In silico screening for β-Catenin inhibitors in colorectal cancer

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ABSTRACT Colorectal cancer (CRC) remains a major global health burden with high mortality in advanced stages, highlighting the urgent need for more effective and safer therapies. Aberrant β-catenin stabilization and nuclear accumulation promote oncogenic transcriptional programs and remains an attractive yet challenging therapeutic target. Here, an *in silico* screen of 25 naturally derived compounds was performed against β-catenin (PDB: 1JDH) using AutoDock Vina 1.2.5. Ligands and receptors were prepared in PyMOL 3.0 and AutoDockTools 1.5.7. Blind docking was conducted using a whole-protein search space centered at x = −2.857, y = 9.859, z = 40.811 with a box size of approximately 107 × 59 × 121 Å in triplicate runs. Binding poses and interaction patterns were visualized in PyMOL 3.0 and BIOVIA Discovery Studio 2024 (3D and 2D interaction maps). Nine compounds achieved predicted binding affinities (ΔG_bind) of ≤ −7.0 kcal/mol, led by silibinin (−9.9 kcal/mol), followed by quercetin (-7.8 kcal/mol), luteolin (-7.7 kcal/mol), ellagic acid (-7.5 kcal/mol), garcinol (-7.5 kcal/mol), betulinic acid (-7.4 kcal/mol), ursolic acid (-7.4 kcal/mol), derricin (-7.0 kcal/mol) , and epigallocatechingallate (EGCG) (-7.0 kcal/mol). Silibinin showed a consistent predicted pose with multiple hydrogen-bond and pi-alkyl hydrophobic contacts within a putative pocket. Drug-likeness analysis using Lipinski's Rule of Five indicated that most of the top hit ligands complied with criteria for molecular weight, hydrogen bond donors/acceptors, and lipophilicity, suggesting favorable oral bioavailability, while EGCG exceeded the recommended limits for hydrogen bond donors and acceptors, and garcinol surpassed the molecular weight and cLogP thresholds. Additionally, ADMET predictions highlighted potential concerns for quercetin due to a predicted mutagenic/tumorigenic risk. Overall, by applying a curated ligand set under a single standardized docking–ADMET workflow, this study reports novel screening outputs, including docking scores, predicted binding poses, and residue-level interaction profiles, together with an ADMET-informed prioritization. Based on these *in silico* results, silibinin emerged as the leading scaffold for prioritized experimental validation.

KEYWORDS: Colorectal cancer; Wnt/β-catenin signaling; Molecular docking; *In silico* screening; silibinin; ADMET
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INTRODUCTION

Colorectal cancer (CRC) ranks as the third most prevalent cancer worldwide and is the second leading cause of cancer-related mortality (World Health Organization, 2023). In Malaysia, CRC is the second most common cancer, with a higher prevalence among men (Malaysia National Cancer Registry, 2019). Common clinical manifestations include alterations in bowel habits such as persistent diarrhea or constipation, unexplained weight loss and abdominal pain (Skalitzky *et al*., 2023). Iron-deficiency anemia is also a recognized presentation in CRC and may contribute to fatigue (Chardalias *et al*., 2023). The majority of CRC-associated mortality arises from late-stage diseases, with approximately 22% of patients diagnosed after metastasis has already occurred. Outcomes for stage IV CRC remain unfavorable, as reflected by a 5-year survival rate of about 14%, in contrast to survival rates of roughly 71% for regional disease and 90% for localized tumors, despite ongoing improvements in therapeutic strategies (Ilyas, 2023).

Current standard treatments encompass surgical resection for localized disease, frequently combined with adjuvant or neoadjuvant chemotherapy and/or radiotherapy, while advanced or metastatic cases may also benefit from targeted biological agents and immunotherapy (Fadlallah *et al*., 2024). Early detection and intervention improve CRC outcomes, but survival in advanced or metastatic cases remains poor, highlighting the need for new treatments (Xie *et al*., 2020). Resistance

to chemotherapy, limited efficacy of targeted drugs, tumor heterogeneity, and adverse side effects hinder disease control and patient quality of life, underscoring the need for safer, more effective therapies (Ashique *et al*., 2024; Xie *et al*., 2020).

However, the discovery and development of new therapeutic agents is an inherently lengthy and costly process, often requiring years of iterative chemical synthesis, biological testing, and clinical evaluation before a viable drug reaches the market (Wouters *et al*., 2020). Traditional high-throughput screening of large compound libraries demands substantial financial investment, extensive laboratory infrastructure, and significant human resources (Flak, 2009). In recent years, *in silico* approaches, such as molecular docking, virtual screening, and computational modelling of ligand–protein interactions have emerged as powerful tools to accelerate the identification of promising drug candidates (Pinzi & Rastelli, 2019). By enabling the rapid screening of vast chemical databases against validated molecular targets, these computational methods can efficiently prioritize compounds with favourable binding affinities and pharmacokinetic properties for subsequent *in vitro* and *in vivo* validation. This integration of computational drug discovery into early research stages offers the potential to reduce both the time and cost associated with traditional screening pipelines while improving the precision of therapeutic development.

Among the molecular pathways implicated in CRC pathogenesis, the Wnt signalling cascade has attracted considerable attention as a promising therapeutic target (Li *et al*., 2024). Aberrant activation of this pathway, often due to mutations in the APC gene or β-catenin itself, drives uncontrolled cell proliferation, evasion of apoptosis, and tumour progression (Yu *et al*., 2019). β-catenin functions as a central transcriptional co-activator within this signalling axis, and its accumulation in the nucleus is a hallmark of CRC cells with dysregulated Wnt activity. Targeting β-catenin directly, or disrupting its interactions with key transcriptional partners, offers a strategic approach to halt downstream oncogenic gene expression. Nevertheless, β-catenin has historically been considered a "difficult-to-drug" protein due to the absence of deep binding pockets, necessitating innovative design strategies and computational screening methods to identify small molecules or biologics capable of effectively modulating its activity (Oladeji *et al*., 2025). Accordingly, this study evaluates a newly curated panel of 25 natural compounds *in silico* using a standardized docking and ADMET pipeline, producing a comparable residue-level interaction profile and a prioritized shortlist of candidate β-catenin inhibitors for experimental follow-up.

METHODOLOGY

Ligand Selection and Preparation

A total of twenty-five naturally derived compounds with reported anticancer activity were retrieved from the PubChem database in 3D conformers SDF format. The selected ligands include quercetin, silibinin, luteolin, ellagic acid, garcinol, betulinic acid, ursolic acid, derricin, epigallocatechingallate (EGCG), apigenin, curcumin, naringenin, berberine, aristolochic acid a, genistein, resveratrol, cannabidiol, lycopene, capsaicin, gingerol, thymoquinone, indole-3-carbinol, eucalyptol, butyrate and sulforaphane. The structures were converted to PDB file using PyMOL 3.0 by Schrödinger and subsequently prepared in AutoDock Tools 1.5.7. Ligands were optimized by adding Gasteiger charges, merging non-polar hydrogens, identifying aromatic carbons, and defining rotatable bonds before conversion to PDBQT format.

Protein Structure Preparation and Docking Simulations

The 3D crystal structure of β-catenin (PDB ID: 1JDH) was obtained from the Protein Data Bank. Co-crystallized ligands and water molecules were removed using PyMOL 3.0, and the structure was refined by adding Kollman charges and polar hydrogens. The prepared receptor was saved in PDBQT format for docking studies. Molecular docking was performed using AutoDock Vina 1.2.5 (Trott & Olson, 2010). Blind docking was performed using a grid box encompassing the entire protein structure to account for potential binding sites. The docking search space was defined in AutoDockTools 1.5.7 by generating a grid box that encompassed the target binding surface. The grid box was centered at x = −2.857, y = 9.859, z = 40.811 with a spacing of 1.000 Å. The number of grid points was set to 108 × 60 × 122 along the x-, y-, and z-axes, respectively, corresponding to a search volume of approximately 107 × 59 × 121 Å. The grid definition (grid.txt) was used to populate the Vina configuration file (config.txt) containing the receptor, ligand, grid center, and box size parameters. Docking runs were performed using AutoDock Vina 1.2.5 via command prompt. The docking used the default exhaustiveness (8). Each protein–ligand docking was performed in triplicate, and the most negative binding affinity (ΔG_bind, kcal/mol) among replicate runs was reported as the representative docking score for each ligand.

Visualization of Interactions

Docked complexes were visualized in PyMOL 3.0 by loading the docking output together with the β-catenin structure (PDBQT format) and rendering the protein in surface representation to highlight putative binding pockets. Each protein–ligand complex was exported in PDB format and further analyzed in BIOVIA Discovery Studio 2024 to characterize binding interactions, identify participating amino acid residues, and measure protein–ligand bond distances (Å).

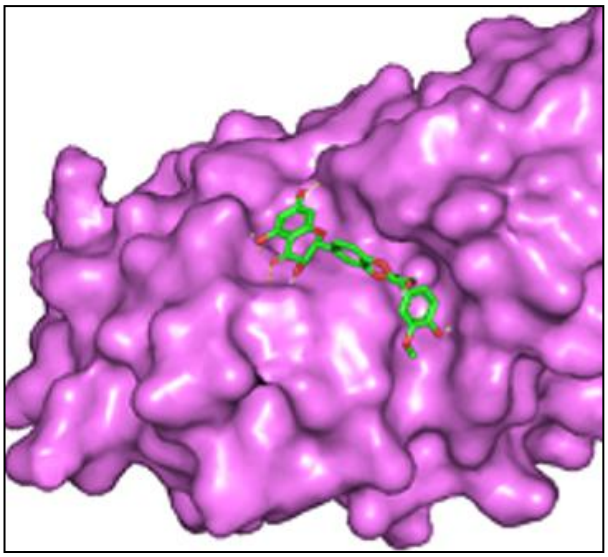
Drug-Likeness and ADMET Prediction

Drug-likeness was evaluated according to Lipinski's Rule of Five. For each compound, canonical SMILES were retrieved from PubChem (via the compound CID) and submitted to SwissADME (Daina *et al.*, 2017) for drug-likeness assessment. Pharmacokinetic properties (ADME) were predicted using admetSAR (Cheng *et al.*, 2012) based on the same PubChem-derived SMILES. Toxicity profiles were subsequently assessed using Osiris Property Explorer.

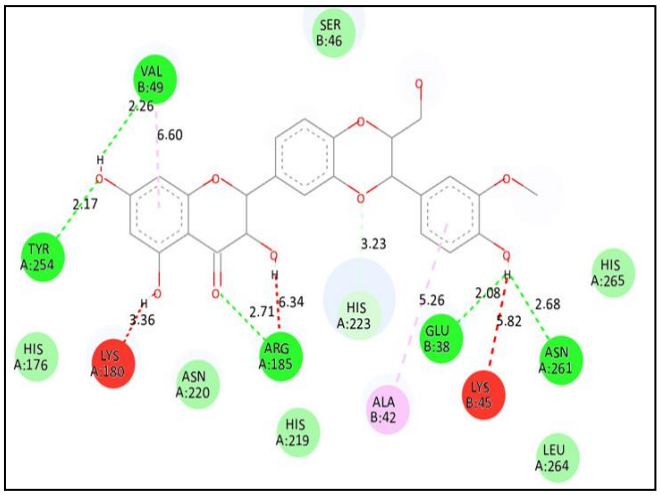
RESULT AND DISCUSSION

This study identified potential natural compounds predicted to interact with β-catenin, a key protein in CRC progression. Molecular docking of 25 ligands showed that silibinin had the most favourable predicted binding affinity (–9.9 kcal/mol), followed by quercetin (-7.8 kcal/mol), luteolin (-7.7 kcal/mol), ellagic acid (-7.5 kcal/mol), garcinol (-7.5 kcal/mol), betulinic acid (-7.4 kcal/mol), ursolic acid (-7.4 kcal/mol), derricin (-7.0 kcal/mol), and EGCG (-7.0 kcal/mol), all with docking scores equal to or below –7.0 kcal/mol. Silibinin exhibited a slightly more favorable predicted binding energy toward β-catenin (−9.9 kcal/mol) compared with values reported across a number of recent β-catenin molecular docking studies (Egbuna *et al.*, 2023; Oladeji *et al.*, 2025; Wang *et al.*, 2025). Silibinin formed four predicted hydrogen bonds with β-catenin (notably at residues Val49[B], Glu38[B], Asn261[A], Arg185[A]) and one predicted pi-alkyl hydrophobic interaction (Ala42[B]), supporting a plausible binding pose within the putative pocket (Figure 1). Hydrogen bonds are generally considered to facilitate protein–ligand binding and contribute to binding affinity (Chen *et al.*, 2016). Hydrogen bonds formed between neighboring segments of a folded polypeptide chain help stabilize the three-dimensional structure of proteins (Alberts *et al.*, 2002). Residues near the ligand contribute to interaction strength, with two predicted hydrogen bonds (Glu-38: 2.08 Å; Val-49: 2.26 Å) displaying donor–acceptor distances of <2.5 Å, indicative of strong hydrogen bonding

due to reduced separation and enhanced electrostatic interactions (Saunders *et al.*, 2019). Collectively, these interactions suggest that silibinin may engage the β-catenin's armadillo groove and could potentially influence β-catenin-cofactor interactions.



(a) Three-dimensional surface view of β-catenin (purple) with silibinin (green) nestled within a binding cavity, visualized in PyMOL 3.0.



(b) Two-dimensional schematic of the silibinin–β-catenin interactions, visualized in BIOVIA Discovery Studio 2024. Conventional hydrogen bonds are shown as bright green dashed lines; van der Waals in medium green; carbon-hydrogen bonds in pale green, pi-alkyl interaction in pink; and unfavourable donor-donor interactions in red (distances in Å).

Figure 1. Figure showing the binding poses of silibinin.

Drug-likeness analysis using Lipinski's Rule of Five indicated that most top-ranked ligands complied with the criteria for molecular weight, hydrogen bond donors/acceptors, and lipophilicity, suggesting favorable oral bioavailability. However, EGCG exceeded the recommended limits for hydrogen bond donors and acceptors, while garcinol violated the molecular weight and cLogP criteria. *In silico* ADMET predictions suggested generally favorable intestinal absorption, permeability, and lack of inherent toxicity for several top-ranked compounds, including silibinin, ellagic acid, betulinic acid, ursolic acid, and EGCG while quercetin showed potential concerns (predicted mutagenic/ tumorigenic risk). These *in silico* ADMET predictions, together with the docking results, support the drug-like potential of the top-ranked compounds. Among them, silibinin showed the most favorable predicted binding affinity and a consistent interaction profile, alongside acceptable drug-likeness and predicted ADMET characteristics, making it a strong candidate for further investigation as a β-catenin–targeting agent. While computational ADMET tools provide useful early guidance, their outputs remain model-based and may vary from *in vivo* outcomes. Accordingly, follow-up studies such as direct binding assays and cell-based Wnt/β-catenin functional assays, with subsequent *in vivo* evaluation as appropriate, are recommended to confirm target engagement and anticancer activity.

CONCLUSION

This *in silico* screening study identified several naturally derived compounds with predicted activity against the Wnt/β-catenin pathway in colorectal cancer (CRC). Among the evaluated ligands, silibinin exhibited the most favorable predicted binding affinity toward β-catenin and demonstrated overall desirable drug-like characteristics within the applied molecular docking and ADMET workflow. Lipinski-based drug-likeness analysis indicated that most compounds possessed generally acceptable oral drug-like properties, although EGCG and garcinol showed notable

deviations from the rule-of-five criteria, while ADMET screening highlighted a potential mutagenic and tumorigenic liability for quercetin. Collectively, these findings suggest that small-molecule natural products can plausibly engage β-catenin despite its challenging structural topology and offer a rational starting point for further investigation.

By assessing a newly curated panel of 25 natural compounds using a standardized docking and ADMET pipeline, this study provides residue-level insights into ligand–β-catenin interactions and an ADMET-guided prioritization of candidate inhibitors to support experimental follow-up. Silibinin, together with other top-ranked compounds, therefore, represents a promising lead for subsequent evaluation in CRC-relevant models. Future studies will focus on experimental validation, including biochemical assays to confirm β-catenin binding and cell-based assays to evaluate modulation of Wnt/β-catenin signaling, followed by in vivo investigations where appropriate. Overall, the integration of molecular docking with ADMET profiling establishes an efficient computational framework for prioritizing candidate CRC therapeutics targeting Wnt/β-catenin signaling and provides a practical foundation for downstream experimental validation and lead optimization.

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