RESEARCH PAPER

Network Pharmacology, Molecular Docking And Molecular Dynamic Studies Of Phytoconstituents Of Tabernaemontana Divaricata For The Hepatoprotective Activity

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Abstract

The present study was undertaken to evaluate the hepatoprotective potential of tabernaemontana divaricata using an integrated approach involving phytochemical analysis, in silico studies, molecular docking, molecular dynamics simulations, and mechanistic insights into hepatotoxicity. The liver plays a crucial role in metabolism, detoxification, and homeostasis, making it highly susceptible to damage caused by drugs, toxins, and oxidative stress. Carbon tetrachloride (ccl_4)-induced hepatotoxicity, a well-established experimental model, was considered to understand the mechanisms of liver injury mediated by free radicals and lipid peroxidation. Phytochemical investigation revealed the presence of bioactive compounds such as alkaloids, flavonoids, and phenolic constituents, which are known for their antioxidant and anti-inflammatory properties. Admet and drug-likeness screening identified potential lead compounds with favorable pharmacokinetic profiles. Network pharmacology analysis demonstrated a multi-target mechanism of action involving key proteins associated with oxidative stress, inflammation, and apoptosis. Molecular docking studies showed strong binding affinities of selected phytoconstituents with targets such as tnf-α, nrf2, and caspase-3, while molecular dynamics simulations confirmed the stability of these interactions. The findings suggest that the hepatoprotective activity of tabernaemontana divaricata is mediated through antioxidant defense, inhibition of inflammatory pathways, and prevention of hepatocellular apoptosis. Overall, this study provides scientific validation for the traditional use of the plant and highlights its potential as a promising candidate for the development of novel hepatoprotective agents.

Keywords: Hepatoprotective, Tabernaemontana Divaricata, Oxidative Stress, Molecular Docking, Network Pharmacology, Admet, Liver Injury.

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Introduction

The main site of xenobiosis in the body takes place through the liver.[1] alcoholic addiction, use of medicines, formation of free oxidative free radicals are some of the leading causes of death for liver damage.[1,2] Hepatotoxicity is defined as liver damage caused by exposure to toxic substances such as drugs, chemicals and other toxins that impair liver function and may lead to liver failure.[3] Treatment of liver diseases with the presently available medications poses the patient to face the severe adverse effects and presently this situation is being overcome by the use of traditional medicines that are part of plant source.[4,5,6] It is the need of the hour to discover new drugs with no adverse effect[6,7].so the present study is undertaken to investigate the phytoconstituents of *Tabernaemontana divaricata* against hepatotoxicity using *insilico* approach of molecular docking and molecular dynamic simulation studies to identify the hepatoprotective principles of this plant.

Materials and methods

Collection of plant materials and authentication

Tabernaemontana divaricata were collected from P.S.C & K.V.S.C Govt College, Nandyal, India. Identified by plant taxonomist Prof. Smt V.J.Sailajarani, plant taxonomist, Department of Botany, was submitted in the concerned herbarium for future reference.

Identification and Screening of Phytoconstituents:

The compilation of phytcochemicals was done by literature survey through available LC-MS data. Pub Chem data base was used to determine molecular weight, chemical structure and canonical SMILES. Swiss ADME was used was used to evaluate the Lipinski rule of five, bioavailabiliy score, GI absorption and ADMET lab2.0 for determining the Hepatotoxicity, AMES toxicity and Blood brain barrier permeability. The selection of compounds was based on considering Lipinski compliance, bioavailability score ≥0.55, non toxic profile and the compounds satisifying these criteria are considered as bioactive candidates.

Network Pharmacology study

This was undertaken for target prediction of selected compounds by pasting **SMILES** in Swiss Target Prediction, organism selected is ***Homo sapiens***. Probability based targets were obtained and targets with higher probability scores were selected and finally redundant targets were removed using Microsoft Excel. Later the hepatoprotective targets were obtained from Gene Cards database. The key words used for this search include **"Liver injury", "Hepatotoxicity", "Drug induced liver injury"**. The targets with relevance score above threshold were selected.the common targets were obtained by uploading the targets in **Venny 2.0** to identify the overlapping genes nad the common targets were considered as therapeutically relevant targets. The Protein-Protien interaction (PPI) analysis was performed using **STRING** database using parameters Homo sapiens for organism at a confidence score of ≥0.7. This has generated the network with Nodes (proteins) and Edges (interactions). The data is exported in TSV format. Network is imported into Cytoscape for visualization and topological analysis followed by identification of Hubgenes using CytoHubba plugin followed by ranking through degree centrality basis.

Network Construction

For making the network construction data is prepared in an excel format consisting of compound-target and target-pathway format and is imported into cytoscape and networks constructed between herb-compound-target network and target-pathway network. Finally, the network analysis is performed using degree, betweenness and closeness.

Molecular Docking Studies[8]

Molecular docking studies involved the ligand preparation after getting them from PubChem in SDF format and later converted into PDB format using Discovery studio and energy minimization is performed using force field. The protein preparation involved removal of water molecules, co-crystallized ligands and addition of polar hydrogens followed by energy minimization

Docking Protocol

Docking is performed using **PyRx (AutoDock Vina)** for this the ligands and proteins are converted to PDBQT format, the grid box is defined around the active site and docking is executed to obtain the binding energies. The compounds are selected based on the lowest binding energy and with favourable interactions. The interactions are analysed based on the type of interactions like hydrogen bonds, π-π interactions, hydrophobic interactions and active site residues.

Molecular Dynamics (MD) Simulation studies[9]. This involves selection of docking complex and generating topology using CHARMM / AMBER force field, and the system solvated in TIP3P water model, the periodic boundary conditions are applied and system is neutralized using Na^+/Cl^- ions. The energy minimization is done to remove steric clashes and equilibriation is done with NVT ensemble (constant temperature at 300K) and NPT ensemble (constant pressure 1 atm). The production is run at 50-100ns simulation. Trajectory analysis is perfomed to analyse parameters like RMSD (stability), RMSF (Flexibility), radius of gyration (compactness) and hydrogen bond analysis.

Results

The selected phytoconstituents were evaluated for their pharmacokinetic and drug-likeness properties using ADMET parameters, including gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, P-glycoprotein (P-gp) substrate activity, cytochrome P450 (CYP) enzyme inhibition, and bioavailability score. All compounds exhibited high GI absorption, indicating good oral bioavailability and suitability for oral administration. BBB analysis revealed that several compounds possess the ability to cross the blood-brain barrier, suggesting potential central nervous system activity, while others remain non-permeable, indicating safer profiles for peripheral targets. Most compounds were identified as non-substrates of P-glycoprotein, implying reduced drug efflux and imporved cellular retention; however, a few compounds showed substrate behaviour, which

may affect their intracellular concentration. The CYP inhibition profile demonstrated that the majority of compounds did not inhibit key metabolic enzymes such as CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4, indicating low risk of drug-drug interactions, although certain compounds, particularly alkaloids like lochnericine, pachysiphine, voacahotine, ibogamine, and coronaridine, exhibited inhibitory effects on CYP2D6 and CYP3A4, suggesting possible involvement in metabolic pathways. The bioavailability scores ranged from 0.55 to 0.85, with higher scores indicating better systemic availability. Overall, these findings suggest that the selected phytoconstituents possess favourable pharmacokinetic profiles and drug-like properties, making them promising candidates for further molecular docking and pharmacological studies.

Drug-likeness Evaluation of selected Phytoconstituents

The drug-likeness of the selected phytoconstituents was assessed using a scoring system ranging from 0 to 1, where higher values indicate better suitability as potential drug candidates. The results revealed a wide variation in drug-likeness scores among the compounds. Several compounds such as retinol acetate (0.8), stigmasterol derivatives (0.78), glycyrrhizin (0.73), glycyrrhetinic acid (0.73), voacangine (0.7), and 3,4-dihydroxyphenylglycol (0.71) demonstrated high drug likeness scores, indicating strong potential for therapeutic application due to favourable physicochemical and pharmacokinetic properties. Moderate scores were observed for compounds like vitamin E (0.48), flavonoids (0.45), haloxazolam (0.4), and ibogamine (0.42), suggesting acceptable but comparatively lower drug-like characteristics. In contrast, several compounds such as lactose (0.11), carbohydrates (0.1), diterpenes (0.09), and proteins (0.18) exhibited low drug-likeness scores, indicating pooer suitability as oral drug candidates, possibly due to high molecular size, low permeability, or unfavourable physicochemical properties. Moderate scores were observed for compounds such as lactose (0.11) , carbohydrates (0.1), diterpenes (0.09), and proteins (0.18) exhibited low drug-likeness scores, indicating poor suitablility as oral drug candidates, possibly due to high molecular size, low permeability, or unfavourable physicochemical properties. Interestingly, one compound (3-buten-1-amine, N,N-dimethyl) showed a score greater than 1(1.3), suggesting an exceptionally high predicted drug-likeness, although such values may require further validation. Overall, the results indicate that a subset of phytoconstituents possesses strong drug-like characteristics and can be prioritized for further molecular docking and pharmacological investigations, while others may require structural modification or may be less suitable for drug development.

Evaluation of Drug-Likeness properties based on Lipinski's Rule of Five

The physicochemical properties and drug-likeness of the selected compounds were evaluated based on Lipinski's rule of five and related parameters, including molecular weight, LogP, hydrogen bond donors and acceptors, molar refractivity (MLOGP), rotatable bond count, and topological polar surface area (TPSA). All compounds exhibited molecular weights below 500 g/mol, satisifying the acceptable limit for oral drugs. The LogP values indicated a balanced range of lipophilicity, suggesting adequate membrane permeability, although a few compounds showed higher lipophilicity, which may influence solubility. The number of hydrogen bond donors (≤5) and acceptors (≤10) for all compounds were within the permissible range, supporting favourable interactions with biological targets. Most compounds also demonstrated acceptable rotatable bond counts (<10), indicating good molecular flexibility, except for a few long-chain fatty acids and sterol derivatives that showed slightly higher values. TPSA values were largely within the acceptable limit (<140 Å²)suggesting good absorption and permeability characteristics. Importantly, all compounds complied with Lipinski's rule, indicating strong drug likeness and suitalbility for oral bioavailability. Overall, these results suggests that the selected compounds possess favourable physicohemical and pharmacokinetic properties, making them promising candidates for further drug development and molecular docking studies.

***In silico* toxicity assessment of selected Phytocompounds**

The toxicity profile of the selected phytocompounds was evaluated using parameters such as AMES TOXICITY, hepatotoxicity, skin sensitization and hERG channel inhibition to assess their safety and suitability as drug candidates. All compounds were found to be non-AMES **toxic**, indicating the absence of mutagenic potential and suggesting genetic safety. Additionally, none of the compounds exhibited hepatotoxicity, highlighting their minimal risk of liver toxicity and favorable safety profile for systemic use. However, a majority of the compounds showed

positive skin sensitization, indicating a potential for causing allergic reactions upon dermal exposure, which may require consideration during formulation. The hERG inhibition analysis revealed a mixed pattern, where some compounds showed **mild to moderate inhibition**, while others were non-inhibitors; since hERG blockade is associated with cardiotoxicity, compounds showing strong inhibition may require further optimization. Overall, the results suggest that while the compounds are generally safe in terms of mutagenicity and hepatotoxicity, caution is needed regarding skin sensitization and potential cardiotoxic effects, and thus, further in vitro and in vivo toxicity studies are recommended to confirm their safety.

Identification of Common Targets Associated with Hepatoprotective Activity

The network-based analysis was performed to identify potential molecular targets involved in the hepatoprotective activity of *Tabernaemontana divaricata*. A Venn diagram was constructed to determine the overlap between predicted phytocompound targets and liver disease-associated genes. The analysis revealed that out of the total targets, **166 were compound-related targets**, while **861 were disease-associated genes**, indicating a broad spectrum of biological interactions. Importantly, **76 common targets** were identified in the overlapping region, representing potential key proteins involved in mediating the hepatoprotective effects of the plant. These shared targets suggest that the bioactive compounds of *Tabernaemontana divaricata* may exert therapeutic effects by modulating multiple signaling pathways associated with liver disorders. The identification of these common targets provides a strong foundation for further studies, including protein–protein interaction (PPI) network analysis, molecular docking, and molecular dynamics simulations, to elucidate the underlying mechanisms of hepatoprotection. Overall, this integrative approach highlights the multi-targeted nature of phytoconstituents and supports their potential role in the treatment of liver diseases.

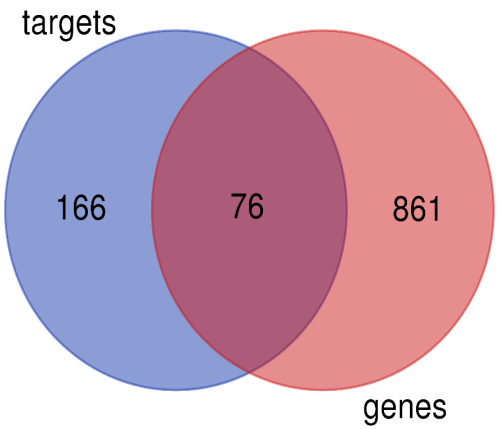

Figure No 1: Identification of Common Targets

Protein–Protein Interaction (PPI) Network Analysis of Common Targets

The protein–protein interaction (PPI) network was constructed to explore the functional relationships among the 76 common targets identified between *Tabernaemontana divaricata* phytoconstituents and liver disease-associated genes. The network revealed a highly interconnected system, indicating strong biological associations and coordinated regulation among the target proteins. Key hub proteins such as AKT1, MAPK1, MAPK14, TNF, ALB, CASP3, BCL2, and PIK3CA exhibited a high degree of connectivity, suggesting their central role in hepatoprotective mechanisms. These hub genes are primarily involved in critical signaling pathways including apoptosis regulation, oxidative stress response, inflammation, and cell survival pathways. The dense clustering observed in the network highlights the multi-target and synergistic nature of phytoconstituents present in *Tabernaemontana divaricata*. Additionally, the presence of signaling molecules related to pathways such as PI3K-Akt, MAPK, and NF-κB further supports the potential of these compounds in modulating liver injury and promoting hepatocellular protection. Overall, the PPI network analysis provides strong evidence that the hepatoprotective activity of Tabernaemontana divaricata is mediated through a complex, multi-target mechanism involving key regulatory proteins and interconnected signaling pathways, which can be further validated through molecular docking, molecular dynamics simulations, and in vitro studies.

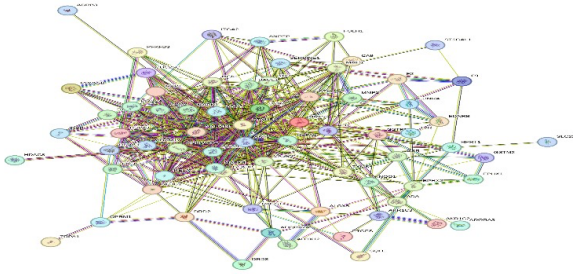

Figure No 2: protein protein interaction and network analysis of common targets.

Molecular Docking:

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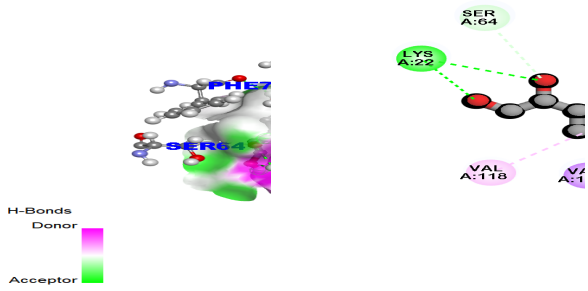
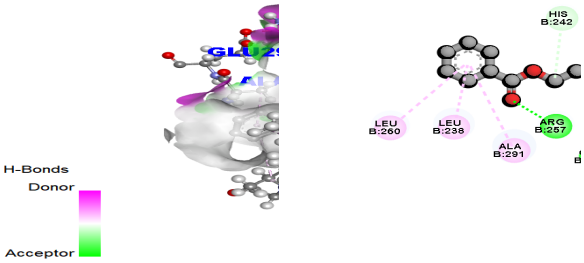
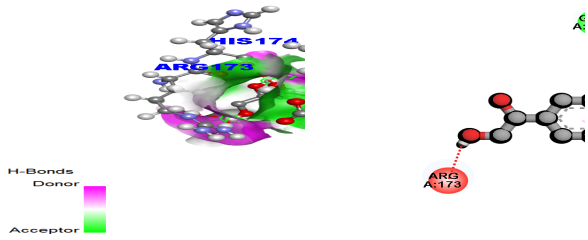


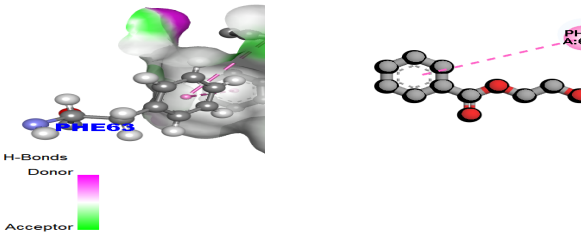


2,2 BENZOATE

BCL2(2W3L)



3,4 PHENYL



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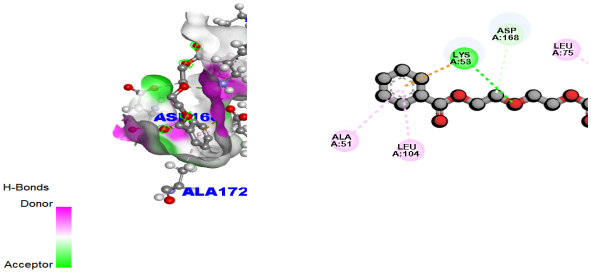
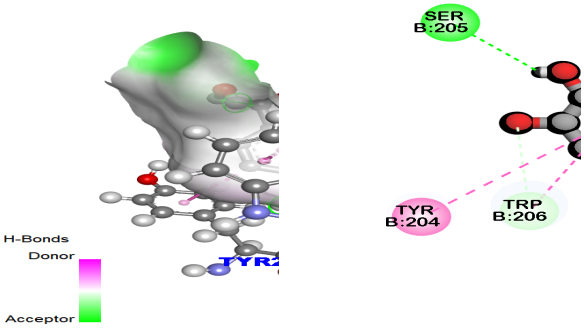


2,2 BENZOATE

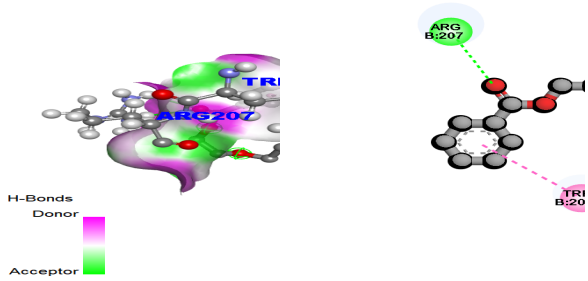
ALB(1AO6)



2,2 BENZOATE

CASP3(1GFW)

3,4 PHENYL



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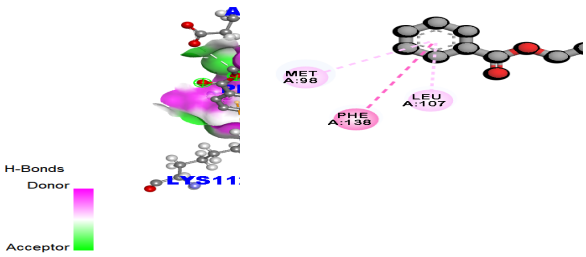
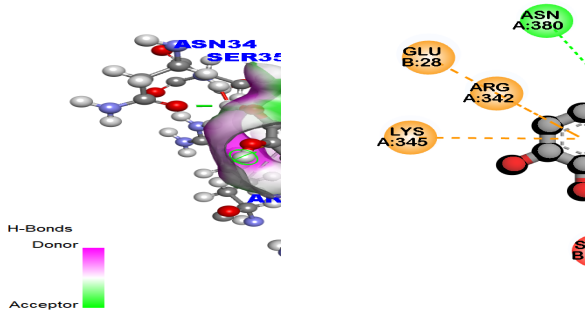
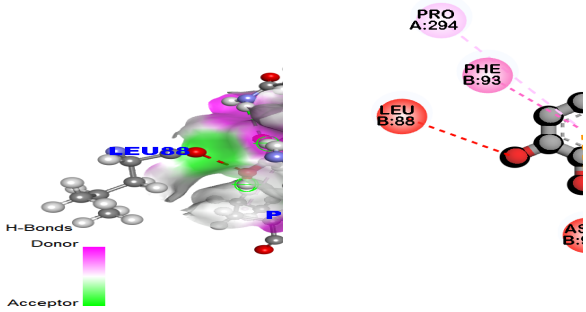


2,2 BENZOATE
GSK3B(1Q5K)



CTNNB1(1JDH)
3,4 PHENYL



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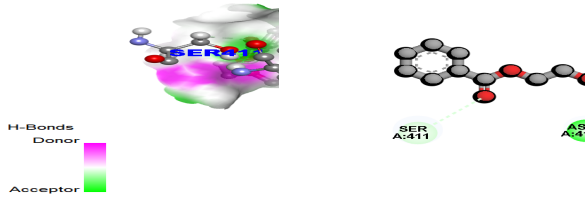

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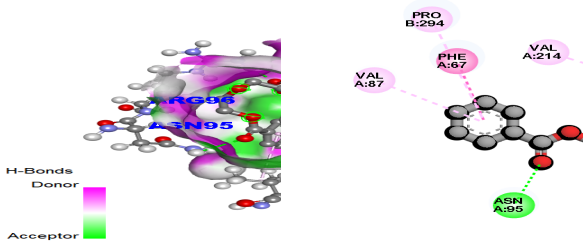
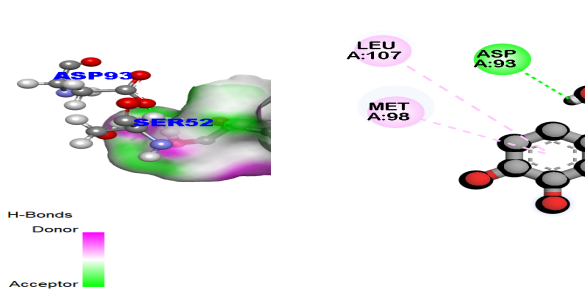
HSP90AA1(3TOZ)



2,2 BENZOATE

MAPK1(2OJJ)



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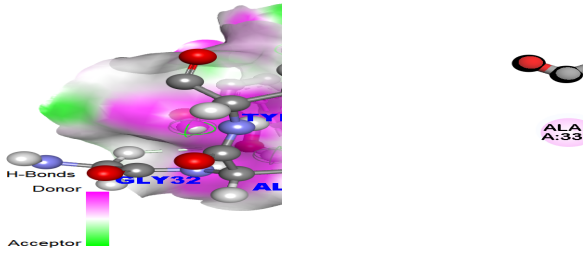

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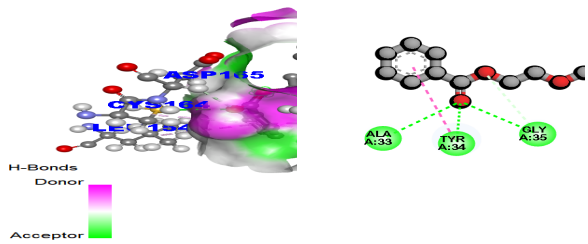
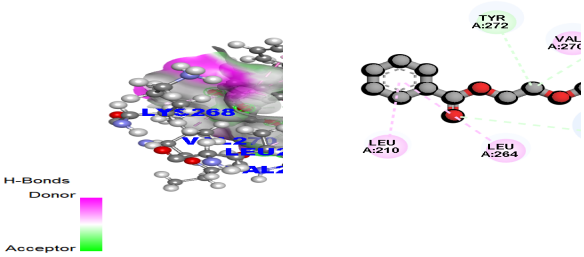
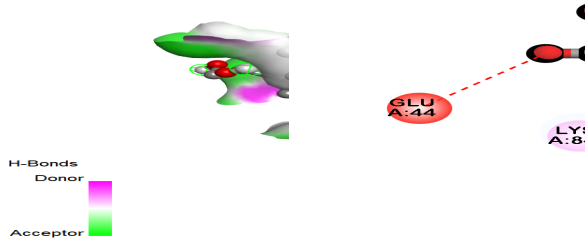


2,2 BENZOATE
KDR(3V2A)



2,2 BENZOATE
STANDARD DRUG(SILYMARIN)
AKT1(3096)



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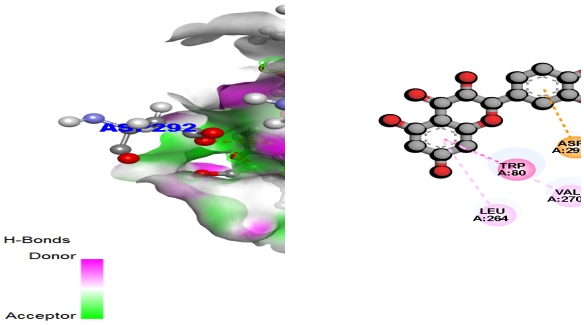
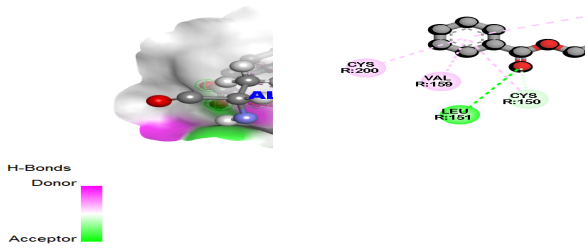


ALB(1AO6)



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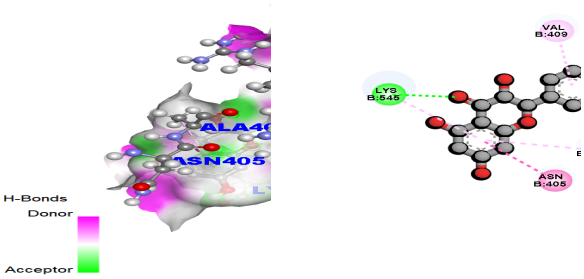
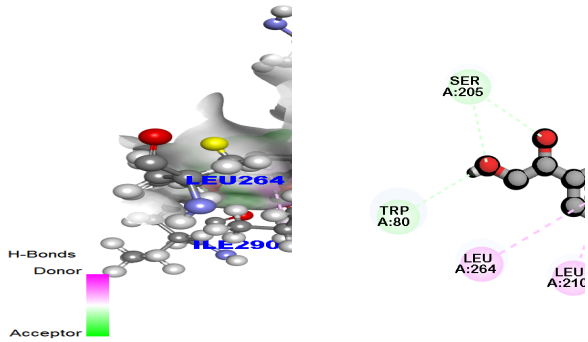
AKT1(3096)



BCL2(2W3L)



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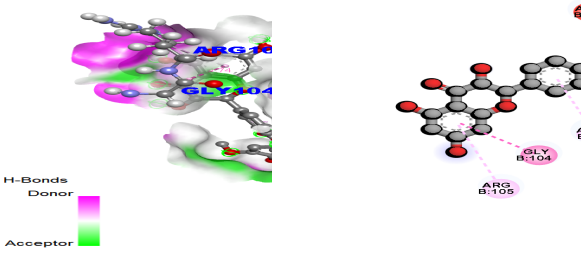


CASP3(1GFW)

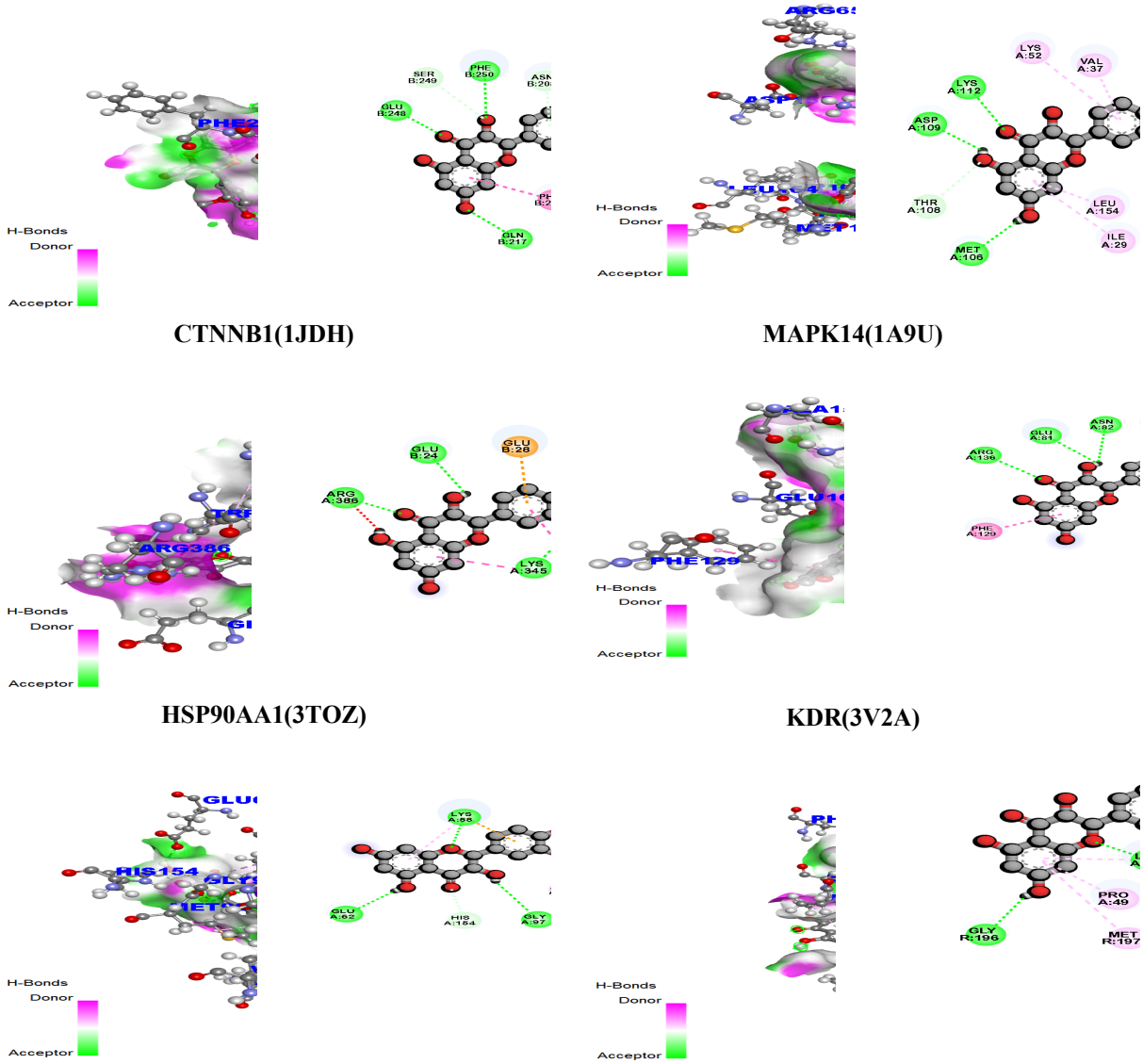


Figure No 3 : Molecular docking and visualiation

6.9 Molecular Dynamics Simulation Analysis

This report is a molecular dynamics (MD) simulation analysis of a protein-ligand complex, carried out using Schrödinger's *Desmond* engine. It provides a detailed look at how the protein and ligand behaved over a 200-nanosecond trajectory under near-physiological conditions.

The simulation setup involved a protein of 367 residues (chain A) with a net charge of +7, solvated in ~5,500 water molecules and neutralized with sodium and chloride ions. The ligand studied is a neutral organic molecule ($C_{19}H_{20}O_5$) with 10 rotatable bonds, allowing conformational flexibility. The system was run in an NPT ensemble at 300 K, ensuring pressure and temperature stability.

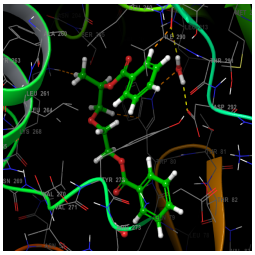
The protein analysis shows that the backbone RMSD stabilized within the acceptable range of 1–3 Å, suggesting the protein maintained structural integrity without major unfolding. RMSF analysis highlights flexible regions, particularly loops and terminal residues, while secondary structure analysis indicates ~25% helices and ~23% strands, reflecting a moderately ordered protein. This balance of rigidity and flexibility is typical of functional proteins, where loops often mediate binding and dynamics.

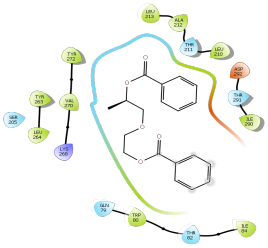
The ligand behavior was also stable, with RMSD values indicating it remained bound in the pocket throughout the simulation. RMSF analysis of individual ligand atoms revealed localized flexibility, consistent with side chains adapting to the binding environment. The torsion profile shows that rotatable bonds sampled multiple conformations, reflecting the ligand's ability to adjust its geometry to fit the protein pocket. Surface property measurements (SASA, PSA, MolSA) suggest moderate polarity and solubility, with no intramolecular hydrogen bonds detected, meaning the ligand's interactions are primarily external with the protein and solvent.

The protein-ligand interactions are central to the analysis. Key residues such as ASN53, TRP80, LYS179, TYR272, ASP292, and LEU210 formed consistent contacts with the ligand. These interactions included hydrogen bonds, hydrophobic contacts, ionic interactions, and water bridges. Notably, residues ASP292 and TYR272 maintained interactions for over 30% of the simulation time, marking them as strong binding hotspots. The timeline of contacts shows that multiple residues contributed simultaneously, creating a stable binding network.

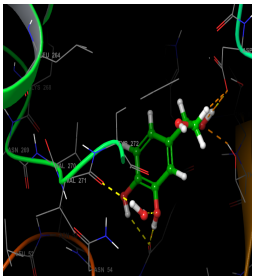
Finally, the ligand properties section confirms that the ligand remained conformationally stable, with a radius of gyration around 4 Å and solvent-accessible surface area fluctuating moderately. This suggests the ligand did not undergo major unfolding or dissociation, reinforcing the conclusion that the binding pocket provided a stable environment.

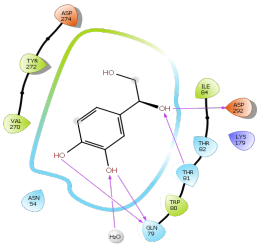
In summary, the simulation demonstrates a stable protein-ligand complex where the protein maintained its structural integrity, and the ligand remained bound through persistent hydrogen bonds and hydrophobic contacts. Flexible regions in both the protein and ligand contributed to dynamic but stable binding. This type of analysis is crucial in drug discovery, as it identifies key residues responsible for ligand affinity and highlights the conformational adaptability of the ligand in the binding site.





2-(2-benzoyloxypropoxy)ethyl benzoate





3,4 dihydroxy phenyl glycol

Figure No 4: Molecular dynamic simulation studies of docked compounds finally selected based on binding affinity.

Table No 1: **Molecular dynamic simulation studies of docked compounds.**

COMPOUNDS	1A9U		1AO6		1GFW		1JDH		1Q5K		2OJJ		2W3L		3O96		3T0Z		3V2A	
	D/S	D/E	D/S	D/E	D/S	D/E	D/S	D/E	D/S	D/E	D/S	D/E	D/S	D/E	D/S	D/E	D/S	D/E	D/S	D/E
4-[(1S)-1,2-dihydroxyethyl]benzene-1,2-diol	-5.0711	-40.0667	-5.0711	-40.0667	-3.5533	-21.2044			-4.8099	-30.6066	-6.9199	-47.3666	-4.7977	-31.9122	-6.5599	-43.2477	-4.7255	-29.1222	-5.2222	-41.6144
2-(2-benzoyloxypropoxy) ethyl benzoate	-5.0778	-54.4422	-5.0778	-54.4422	-2.4766	-28.3966			-4.1533	-38.9500	-6.2788	-61.0299	-4.9533	-44.0366	-7.5977	-66.6099			-4.1311	-44.9400

Discussion

The present investigation was undertaken to explore the hepatoprotective potential of *Tabernaemontana divaricata* through an integrated approach involving phytochemical screening, network pharmacology, molecular docking, molecular dynamics simulations, and mechanistic understanding of hepatotoxicity. The findings of this study are discussed in relation to established concepts of liver physiology, pathogenesis of liver injury, and emerging therapeutic strategies.

- The liver is a vital organ responsible for metabolic homeostasis, detoxification, protein synthesis, and bile secretion, making it highly vulnerable to xenobiotic-induced injury.[10] Hepatic injury manifests through various pathological processes such as steatosis, necrosis, apoptosis, inflammation, and fibrosis, depending on the severity and duration of the insult . These processes collectively contribute to progressive liver diseases including cirrhosis and hepatic failure, which remain major causes of morbidity and mortality worldwide [11.]
- Among various experimental models, carbon tetrachloride (CCl_4)-induced hepatotoxicity remains one of the most widely used and validated models for studying liver injury. The hepatotoxic effects of CCl_4 are primarily mediated through its biotransformation by cytochrome P450 enzymes, particularly CYP2E1, leading to the generation of trichloromethyl radicals (CCl_3•) and trichloromethyl peroxy radicals (CCl_3OO•)[12]. These reactive intermediates initiate lipid peroxidation, disrupt membrane integrity, and impair cellular function, ultimately resulting in hepatocyte necrosis and fatty degeneration. This mechanism aligns with the findings of Recknagel et al. (1989), who emphasized the central role of free radical-mediated oxidative stress in chemically induced liver injury[13].
- Oxidative stress plays a pivotal role in the pathogenesis of hepatotoxicity. It arises from an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, leading to cellular damage [14,15]. ROS such as superoxide anions and hydroxyl radicals can damage lipids, proteins, and nucleic acids, thereby compromising cellular integrity. The liver is equipped with endogenous antioxidant systems, including glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT), which act synergistically to neutralize ROS[16] . However, depletion of these antioxidants during toxic insult enhances susceptibility to liver [17]
- In the present study, the hepatoprotective potential of *Tabernaemontana divaricata* can be attributed to its rich phytochemical composition, including alkaloids, flavonoids, phenolic compounds, and triterpenoids . Alkaloids such as voacangine and coronaridine, along with flavonoids like quercetin and kaempferol, are known to possess strong antioxidant and anti-inflammatory properties. Phytochemical studies by Van Beek et al. (1984) have identified several indole alkaloids in *T. divaricata*, providing a biochemical basis for its pharmacological activities .
- Flavonoids and phenolic compounds play a crucial role in scavenging free radicals and inhibiting lipid peroxidation, thereby protecting hepatocytes from oxidative damage. Kulkarni et al. (2012) demonstrated significant antioxidant activity of *T. divaricata*, supporting its potential hepatoprotective role . Similarly, Gupta et al. (2015) reported anti-inflammatory activity of the plant, which is essential in preventing inflammation-mediated liver injury . These findings are consistent with studies on other hepatoprotective plants such as *Phyllanthus*[rr21] *Andrographis paniculata*[22], which exert their effects primarily through antioxidant and anti-inflammatory mechanisms .
- The ADMET and drug-likeness analysis performed in this study ensured that selected phytoconstituents possess favorable pharmacokinetic and toxicity profiles[23] . Lipinski's rule of five serves as an important criterion for predicting oral bioavailability, and compounds satisfying these criteria are more likely to be successful drug candidates. This screening step enhances the translational potential of the identified phytoconstituents.
- Network pharmacology analysis revealed that the hepatoprotective activity of *T. divaricata* is mediated through a multi-target mechanism. The identification of overlapping targets between phytoconstituents and liver disease-related genes highlights the complex interaction between bioactive compounds and biological systems[24,25]. Protein–protein interaction (PPI) analysis further identified key hub genes involved in inflammation, apoptosis, and oxidative stress, emphasizing the network-based therapeutic approach.
- Molecular docking studies demonstrated strong binding affinities of selected compounds with key proteins such as TNF-α, Nrf2, and caspase-3. TNF-

α is a pro-inflammatory cytokine involved in liver inflammation and apoptosis, while Nrf2 regulates antioxidant defense mechanisms, and caspase-3 plays a central role in apoptosis. Favorable interactions, including hydrogen bonding and hydrophobic interactions, suggest that these phytoconstituents can effectively modulate these targets and prevent liver injury [26] .

- Molecular dynamics (MD) simulations provided further validation of docking results by assessing the stability of ligand–protein complexes over time. Parameters such as RMSD, RMSF, and hydrogen bonding confirmed the stability and reliability of these interactions[27]. Stable interactions indicate that the compounds can effectively bind to target proteins under physiological conditions.
- Drug-induced liver injury (DILI) remains a significant clinical challenge, with mechanisms involving direct toxicity, immune-mediated reactions, and metabolic idiosyncrasies[28,29] . Risk factors such as age, sex, genetic variability, and alcohol consumption further influence susceptibility to hepatotoxicity[30]. The ability of *T. divaricata* to counteract oxidative stress and inflammation suggests its potential role in mitigating DILI.
- In vitro models such as HepG2 cell lines and MTT assay are widely used to evaluate hepatoprotective activity. Mosmann (1983) developed the MTT assay as a reliable method for assessing cell viability, while Wilkening et al. (2003) demonstrated the suitability of HepG2 cells for hepatotoxicity studies . These models provide valuable insights into cellular responses to toxic insults and protective effects of compounds.
- Furthermore, the integration of in silico and experimental approaches enhances the efficiency and reliability of drug discovery. Gupta et al. (2018) emphasized that combining computational predictions with experimental validation improves the success rate of identifying potential therapeutic agents . This approach is particularly relevant in the context of herbal drug research, where multiple bioactive compounds contribute to therapeutic effects.
- Overall, the hepatoprotective activity of *Tabernaemontana divaricata* can be attributed to multiple mechanisms, including antioxidant activity, anti-inflammatory effects, inhibition of apoptosis, and modulation of key signaling pathways. The presence of diverse phytoconstituents enables a synergistic effect, enhancing therapeutic efficacy. The findings of this study provide scientific validation for the traditional use of *T. divaricata* and highlight its potential as a promising candidate for the development of novel hepatoprotective agents.

Conclusion

The present study demonstrates that *Tabernaemontana divaricata* possesses significant hepatoprotective potential, which can be attributed to its rich phytochemical composition, particularly alkaloids, flavonoids, and phenolic compounds. The integrated approach combining ADMET screening, network pharmacology, molecular docking, and molecular dynamics simulations revealed that the selected phytoconstituents exhibit favorable pharmacokinetic properties and strong binding affinity towards key targets involved in liver injury, such as TNF-α, Nrf2, and caspase-3. The findings suggest that the hepatoprotective activity of *T. divaricata* is mediated through multiple mechanisms, including antioxidant activity, inhibition of oxidative stress, suppression of inflammatory pathways, and prevention of apoptosis. Furthermore, the study supports the role of oxidative stress as a central factor in hepatotoxicity and highlights the importance of natural antioxidants in liver protection. Overall, this research provides scientific validation for the traditional use of *Tabernaemontana divaricata* and establishes it as a promising candidate for the development of novel hepatoprotective agents, warranting further in vivo and clinical investigations.

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