with Folin–Denis Reagent and Folin–Ciocalteu Reagent: Modification and Comparison. *Russ J Bioorg Chem* 2022;48:1519–25. <https://doi.org/10.1134/S1068162022070214>.
- [41] Mallapur SP, Unger BS, Patil MB, et al. Insight on toxicity mechanisms of *Datura metel* L. seeds: a 28-day repeated dose study, *in-silico* studies, and effect of *Shodhana* a traditional detoxification method. *Drug and Chemical Toxicology* 2026;49:284–303. <https://doi.org/10.1080/01480545.2025.2581707>.
- [42] Hopkins AL. Network pharmacology: the next paradigm in drug discovery. *Nat Chem Biol* 2008;4:682–90. <https://doi.org/10.1038/nchembio.118>.
- [43] Wasim M, Zia SR, Ahmad S. Molecular Docking Analysis Reveals the Promising Role of Apigenin as a Potential Treatment for Neurological Disorders. *Clin Neuropharm* 2024;47:176–80. <https://doi.org/10.1097/WNF.0000000000000608>.
- [44] Amin F, Ibrahim MAA, Rizwan-ul-Hasan S, et al. Interactions of Apigenin and Safranal with the 5HT1A and 5HT2A Receptors and Behavioral Effects in Depression and Anxiety: A Molecular Docking, Lipid-Mediated Molecular Dynamics, and In Vivo Analysis. *Molecules* 2022;27:8658. <https://doi.org/10.3390/molecules27248658>.
- [45] Riyad P, Purohit A, Sen K, et al. HMG – CoA reductase inhibition mediated hypocholesterolemic potential of myricetin and quercetin: *in-silico* and *in-vivo* studies. *CyTA - Journal of Food* 2023;21:115–25. <https://doi.org/10.1080/19476337.2022.2162976>.
- [46] Ignasius Agyo Palmado, Sulistyanaengci Winarto, Honey Dzikri Marhaeny, et al. The Effect of Quercetin on Coenzyme HMG-CoAR, ABCA1 Transporter, Dyslipidemia Profile and Hepatic Function in Rats Dyslipidemia Model. *JFIKI* 2024;11:274–90. <https://doi.org/10.20473/jfiki.v11i32024.274-290>.
- [47] Noshadi N, Bonyadian A, Hojati A, et al. The effect of quercetin supplementation on the components of metabolic syndrome in adults: A systematic review and dose–response *meta*-analysis of randomized controlled trials. *Journal of Functional Foods* 2024;116:106175. <https://doi.org/10.1016/j.jff.2024.106175>.
- [48] Koushki M, Farahani M, Yekta RF, et al. Potential role of resveratrol in prevention and therapy of diabetic complications: a critical review. *Food & Nutrition Research* 2024;68. <https://doi.org/10.29219/fnr.v68.9731>.
- [49] Pan X, Xue G, Zhao M, et al. Resveratrol ameliorates high-fat diet-induced insulin resistance via the DDIT4/mTOR pathway in skeletal muscle. *Biomedical Reports* 2025;22:1–14. <https://doi.org/10.3892/br.2025.1977>.
- [50] Dror RO, Dirks RM, Grossman JP, et al. Biomolecular Simulation: A Computational Microscope for Molecular Biology. *Annu Rev Biophys* 2012;41:429–52. <https://doi.org/10.1146/annurev-biophys-042910-155245>.
- [51] Khanal P, Patil BM, Unger BanappaS. Zebrafish shares common metabolic pathways with mammalian olanzapine-induced obesity. *Futur J Pharm Sci* 2020;6:36. <https://doi.org/10.1186/s43094-020-00049-7>.

- [52] Determination of Total Flavonoid Content and Phenolic Content, Antioxidant Assay, and Antiepileptic Activity of *Achillea millefolium* Extract - Sonia Hayat, G. A. Miana, Madiha Kanwal, Zartab Ahsan, Muhammad Junaid Tariq, 2025 n.d. <https://journals.sagepub.com/doi/full/10.1177/1934578X251319221> (accessed March 27, 2026).
- [53] Ali SI, Gopalakrishnan B, Venkatesalu V. Pharmacognosy, Phytochemistry and Pharmacological Properties of *Achillea millefolium* L.: A Review: *Achillea millefolium* L.: A review. *Phytother Res* 2017;31:1140–61. <https://doi.org/10.1002/ptr.5840>.