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In silico molecular docking and virtual screening of natural compounds against Tubulin-7-Aminonoscapine complex (6Y6D) for estimation of anticancer potential

Snehal Kashid[1]*, Prashali Shinde[2], Reshma Pawar[3] & Ashish Mishra[4]

[1]Department of Pharmacognosy; [2]Department of Pharmaceutical Chemistry; & [3]Department of Pharmacology, College of Pharmacy, Akluj-413 101, Maharashtra, India

[4]Department of Pharmacology, Apex Institute of Pharmacy, Samaspur Chunar-231 304, Uttar Pradesh, India

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This comprehensive study integrated *in silico* molecular docking and pharmacokinetic profiling to elucidate the interactions and properties of eight bioactive compounds (nimbin, azadirachtin, betasitosterol, nimbidinin, chavicol, chavibetol, curcumin, and zingiberene) from natural sources with protein 6Y6D. SwissADME and SwissTargetPrediction are used for pharmacokinetic screening, whereas, autodock vina is used for molecular docking and virtual screening. Protox3 software was used to establish each phytoconstituent's specific toxicity profile. According to the results, nimbidinin and nimbin exhibit extraordinary binding affinity without toxicity and have the potential to be further screened as lead compounds. Azadirachtin, on the other hand, exhibits the highest binding affinity with a moderate toxicity profile. Furthermore, zingiberene and chavicol exhibit low binding affinities. A detailed investigation reveals the binding modes and molecular interactions with different amino acids. Our findings may facilitate the development of novel drugs and provide a foundation for further experimental validation.

Keywords: Anticancer, Autodock vina, Molecular docking, PDB ID:6Y6D, Phytoconstituents

Molecular docking is a computational technique used to predict the preferred orientation of a small molecule, or ligand, when bound to a specific protein. This method has become a crucial tool in drug discovery and development, allowing researchers to identify potential lead compounds and understand their binding mechanisms. Protein-ligand interactions play a vital role in various biological processes, and deciphering these interactions is essential for understanding disease mechanisms and developing effective treatments[1-3]. The PDB (protein data bank) ID: 6Y6D represents the Tubulin-7-Aminonoscapine complex, providing valuable information about the binding site and molecular interactions. Noscapine, a natural alkaloid and FDA approved anti-tussive agent. This drug also possesses weak anticancer potential. As per the report 7A-aminonoscapine binds to tubulin's colchicine site which ultimately causes mitotic arrest[4]. In this study, we utilize molecular docking techniques to investigate the binding modes of selected ligands, including nimbin, azadirachtin, betasitosterol, nimbidinin, chavicol, chavibetol, curcumin, and zingiberene, to the protein structure 6Y6D. These ligands, derived from natural products, have shown promising biological activities and potential therapeutic applications. Nimbin and nimbidinin, isolated from *Azadirachta indica*, possess anti-inflammatory and antimicrobial properties. Azadirachtin, also from *Azadirachta indica*, exhibits insecticidal and anticancer activities[5]. Betasitosterol, a phytosterol, has been shown to have anti-inflammatory and antitumor effects[6]. Chavicol and chavibetol, found in *Piper betle*, exhibit antioxidant and antimicrobial properties[7]. Curcumin, a polyphenol from *Curcuma longa*, has potent anti-inflammatory, antioxidant, and anticancer activities. Zingiberene, a sesquiterpene from *Zingiber officinale*, displays anti-inflammatory and antimicrobial properties[8].

In silico SwissTargeting computational method used to predict the bioactivity of small molecules against specific targets, such as proteins or enzymes. It has become a powerful tool in the field of cheminformatics and drug discovery, enabling researchers to efficiently identify promising lead compounds and accelerate the development of new treatments[9]. Additionally *in silico* approach for absorption, distribution, metabolism, excretion, and toxicity (ADMET) predictions to evaluate the potential of selected ligands as therapeutic agents.

*Correspondence:
E-mail: snehalkashid1231@gmail.com

This integrated approach streamlines the drug discovery process, reducing the need for costly and time-consuming experimental assays, and identifies promising candidates for further validation[10]. By analysing the protein-ligand interactions and binding affinities of these selected ligands, we aim to gain insights into the molecular mechanisms underlying their biological activities and identify potential therapeutic applications. This research contributes to the growing field of structural biology and drug discovery, highlighting the importance of molecular docking and protein-ligand interactions in understanding biological systems and developing novel treatments[11]. The current research work emphasized on screening of selected phytoconstituents against Tubulin-7-Aminonoscapine complex and highlighting the importance of *in silico* screening and its pharmacophore.

Materials and Methods

Network pharmacology

Target prediction

Based upon literature search from pubchem, google scholar, springer link, science direct, and web of science data eight potential constituents from natural sources were selected such asnimbin, nimbidinin, azadirachtin, and betasitosterol from *Azadirachta indica.* Chavibetol and chavicol from *Piper betle* while, curcumin, zingiberene from *curcuma longa* and *Zingiber officinale*, respectively.

Target prediction for ligand

We used SwissTargetPrediction, binding DB and Puchem for screening the other potential ligand target, *in silico*. We developed this tool to identify targets of bioactive compounds in different species. We retrieved the conical smile data from Pubchem for the purpose of ligand target prediction [12-14].

Moreover the disease gene was screened using MalaCards (https://www.malacards.org) and GeneCards Version 5.21 softwares[15].

Network analysis

The STRING (https://string-db.org) and EnrichR (https://maayanlab.cloud/Enrichr) tools are used for the protein–protein interaction, enrichment analysis, and gene ontology enrichment analysis. The functional gene network is built using the Cytoscape interface (https://cytoscape.org)[16].Additionally cytoNCA software was utilized to investigate closeness, degree and betweenness. Several disease association genes with ligand were calculated considering the p-value significance. Cancer, COVID-19, asthma, respiratory disease, anti-bacterial, and hormonal diseases gene were considered during screening.

Molecular docking analysis

Computerized tools, such as iterative molecular docking, estimate the binding affinity of ligands to receptor proteins. While it may have applications in pharmacological research, it has evolved into a powerful tool for pharmaceutical development. To achieve this, we employ virtual screening using extensive collections of compounds[2,17-19].

Software and database systems

We conducted a computational analysis using MGL tools 1.5.6 and 4.2.6 of Autodock Vina, available at https://vina.scripps.edu[20]. We use this software to predict the interaction between selected ligands and proteins, naming the tubulin-7-aminonoscapine complex. We converted the ligand from SDF to PDB using OpenBabel 3.1.1 (http://openbabel.org) and used BIOVIA Discovery Studio (https://discover.3ds.com) for protein preparation and visualization. We conducted docking sessions on the Windows 10 operating system[21-22].

Protein Selection

Based on the literature from PubMed, Springer Link, and Science Direct Services, we selected the protein PDB ID: 6Y6D. The 3D structure of selected protein PDB ID: 6Y6D was extruded from protein data bank in PDB format accessible at http://www.rcsb.org.its chains and structure was observed in (Fig. 1).

Protein preparation

We introduced the Kollman charges, performed polar hydrogen atom addition, and eliminated water molecules and files saved in PDB format using the software BIOVIA Discovery Studio.

Selection of ligands

We picked out the three-dimensional shapes of ligands from Pubchem (https://pubchem.ncbi.nlm.nih.gov), such asnimbin, zingiberene, azadirachtin, betasitosterol, chavibetol, chavicol, curcumin, and nimbidinin (Table 1). We converted the ligand from SDF to PDB using open babel 4, and then docked these 8 ligands against proteins[23].

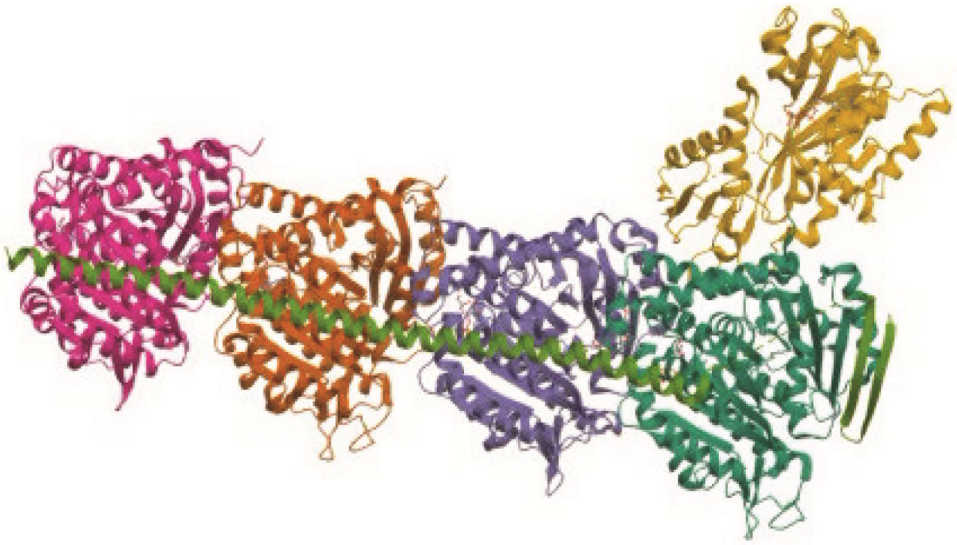


Fig. 1 — Chain A, B, C, D, E, and F of PDB ID: 6Y6D

Table 1 — *In silico* Pharmacokinetic profile of selected compounds

Compound	GI absorption	Metabolism CYP Inhibitors for					Membrane Transporters		Drug likeness	LD_{50} mg/kg
		1A2	2C19	2C9	2D6	3A4	BBB	P-gp Substrate		
Azadirachtin	Low	No	No	No	No	No	No	Yes	No	2000
Beta-sitosterol	Low	No	No	No	No	No	No	No	Yes	890
Chavibetol	High	Yes	No	No	No	No	Yes	No	Yes	1230
Chavicol	High	Yes	No	No	No	No	Yes	No	Yes	870
Curcumin	High	No	No	Yes	No	Yes	No	No	Yes	2000
Zingiberene	Low	No	No	No	Yes	Yes	No	No	Yes	1680
Nimbidinin	High	No	No	No	Yes	No	No	Yes	Yes	600
Nimbin	High	Yes	No	No	No	No	Yes	No	Yes	1000

Docking Procedures

Autodock Vina is a commonly used molecular docking computer program. We established a PDBQT format for both the ligand and the protein elements. The command prompt is an essential stage subsequent to grid setting in the docking procedure. The XYZ coordinates for Protein PDB ID: 6Y6D are 15.751136, 38.857401, and 19.220014, respectively, with a radius of 90. We set the exhaustiveness level at 8, which is widely accepted. BIOVIA Discovery Studio achieves the graphical representation of the protein-ligand binding interaction[24].

ADME profiling

We determined the absorption, distribution, metabolism, and excretion of each ligand *in silico* using the software SwissADME (http://www.swissadme.ch/index.php). We retrieved the conical smile data from Pubchem to predict the pharmacokinetic properties of phytoconstituents[25-27].

Toxicity profiling

The selected ligands were screened for *in silico* toxicity prediction using the ProToxII obtain from https://tox.charite.de/protox3. The various toxicities were predicted such as hepatotoxicity, respiratory toxicity, nephrotoxicity, cardio-toxicity, carcinogenicity, immuno-toxicity, mutagenicity, cytotoxicity along with LD_{50}[28] (Table 2).

Results and Discussion

Network pharmacology

Total eight phytoconstituents were extracted from pubchem and the common genes were found *via* screening the compound through SwissTargetPrediction and gene disease from genecard and malacard. Curcumin showing (71) highest common gene while azadirachtin shows only eight common genes. Most of the phytoconstituents shows common gene with cancer disease gene and hence further screening was carried out considering disease gene data.

The network between the genes was generated using STRING and the figures were observed below in (Fig. 2A-G).

Prime target detected *via* closeness and found several target gene amongst them top five genes for Azadirachtin are PPARD, ESRRA, PKM, NR3C1, GLI1. For betasitosterol ESR1, CYP19A1, CYP17A1, HMGCR, NR3C1 genes were found. In case of chavibetol SRC, KDR, PARP1, MAPK14, and PGR gene were found as prime target. For Chavicol single gene (ACACA) was found as target. In case of curcumin more than 15 targets were observed but AKT1, EGFR, STAT3, BCL2 and GSK3B are showing highest closeness. Nimbidinin shows target gene STAT3, IL6, EGFR, HSP90AA1 and MMP9 while nimbin shows PARP1, HSP90AA1, FASN, HDAC1, and HDAC4 as target gene.

Molecular docking

Selected protein

Using computational tool naming molecular docking, the 4D structure of a chosen protein was

Table 2 — *In silico* Toxicity prediction of selected compounds

compound	Hepatotoxicity	Respiratory toxicity	Nephrotoxicity	Cardiotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
Azadirachtin	No	Yes	Yes	No	No	Yes	No	No
Betasitosterol	No	Yes	No	No	No	Yes	No	No
Chavibetol	No	No	No	No	No	No	No	No
Chavicol	No	No	No	No	No	No	No	No
Curcumin	No	No	Yes	Yes	No	Yes	No	No
Zingiberene	No	No	No	No	No	No	No	No
Nimbidinin	No	Yes	No	No	No	Yes	No	No
Nimbin	No	Yes	No	No	Yes	Yes	No	No

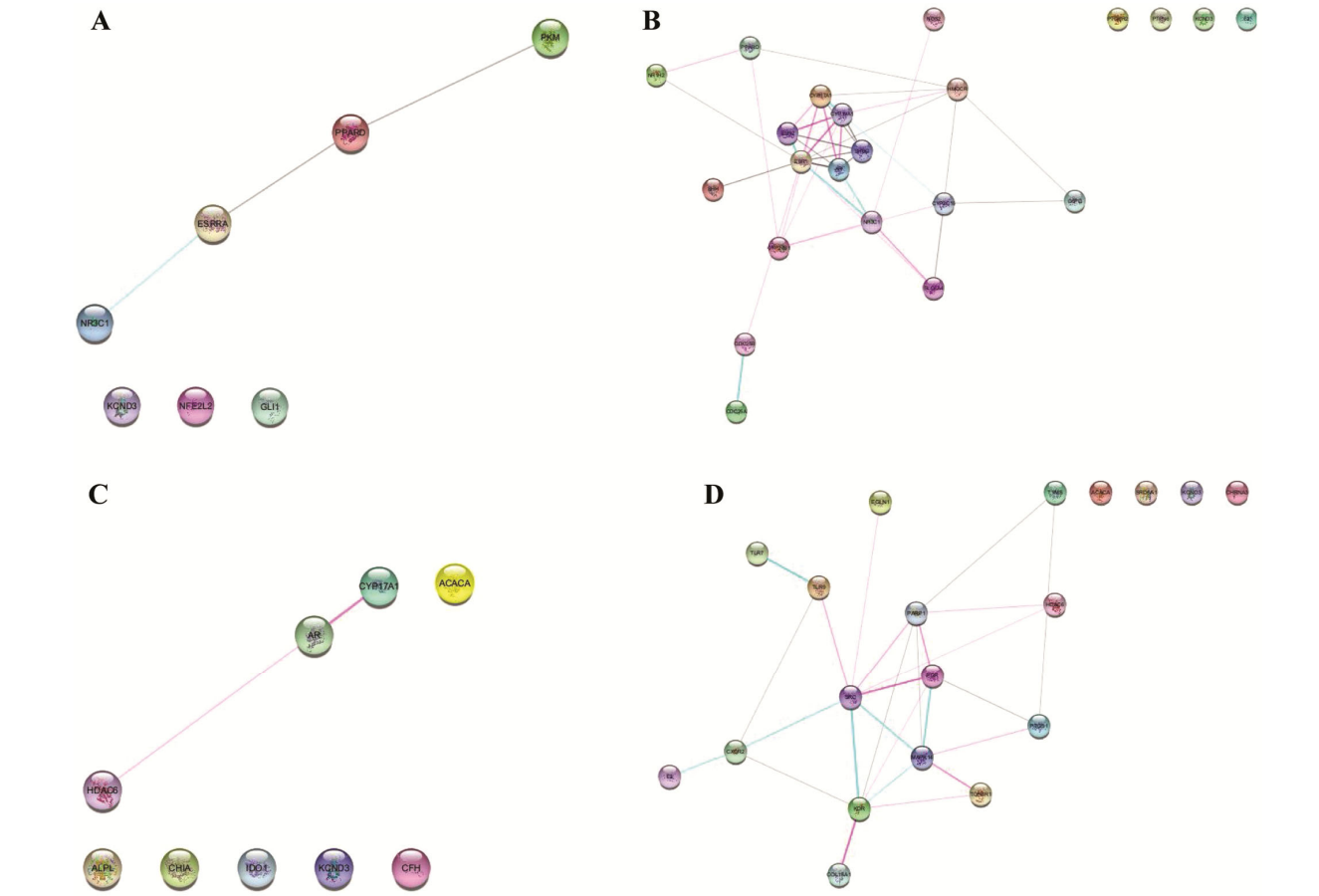


Fig. 2 — Network of (A) Azadirachtin; (B) Beta-sitosterol; (C) Chavicol; (D) Chavibetol; (*contd.*)

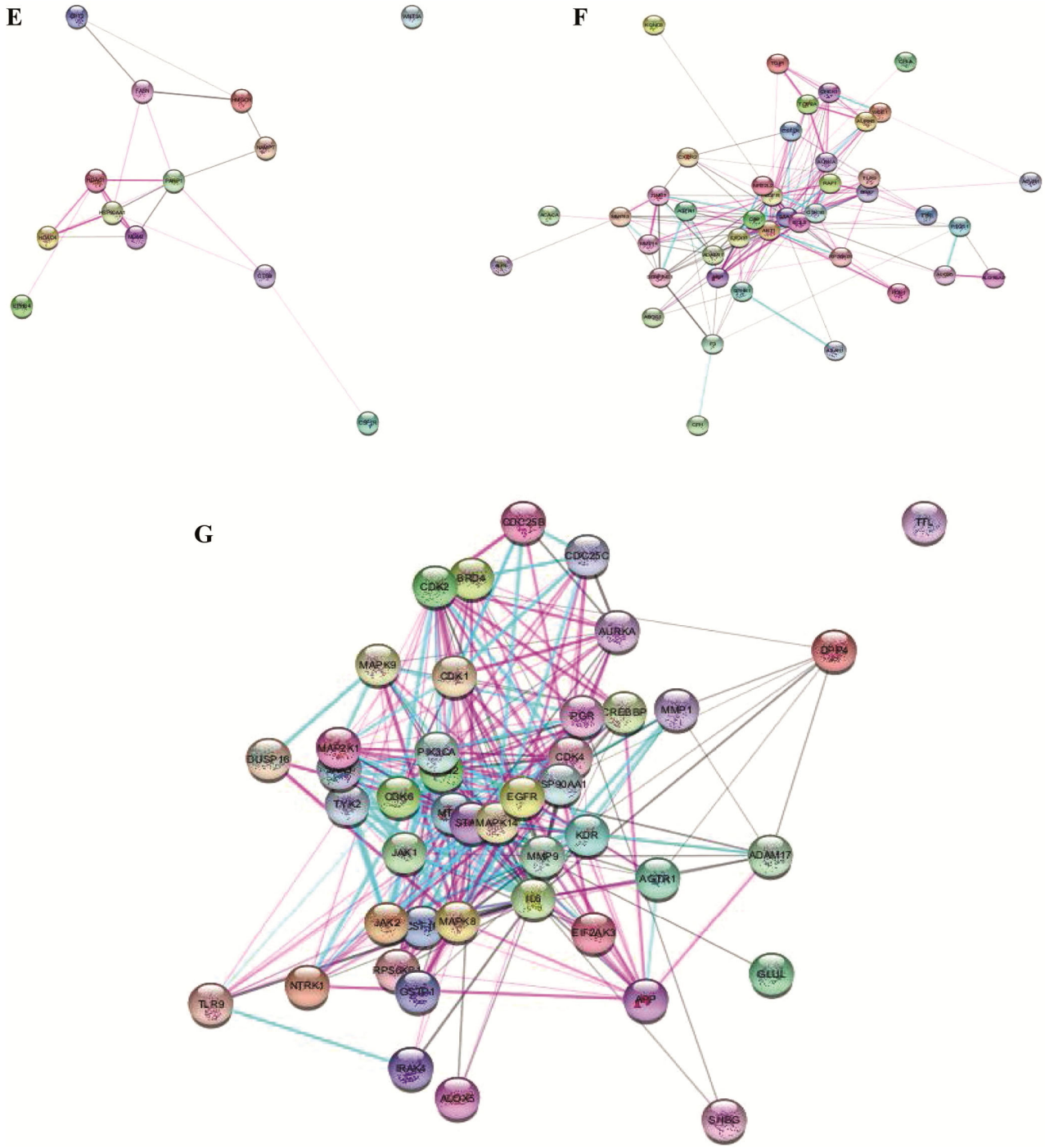


Fig. 2 — (E) Nimbin; (F) Curcumin; and (G) Nimbidinin generated by STRING (*contd.*)

obtained from PDB and its anti-cancer potential investigated. In current study PDB ID: 6Y6D (Tubulin-7-Aminonoscapine complex) was targeted against eight ligands. This protein shows 6 chains (A to F) amongst them all chain except chain E possesses active sites.

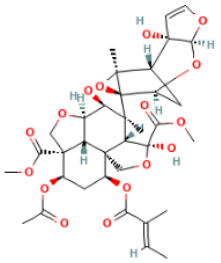
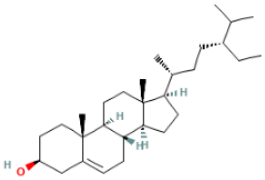
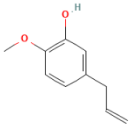
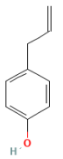
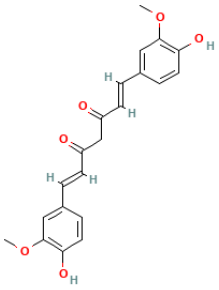
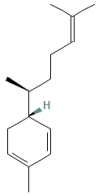
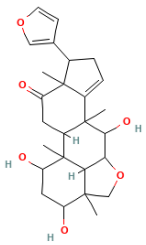
Selection of ligand

Eight ligands were retrieved from pubchem and its CID number, conical smiles and 2D structure were given in (Table 3).

Docking interaction

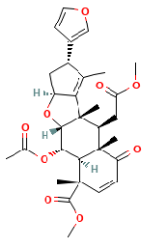
The molecular docking interaction of protein 6Y6D against selected 8 bioactive compounds was presented

Table 3 — List of selected compounds and their details

Compound name	Compound CID	Conical smiles	2D structure
Azadirachtin	5281303	CC=C(C)C(=O)OC1CC(C2(COC3C2C14COC(C4C(C3O)(C)C56C7CC(C5(O6)C)C8(C=CO C8O7)O)(C(=O)OC)O)C(=O)OC)OC(=O)C	
Beta-sitosterol	222284	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C	
Chavibetol	596375	COC1=C(C=C(C=C1)CC=C)O	
Chavicol	68148	C=CCC1=CC=C(C=C1)O	
Curcumin	969516	COC1=C(C=CC(=C1)C=CC(=O)CC(=O)C=CC2=CC(=C(C=C2)O)OC)O	
Zingiberene	92776	CC1=CCC(C=C1)C(C)CCC=C(C)C	
Nimbidinin	169088	CC12COC3C1C(C(CC2O)O)(C4CC(=O)C5(CCC=C5C4(C3O)C)C6=COC=C6)C)C	

(*contd.*)

Table 3 — List of selected compounds and their details (*contd.*)

Compound name	Compound CID	Conical smiles	2D structure
Nimbin	108058	CC1=C2C(CC1C3=COC=C3)OC4C2(C(C5(C(C4OC(=O)C)C(C=CC5=O)(C)C(=O)OC)C)CC(=O)OC)C	

in (Table 4). This table comprises 2D and 3D interactions of ligands against protein. The protein possesses six chains; among them, ligands frequently bind to chains A, B, C, and E. Chain C residue of protein 6Y6D was bound to chavibetol and zingiberene *via* hydrophobic bond and shows binding affinity of -6.6 kcal/mol and -5.3 kcal/mol, respectively. Additionally, betasitosterol binds to chain C *via* hydrogen and hydrophobic bond, showing a binding affinity of -10.6 kcal/mol. Chain B residue of protein binds to chavicol and curcumin *via* hydrophobic and electrostatic bond, showing binding affinity of -4.8 kcal/mol and -7.8 kcal/mol, respectively. Azadirachtin binds to multiple chain residues such as A, B, and E *via* electrostatic bond and hydrogen bond with excellent binding affinity (-13.5). Nimbin binds to chain A and E residue *via* hydrophobic and electrostatic bonds and possesses a binding affinity of -11.8 kcal/mol, while nimbidinin binds with A and B chain residue *via* electrostatic bond and shows a binding affinity of -12.3 kcal/mol. Chavicol possesses weak binding affinity, while azadirachtin shows the highest binding affinity.

Moreover previously, the research on the anticancer potential of new amido-thiadiazol-coupled noscapine derivatives was screened against PDB ID 6Y6D. The findings suggested that the synthesised compounds show binding affinity in between -5.418 to -9.679 kcal/mol, while noscapine shows binding affinity-5.304 kcal/mol[29]. The 2D and 3D interaction data were represented in (Table 2) while the comparison between several binding affinities were observed in (Fig. 3).

Several amino acids were involved in this protein ligand interaction such as GLU65, GLU411, GLU58, ASP163, ARG61, ASN206, ALA12, ILE171, VAL78, TYR83, LYS402, LEU405, LYS105, TYR262, ASP98, and LEU248 represented in (Table 5).

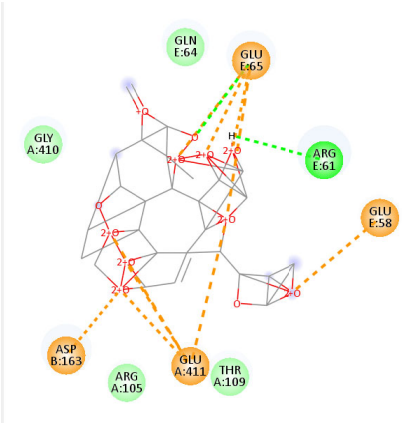
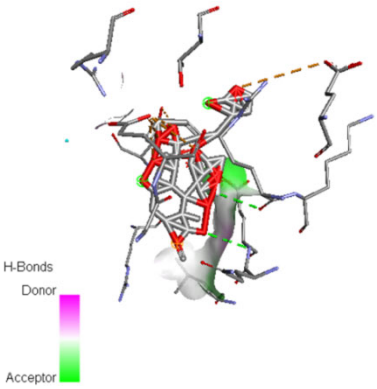
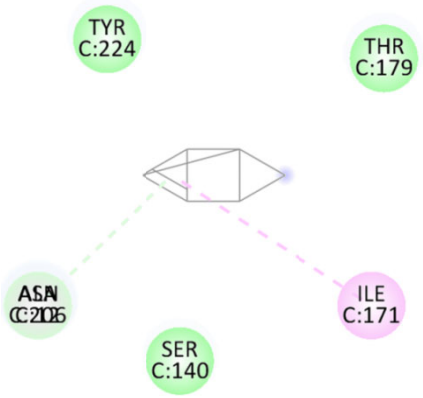
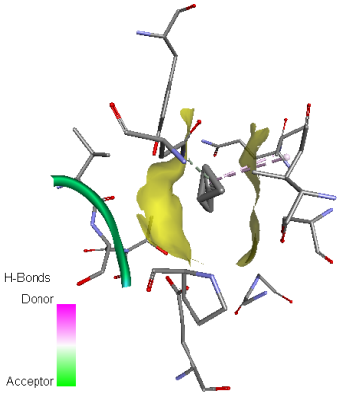
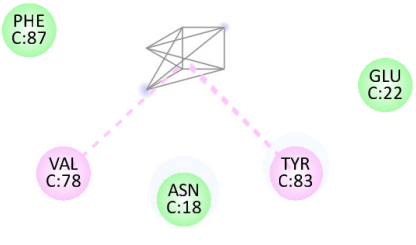
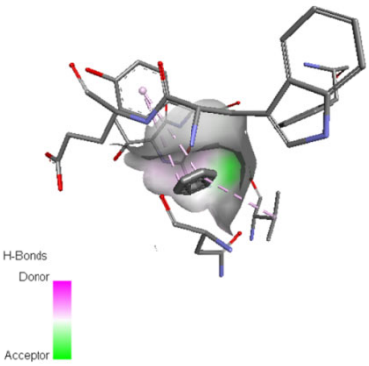
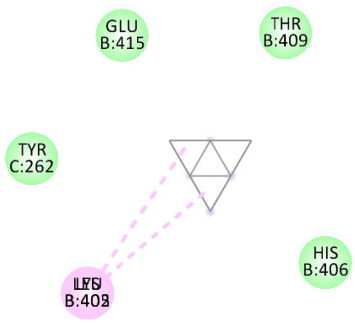
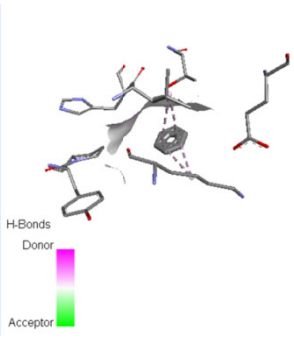
ADME and target prediction

The *in silico* ADME and target prediction were carried out using conical smiles of selected compounds. All compounds show versatile targets and can be screened further for several pharmacological actions *via in silico*, *in vitro*, and *in vivo* approaches. The predicted target and ADME profile of selected compounds was represented in (Table 6).

The pharmacokinetic profiles of eight bioactive compounds from natural sources were evaluated, revealing diverse absorption, metabolism, and transport characteristics. Azadirachtin, Beta-sitosterol and Zingiberene showed low absorption while others show high absorption. CYP inhibition assays revealed that Chavibetol and Nimbin were potential inhibitors of CYP1A2, while Curcumin inhibited CYP2C9. These findings suggest potential drug-herb interactions and motivate further investigation. Membrane transporter interactions were observed for several compounds, with Azadirachtin, Beta-sitosterol, and Zingiberene identified as P-gp substrates. This highlights the importance of considering transporter-mediated efflux in the pharmacokinetics of these compounds. The LD50 values revealed varying toxicity profiles, with Azadirachtin and curcumin exhibiting the lowest toxicity (2000 mg/kg) and Nimbidinin showing the highest toxicity (600 mg/kg). Overall, this study provides valuable insights into the pharmacokinetic properties of bioactive compounds from natural sources, highlighting their potential for drug development and herb-drug interactions.

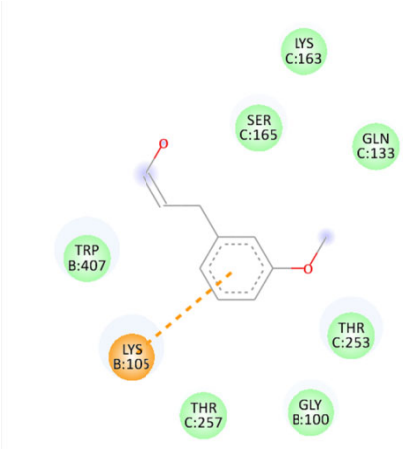
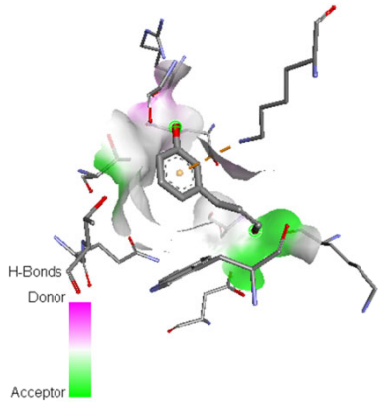
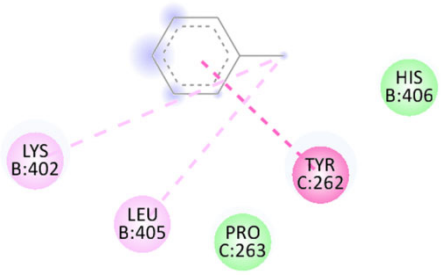
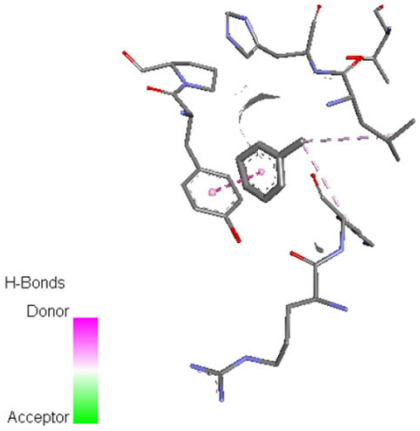
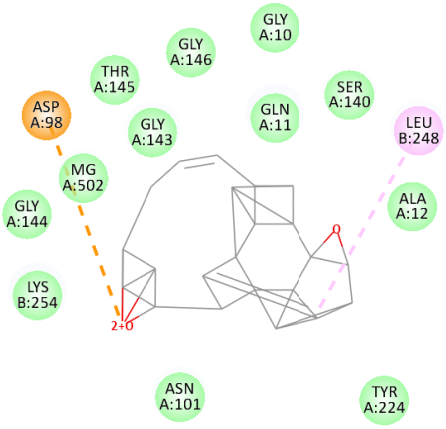
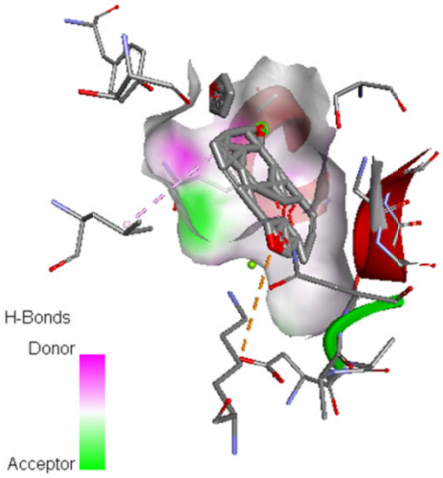
In silico toxicity prediction suggests that zingiberene, chavibetol, and chavicol were relatively non-toxic. Although curcumin exhibits slight toxicity, particularly to the immune system and kidneys, it was generally regarded as harmless. Nimbin and azadirachtin, however, had wide toxicity profiles.

Table 4 — 2D and 3D interaction of ligand against PDB ID: 6Y6D

Compound	2D Interaction	3D Interaction
Azadirachtin		
Beta-sitosterol		
Chavibetol		
Chavicol		

(*contd.*)

Table 4 — 2D and 3D interaction of ligand against PDB ID: 6Y6D (*contd.*)

Compound	2D Interaction	3D Interaction
Curcumin		
Gingiberene		
Nimbidinin		

(*contd.*)

Table 4 — 2D and 3D interaction of ligand against PDB ID: 6Y6D (*contd.*)

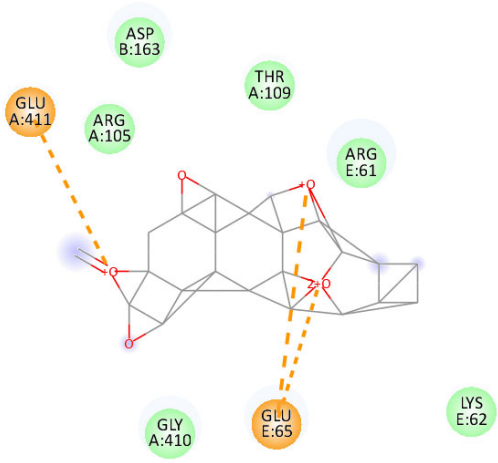
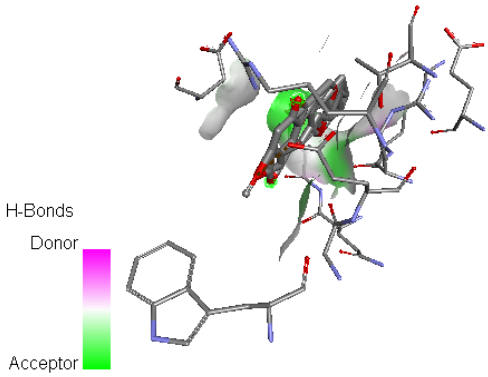
Compound	2D Interaction	3D Interaction
Nimbin		

Table 5 — Binding affinity and amino acid residue of selected compound

Sr. no	Compound Name	Binding affinity (kcal/mol)	Bond	Type	Amino acid residues
1	Azadirachtin	-13.5	Electrostatic Hydrogen Bond	Attractive Charge, Conventional Hydrogen Bond	GLU65, GLU411, GLU58, ASP163, ARG61
2	Beta-sitosterol	-10.6	Hydrogen Bond Hydrophobic	Pi-Donor Hydrogen Bond, Pi-Alkyl	ASN206, ALA12, ILE171
3	Chavibetol	-6.6	Hydrophobic	Alkyl, Pi-Alkyl	VAL78, TYR83
4	Chavicol	-4.8	Hydrophobic	Alkyl	LYS402, LEU405
5	Curcumin	-7.8	Hydrogen Bond, Electrostatic	Pi-Cation Pi-Donor Hydrogen Bond	LYS105
6	Zingiberene	-5.3	Hydrophobic	Pi-Pi T-shaped, Alkyl	TYR262, LYS402, LEU405
7	Nimbidinin	-12.3	Electrostatic, Hydrophobic	Attractive Charge, Alkyl	ASP98, LEU248
8	Nimbin	-11.8	Electrostatic	Attractive Charge	GLU65, GLU411

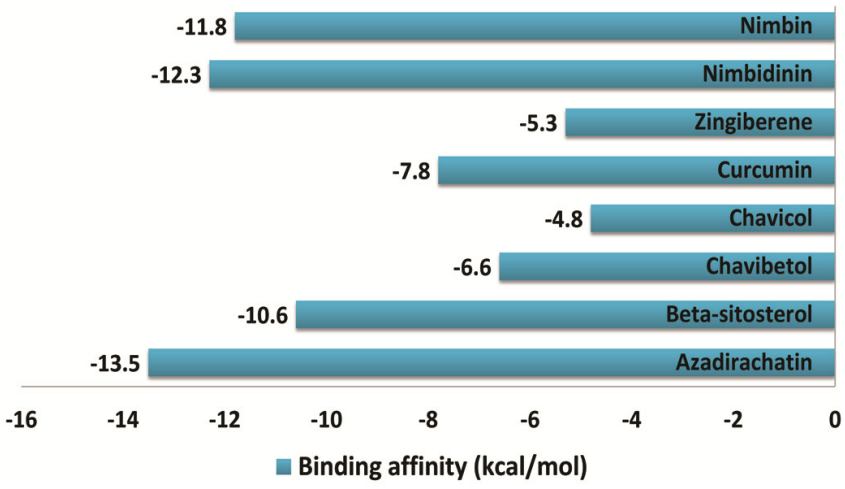
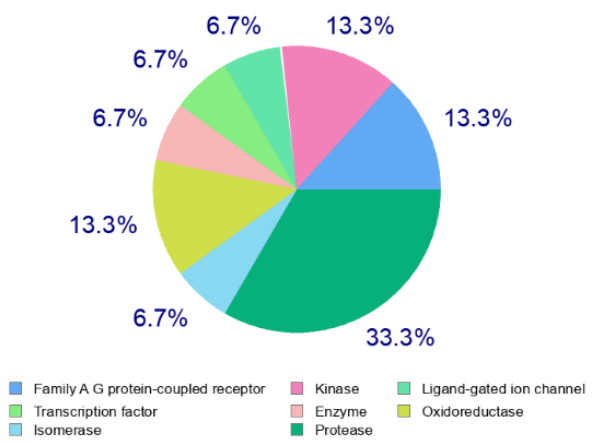
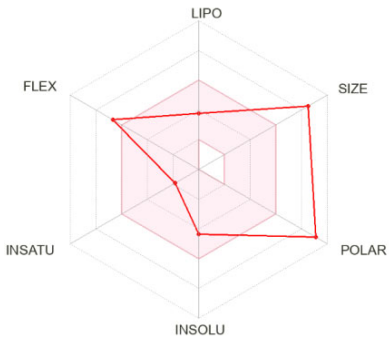
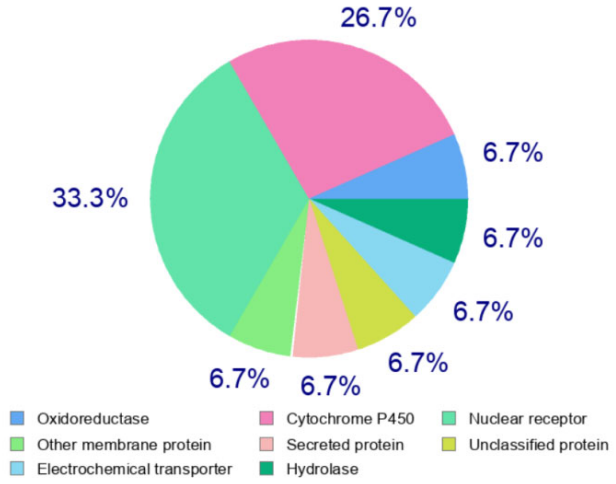
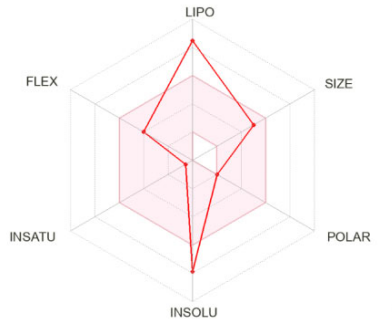
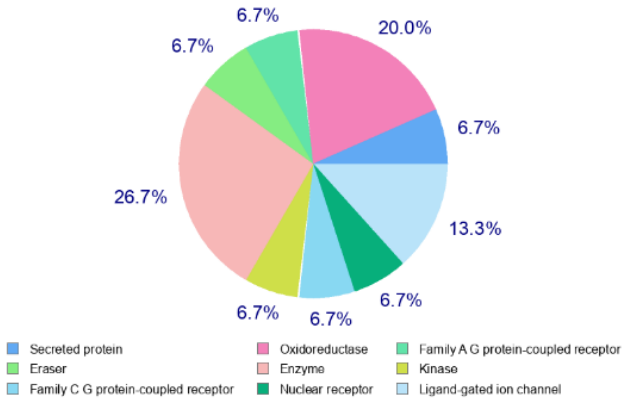
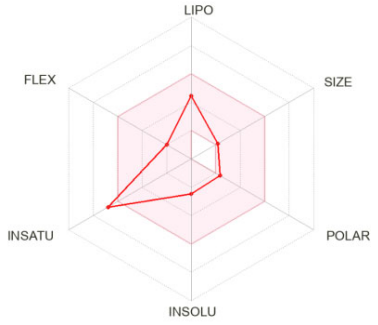


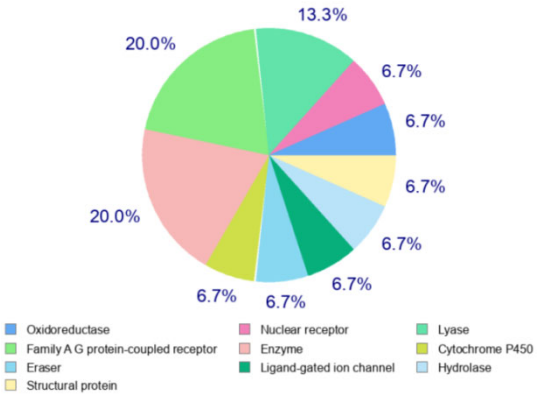
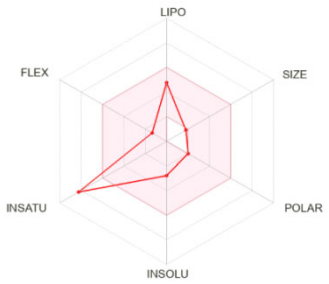
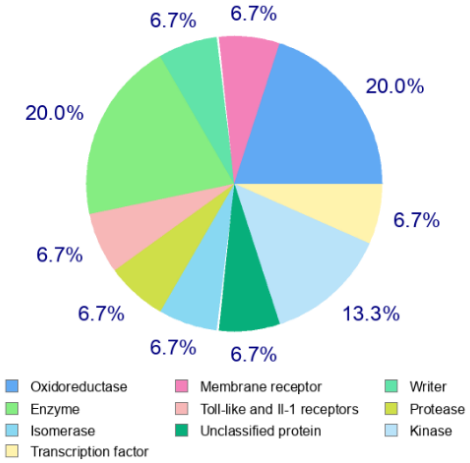
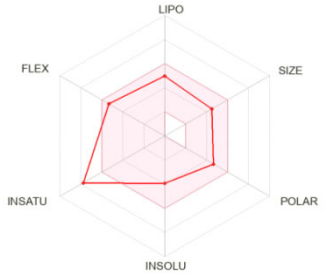
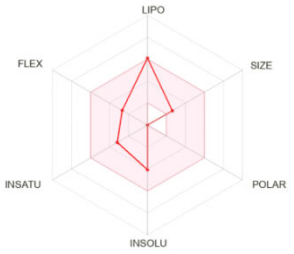
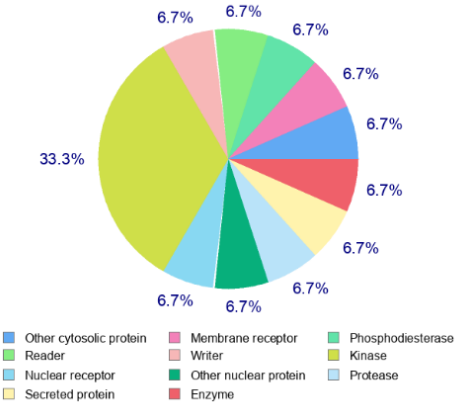
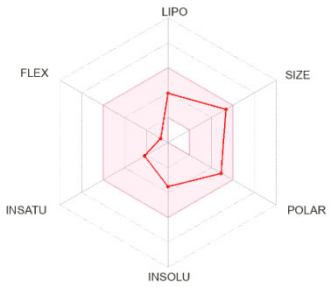
Fig. 3 — Comparative binding affinity of selected ligands against PDB ID: 6Y6D

Table 6 — Target prediction and ADME of selected compounds

Compound name	Target prediction	ADME
Azadirachtin		
Beta-sitosterol		
Chavibetol		

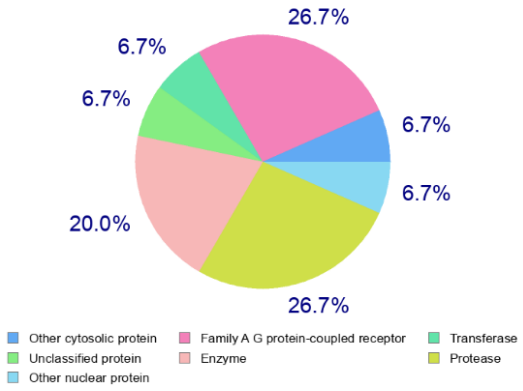
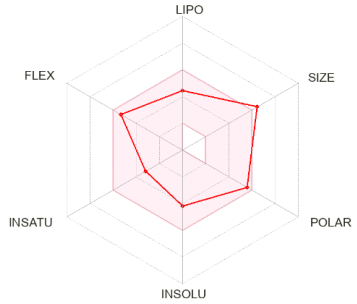
(*contd.*)

Table 6 — Target prediction and ADME of selected compounds (*contd.*)

Compound name	Target prediction	ADME
Chavicol		
Curcumin		
Zingiberene	-	
Nimbidinin		

(*contd.*)

Table 6 — Target prediction and ADME of selected compounds (*contd.*)

Compound name	Target prediction	ADME
Nimbin		

Nimbidinin and betasitosterol demonstrate the immune and respiratory systems.

Conclusion

From network pharmacology and target prediction, it was found that several anticancer genes are common in all the constituents. Additionally, NR3C1 is a common gene found in azadirachtin and betasitosterol, which is a glucocorticoid receptor. STAT3, EGFR are common genes found in curcumin and nimbidinin, which are signal transducer and activator of transcription 3 receptor and epidermal growth factor receptor, respectively. HSP90AA1, which is the heat shock protein HSP 90-alpha receptor common gene found in Nimbidinin and nimbin. While PARP1 is a poly [ADP-ribose] polymerase 1 receptor common gene observed in nimbin and chavibetol. Considering network pharmacology analysis, all the constituents were further screened for their anticancer potential *via in silico* molecular docking studies.

Strong binding affinities were found by molecular docking, particularly for azadirachtin, nimbidinin, and nimbin, suggesting potential anticancer action. Significant interactions involving various binding affinities between protein 6Y6D and the chosen bioactive substances were found in the investigation. The maximum binding affinity was shown by azadirachtin (-13.5 kcal/mol), which was followed by nimbin (-11.8 kcal/mol) and nimbidinin (-12.3 kcal/mol). Significantly, the protein's chain C exhibited significant binding to betasitosterol, zingiberene, and chavibetol, suggesting a possible binding site. According to these results, the bioactive compounds that have been chosen, in particular azadirachtin, nimbidinin, and nimbin, may be useful as lead compounds for further research into developing anticancer medicines that target protein 6Y6D.

Further experimental validation and optimization are necessary to explore their therapeutic potential. Pharmacokinetic evaluation showed diverse absorption, metabolism, and transport characteristics, highlighting potential drug-herb interactions and the importance of considering transporter-mediated efflux. The compounds exhibited varying toxicity profiles, with Azadirachtin and Curcumin showing the lowest toxicity. Overall, this study provides a holistic understanding of the selected bioactive compounds, underscoring their potential for drug development and highlighting the need for further investigation into their therapeutic applications and safety profiles. The findings of this study can inform the development of novel anticancer agents and highlight the importance of considering pharmacokinetic properties in the development of herbal-based therapies.

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Conflict of interests

All authors declare no conflict of interest.

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