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In-silico characterization of phytochemicals identified from *Vitex negundo* (L) extract as potential therapy for Wnt-signaling proteins

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Abstract

Background: Colorectal cancer is the third most diagnosed disease in the world population and current chemotherapy has been used for targeting the cell proliferation and metastasizing ability of tumor cells. Potent chemotherapeutic drugs for colorectal cancer are capecitabine, fluorouracil, irinotecan, etc. which have toxic effects in normal tissues and adverse effects in multiple organs leading to major obstacles in clinical use. The aim of the study is the use of plant-derived compounds that improve the effectiveness of chemotherapeutics with lower and alleviate toxic side effects and reduce the risk of tumor progression.

Results: The current study is performed using *Vitex negundo* leaf which has been demonstrated to have positive effects against colorectal cancer. The use of computational approaches will help improve the identification and screening of lead molecules using AutoDock 4.2 and AutoDock Vina. Using computational approaches will help to improve lead identification and screening. Herein, we have retrieved six phytochemicals from published literature and investigated their inhibitory effect with Wnt-associated signaling proteins. Authentication of phytochemicals and Wnt-associated signaling proteins was done using AutoDock4.2.

Conclusions: The results are screened based on the number of hydrogen bonds, binding energy, and interacting amino acids. The Isoorientin, luteolin, and Chrysophanol get the highest binding energy with target receptors. The binding energy is calculated with all target receptors from the range of -6.0 to -8.9 kcal/mol. The *In-silico* drug likeness properties are predicted to be the best interacting compounds based on Lipinski Rule of 5 and ADMET analysis. Hence, we propose that Isoorientin, luteolin, and Chrysophanol are the potential inhibitors of Wnt signaling inhibitors, and preclinical studies are needed to confirm the promising therapeutic ability of colorectal cancer.

Keywords: WNT, Colorectal cancer, Pharmacophore, Docking, Drug likeness

Background

Cancer is an uncontrolled growth and spread of abnormal cells in the body which eventually leads to the host's death. Over 1.7 million new cancer cases were diagnosed in 2019 [1]. 63% of cancer deaths were observed in low- and middle-income countries [2, 3]. Population growth and several risk factors can simultaneously act

to initiate and promote cancer growth eventually leading to death [4]. Colorectal cancer (CRC) is the third most observed disease that leads to mortality with 1.4 million new cases annually worldwide [5]. Several risk factors influence to cause of the disease based on lifestyle, diet content, environmental factors, lack of exercise, excess consumption of alcohol, diabetes, and other chronic observations in the intestinal region that may lead to cause disease [6–8]. There are several genes and proteins that directly influence the regulation of gene expression and cause disease. In several cancer types, there are gene transcriptional regulators that

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phosphorylate, co-phosphorylates, polymerizes signal transduction such as WNT signaling protein, Notch, TGF β -signalling cascades, Hedgehog, JAK-STAT, RAS-MAPK, PI3K-Akt, P53 signaling cascades initiates the progression of CRC [9–12].

The Wnt signaling pathway is an integral of both canonical and noncanonical signaling transduction pathways that regulate gene expression, cell fate differentiation, cell migration, embryogenesis, and tissue homeostasis [13–15]. The WNT pathway is involved in various cancer types such as colorectal cancer, breast, myeloma, and gastrointestinal tumors [16]. The WNT signaling pathway begins with membranal proteins that pass signals to the cell surface receptors which results in the fusion of integral proteins. Depending on the β -catenin signaling receptor the pathway is divided into two groups such as canonical or dependent of β -catenin receptor and non-canonical or independent of the β -catenin receptor [17]. In the absence of β -catenin receptor, the signaling cascade receptors the glycoprotein that bind to Frizzled receptors and LRP5/6 coreceptor complexes is ubiquitinated by ZNRF3 and RNF43 which induces the dual phosphorylation of LRP6 by CK1 and GSK3- β and this allows for the translocation of a protein complex containing Axin, APC, and GSK-3 β phosphorylates CK1 and β -catenin receptors which degrades the proteolytic activity with β -TrCP. But in the case of the β -catenin receptor is present in the pathway which activates LRP5/6 coreceptors that are bound with Frizzled protein and activates Dishevelled (Dvl) by sequential phosphorylation, and polymerization which displaces GSK-3 β from APC/Axin which traps endosome sequestration [18, 19]. The subsequent transcriptional factors displace the regulation of proteins that may lead to point mutation and cause human tumors, neurodegenerative disease, and diabetes [20].

Based on the observation of the disease progression and molecular treatment, there are a smaller number of chemotherapeutic drugs that can help prevent the disease. But still, there is more demand to identify the novel therapeutic drug molecules to control the disease. In the present study, we used phytochemicals chemical isolates from *V. negundo* (L) extract as potential chemotherapeutic properties for colorectal cancer. *V. negundo* is a large evergreen, climbing, much-branched shrub found throughout India [21]. The plant parts such as leaf, bark, and root are used as indigenous medicines for the treatment of eye disease, toothache, inflammation, leukoderma, enlargement of the spleen, skin ulcers, etc. [22]. The *V. negundo* (L) extract is a rich source of flavonoids, phenolic derivatives, tannins, and glycosides, that have anti-cancer, anti-inflammatory, antiseptic, astringent properties [23].

In this study, we selected WNT signaling proteins such as Axin, APC, β -catenin, GSK-3 β , and Dishevelled (DSH) proteins which have significant roles in signal transduction that leads to colorectal cancer. We also used phytochemical compounds identified from the *V. negundo* (L) extract to understand the better inhibitory effect with WNT signaling proteins as potential chemotherapeutic properties. We used computational drug discovery approaches to select the compounds and the target receptors based on the methodology and studied protein–ligand interaction using computational drug discovery software for preliminary screening of best chemotherapeutic compounds for colorectal cancer.

Methods

Study design

Briefly describing the Wnt signaling proteins such as Axin, APC, β -catenin, GSK-3 β , and Dishevelled (DSH) protein sequences are selected from the GenBank database and the sequence templates are identified using BLASTp. The best hits are selected and used homology modeling using the Swiss Model. The energy minimization is performed using SPDBV (Swiss PDB Viewer) and stereochemical activity of amino acid interactions are predicted using SAVES. The complexity of protein structure is predicted by Procheck and Ramachandran plot is predicted by RAMPAGE. The active site amino acids are predicted by the CastP calculation server to understand the ligand-binding site. *V. negundo* leaf sample phytochemicals are selected from literature and further used for drug-likeness prediction using Molinspiration. The pharmacokinetic analysis is applied to understand the biological activity of phytochemicals. Finally, Molecular docking is performed using AutoDock 4.2.6 and virtual screening is performed using AutoDock Vina. The best protein–ligand interaction molecules are visualized using BIOVIA Discovery Studio 2021 client.

Selection of phytochemicals from *V. negundo* (L) for inhibitory design

There are 6 phytochemicals are selected from *V. negundo* (L) extracts based on the inhibitory property against WNT signaling proteins [24]. The phytochemicals possessing anti-inflammatory, antiseptic, astringent properties were retrieved from an extensive literature survey for ligand preparation to act against WNT signaling proteins. The respective phytochemical structures were retrieved from the PubChem-NCBI database by downloading structure data format (SDF) and then converted into protein databank (PDB) format using Pymol for further analysis. The chemical structures of Silibinin, capecitabine, fluorouracil, irinotecan, oxaliplatin were processes similarly as controls.

Drug-likeness prediction of phytochemicals

Drug likeness properties of the phytochemicals are examined using molinspiration (<https://www.molinspiration.com/cgi-bin/properties>). It offers a broad range of chemoinformatic properties of chemicals based on “Lipinski Rule of 5”. The properties of poor absorption or permeation are more likely when H-bond donors > 5 (expressed as the sum of all OH and NH functional groups), H-bond acceptors > 10 (expressed as the sum of all N and O groups), molecular weight should be in the range from 130 to 725 Da, $\log P > 5$ (or $\text{MiLogP} > 4.15$), Substrates for biological transporters are exceptions to the rule. Veber’s rule suggests good oral bioavailability of compounds based on properties of rotatable bonds ≤ 10 , $\text{TPSA} \leq 140 \text{ \AA}^2$ number of rotatable bonds ≤ 12 . Although “violation” of one rule may not result in poor absorption [25].

ADMET properties

ADME and toxicity of the phytochemicals are predicted using admetSAR online server (<http://lmmd.ecust.edu.cn/admetSar2/>). The ADMET properties are considering the parameters of atoms based on human intestinal absorption (HIA), Human oral bioavailability, Caco-2 permeability, plasma protein binding, blood–brain barrier penetration (BBB), acute toxicity, carcinogenicity, LD_{50} , and Mutagenicity is considered the best active molecules and screen experimentally using molecular docking [5].

Identification of protein targets and homology model construction

The WNT signaling proteins such as APC, Axin, β -catenin, GSK-3 β , and Dishevelled (DSH) sequences were retrieved by running protein BLAST (tool of NCBI) submitted in NCBI. The WNT signaling proteins are query sequences submitted to the SWISS-MODEL server (<https://swissmodel.expasy.org/templates/>) to search for evolutionary-related protein structures using BLAST and HHblits method. The top-ranked templates are alignments are compared based on global model quality estimate (GMQE) and quaternary structure quality estimate (QSQE) to provide the descriptive sets of three-dimensional structures, sequence similarity, and quaternary protein structure. The 3D protein structures with modeling errors and quality estimations are predicted based on QMEAN values. The model protein structures are used to estimate the stereochemical quality of protein structures by SAVES v6.0 server (<https://saves.mbi.ucla.edu/>) and Ramachandran plot of Ψ versus Φ conformational angles of 3D macromolecule measures the torsion angles of $\text{C}\alpha$ (ideal) -N-C β (obs). Active site amino acids were predicted using the CastP calculation server ([\[sts.bioe.uic.edu/castp/calculation.html\]\(http://sts.bioe.uic.edu/castp/calculation.html\)\) based on the delineating measure surface area and surface volume of 3D protein structure \[26\].](http://</p>
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Preparation of modeled proteins and ligands for docking

The homology modeled WNT signaling proteins are used for docking. Before docking, gasteiger charges and polar hydrogens were added to the macromolecules and non-polar and polar hydrogen atoms were merged by adding partial charge using AutoDock 4.2.6. and AutoDock Vina 4.2. For phytochemical structures along with the conventional drugs for comparison, rotatable bonds were determined and non-polar hydrogens were merged with polar hydrogens atoms. Rigid and flexible macromolecules are generated and grid maps are generated by adjusting the grid dimension to $40 \times 40 \times 40$ points and spacing was adjusted to 0.8 $\text{\AA}$ to enable ligand binding. For each grid, amino acid energy is calculated for the entire binding site with equilibrated energy distribution. The configuration file is generated in a text file for running in AutoDock Vina to evaluate the binding affinity of ligand and target proteins. The docking energy of all ligand molecules and WNT signaling proteins were evaluated by using empirical free energy functions and Lamarckian genetic algorithm to predict the best docking conformations of protein–ligand interactions by evaluating the AutoDock energy calculation [27].

Results

Selection of phytochemical models for WNT signaling inhibitor design

Usually, the phytochemicals have one or more medicinal properties, before docking with target receptors, there is a need for evaluating the phytochemicals to qualify the drug-likeness test, i.e., Lipinski rule of 5 for various descriptors. The independent descriptors were determined for each of the 5 phytochemicals using molinspiration and represented in (Table 1). The different descriptors for each phytochemical along with 4 reference drugs were manually compared with standard values and finally, drug-likeness scores were computed in (Table 2). Luteolin has the highest drug score of 0.84 and 5-fluorouracil has the lowest score of 0.06. Ligands have zero violations and these phytochemicals are represented in (Fig. 1), and further screened using docking.

ADMET properties of the phytochemicals

The drug likeness properties such as Lipinski’s rule of 5 includes molecular weight ($\text{MW} < 500 \text{ Da}$), calculated lipophilicity (ALogP), Polar surface area (PSA), number of hydrogen bond acceptors (HBA), and number of hydrogen bond donors (HBD) were used to assess the “drug-like” property of compounds. In silico methods

Table 1 Pharmacophore properties of selected compounds based on Lipinski Rule of 5 and Veber's Rule

Chemicals	miLogP	TPSA	N atoms	MW	H-acceptor	H-donor	Nrotb	Vol
Casticin	2.4	103.6	27	374.35	8	2	5	318.21
Isoorientin	− 0.42	197.3	32	448.38	11	8	3	363.22
Luteolin	1.99	107.2	21	286.24	6	4	1	232.07
4-Hydroxybenzoic acid	0.8	57.53	10	138.12	3	2	1	119.06
Chrysofanol	2.69	74.6	19	254.24	4	2	0	215.17
Silibinin	2.13	155.1	35	482.44	10	5	4	400.86
Capecitabine	0.47	120.6	25	359.35	9	3	7	311.36
5-fluorouracil	− 0.84	58.2	9	130.08	4	2	0	96.91
Irinotecan	3.56	112.5	43	586.69	10	1	5	530.67

miLogP (Octanol–water dissolution) < 5, Topological polar surface area (TPSA) 30–130, Number of Atoms (Natoms) < 80, molecular weight (MW) > 180–500 kDa, hydrogen bond acceptor (nON) > 1–10; hydrogen bond donor (nOHNH) > 1–5, Number of Rotatable Bonds (Nrotb) > 1–12; Volume (Vol) > 180–500

Table 2 Drug likeness score of different phytochemicals predicted based on Lipinski Rule

Ligands	Solubility	Drug likeness	Drug Score
Casticin	− 3.26	1.45	0.26
Isoorientin	− 1.97	− 0.71	0.54
Luteolin	− 2.56	1.9	0.84
4-Hydroxybenzoic acid	− 1.33	− 1.5	0.35
Chrysophanol	− 4.48	− 1.67	0.16
Silibinin	− 3.41	1.64	0.64
Capecitabine	− 3.37	− 21.3	0.16
5-Fluorouracil	− 1.76	− 4.5	0.06
Irinotecan	− 4.5	0.07	0.35

predicted by ADMET properties helps to analyses the novel chemical entities to predict the lead candidates that would be metabolized and cross membranes to active or inactive cellular functions. ALogP is to predict the dissolution of compounds is the water/octanol method that can easily predict the permeability in the cell membrane. TPSA helps to calculate the distribution of fragments from polar residues O and N that can admire the permeability in various cellular membranes include blood–brain barrier distribution, Caco2 permeability, and human intestinal absorption. Plasma protein binding suggests that the significant plasma protein bound to have highly hydrophilic in tissue component to have the best therapeutic index. LD₅₀ predicts the acute toxicity of chemicals to predict the dose amount of a tested molecule to kill 50% of the treated animals within a given period (Table 3).

Identification of protein structure and homology modeling

The homology model construction of WNT signaling proteins was done using the Swiss Modeller server. The proteins sequences with specific templates were

identified using BLAST and HHblits tools, 3D structures are predicted for comparative analysis and represented in Table 4 and the model 3D structures are presented in (Fig. 2). Ramachandran plot is predicted based on Ψ versus Φ conformations based on the angel rotation and the default protein structures are used for active site identification to understand the protein–ligand interactions. Active sites of each protein structure are predicted by the CastP calculation server with the PyMol plugin to measure the molecular surface and molecular volume of the pocket regions where the ligand-binding sites are recognized, the cavity of active sites, and the list of amino acids are mentioned in (Table 5).

Molecular docking study using AutoDock Vina 4.2

AutoDock 4.2 Vina is used to optimizing the protein structure by adding polar hydrogens and gasteiger charges to the atomic charge groups. The ligand modifications are performed through the rotatable bond assignment and calculating energy contributions that can resolve the ligand interaction to protein. The grid maps are generated and facilitate the docking by creating new docking scores with ligand molecules by interacting with proteins. In the present study, we have determined the WNT signaling proteins with target phytochemicals and reference compounds with their binding affinities were evaluated in (Table 6).

The docking interaction of APC protein with phytochemicals is observed based on the binding energy and hydrogen bond formation. The result shows, the Isoorientin has one conventional hydrogen bond with Cys82, 3 Pi donor hydrogen bonds with Asn42, Ser81, and Tyr85, and pi is formed within the active site amino acids and anion interaction with Asp46 amino acid with the interaction energy of − 8.4 kcal/mol, compared with standard drug Silibinin has 4 conventional hydrogen bonds with Arg54, Arg330, Val374, and Asn375 amino acids 1

Pi donor with Phe329 amino acid with the interaction energy of -8.2 kcal/mol. We also compared Luteolin and Chrysophanol have 3 hydrogen bonds within active site amino acids compared to the standard drugs with the interaction energy of -8.0 and -8.7 kcal/mol. Capecitabine also has 3 hydrogen bonds within active site amino acids with the interaction energy of -7.8 kcal/mol and the overall results show the compounds Isoorientin, Luteolin, Chrysophanol have very good potential drug molecules to the APC protein compared with standard chemical structures (Fig. 3).

Similarly, we also performed docking analysis of AXIN protein with phytochemicals and the results show Isoorientin has 1 conventional hydrogen bond with Glu458, 1 Pi anion bonds with Asp459 with the binding energy of -6.9 kcal/mol. Other compounds such as Chrysophanol have 3 conventional hydrogen bonds with Asn426, Gly422, and Asp459 amino acids and interaction energy of -6.3 kcal/mol. Silibinin has 5 hydrogen bonds with Tyr254, Ser471, and Gln478 amino acids and 1 carbon-hydrogen bond with Glu475 amino acids with interaction energy of -8.0 kcal/mol. Other compounds such as Castenin, Luteolin, and Irinotecan has found 3 hydrogen

bonds and interaction energy of -8.4 kcal/mol. Based on the results, Isoorientin, Chrysophanol, Casticin, and Luteolin have potential inhibitors against AXIN protein (Fig. 4).

Another WNT signaling protein such as β -catenin is docked with phytochemicals and reference drug structures. The results show that phytochemicals such as Luteolin have 2 conventional hydrogen bonds with Gln203 and Lys233 amino acids, Pi-cation with Lys242 amino acid with the interaction energy of -7.5 kcal/mol. The Chrysophanol has one conventional hydrogen bond with Phe232 and Pi-cation with Lys242 amino acid. There are sigma and alkyl groups also present in the ligand interactions with the binding energy of -7.1 kcal/mol. The reference compound includes Capecitabine has 5 conventional hydrogen bonds with Ser196, Arg200, Gln203, and Asn206 amino acids and fluorine halogen interaction with Ser234 amino acid. There are van der Waals interactions and alkyl interactions also observed with the ligand interactions with the binding energy of -6.6 kcal/mol. Based on the observation of phytochemicals interactions Luteolin and Chrysophanol have potential inhibitors with target β -catenin protein (Fig. 5).

Table 3 Estimation of ADMET analysis for phytochemicals selected from *V. negundo* (L)

Properties	Casticin	Isoorientin	Luteolin	P-Hydroxybenzoic acid	Chrysophanol	Silibinin	Capecitabine	5-Fluorouracil	Irinotecan
BBB	1.49973	1.49973	1.49973	1.49973	1.49973	1.49973	1.49973	1.49973	1.49973
Buffer solubility (mg/L)	5803.83	5803.83	5803.83	5803.83	5803.83	5803.83	5803.83	5803.83	5803.83
Caco2	22.2815	22.2815	22.2815	22.2815	22.2815	22.2815	22.2815	22.2815	22.2815
HIA	100	100	100	100	100	100	100	100	100
MDCK	204.401	204.401	204.401	204.401	204.401	204.401	204.401	204.401	204.401
Pgp inhibition	Non	Non	Non	Non	Non	Non	Non	Non	Non
Plasma Protein Binding	− 1.0	− 1.0	− 1.0	− 1.0	− 1.0	− 1.0	− 1.0	− 1.0	− 1.0
Pure water solubility (mg/L)	3921.34	3921.34	3921.34	3921.34	3921.34	3921.34	3921.34	3921.34	3921.34
Skin Permeability	− 1.86648	− 1.8665	− 1.8665	− 1.8665	− 1.8665	− 1.8665	− 1.86648	− 1.86648	− 1.86648
SKlogD value	0.96516	0.96516	0.96516	0.96516	0.96516	0.96516	0.96516	0.96516	0.96516
Algae_at	0.0514568	0.05146	0.05146	0.05146	0.05146	0.05146	0.051457	0.051457	0.051457
Ames test	Mutagen	Mutagen	Mutagen	Mutagen	Mutagen	Mutagen	Mutagen	Mutagen	Mutagen
Carcino Mouse	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
hERG inhibition	Medium risk	Medium risk	Medium risk	Medium risk	Medium risk	Medium risk	Medium risk	Medium risk	Medium risk

BBB, blood–brain barrier; HIA, human intestinal absorption; Pgp, phosphoglycoprotein; hERG, human ether-a-go-go related gene

Table 4 WNT signalling proteins and template structures predicted using Swiss Model

Target protein	Length	Template ID	% of identity	Sequence coverage	Total score
APC	2843	5L9W_A	100	1–684	1429
		3NMZ_A	100	303–739	932
		3T7U_A	99.4	405–775	791
Axin	305	2QZ5_A	99.35	151–305	318
		2QZQ_A	87.50	153–304	281
		1UG7_A	100	1–122	238
β-catenin	781	3SL9_A	100	1–167	342
		3SLA_A	100	1–167	342
		1JDH_A	100	3–171	337
DSH	736	5LNP_A	100	1–97	200
		2REY_A	96.97	260–358	205
		4WIP_A	97.94	11–107	200
GSK3β	460	1GNG_A	100	1–378	765
		4PTC_A	100	1–441	874
		4IQ6_A	100	1–426	874

Dishevelled (Dvl) is another target receptor in the WNT signaling pathway. The Dishevelled protein is docked with a selected chemical structure and the interactions are observed based on binding energy and amino

acid interactions. The Isoorientin has 4 conventional hydrogen bonds formed with Arg442, Tyr502 amino acids, Pi-cation interaction with Arg442, and 3 stacked noncovalent interactions between aromatic rings with

Table 5 Active site amino acids of WNT signalling proteins

Protein	Area (SA)	Volume (SV)	Active site amino acids
APC	5143.368	5419.876	Ser410, Ser202, His123, Asp65, Asp126, His248, Cys82, Asp500, Asn501, Thr365
AXIN	339.096	284.884	Asn102, Ala106, Glu107,
β-catenin	6846.710	18,020.249	Asp299, Leu264, Gln302, Arg274, Asn290,
GSK-3β	3206.606	4409.052	Val214, Arg108, Lys205, Arg96, Arg209,
DSH	3798.556	6489.172	Asn33, Tyr153, Asp71, Asp72, Asn28, His66. Asn67, His78

Ares (SA), surface area; Volume (SA), SURFACE VOLUME

Trp444 amino acid and the overall binding energy is -8.4 kcal/mol. The Luteolin compound also has 4 conventional hydrogen bonds and 2 Pi-cation interactions were observed with Glu438, Arg440, Arg442, and Trp444 amino acids respectively. There is Pi-donor hydrogen bond interaction also present with Trp502 amino acid with the overall interaction energy of -8.4 kcal/mol. Chryophanol has 2 conventional hydrogen bond interactions with Arg442, and Tyr502 amino acids, Pi-Cation interaction with Arg440, and Pi-sigma interaction with Leu454 amino acid, and non-covalent interaction with ring structure of Trp444 amino acids with overall interaction of -8.4 kcal/mol. The reference compounds such as Capecitabine, 5-fluorouracil, and Irinotecan also have strong interaction with ligand binding site amino acids Arg442, Trp444, and His490, Thr491, and Val492 amino acids and Arg440, Gln500 amino acids with an

overall binding energy of -8.2 , -5.2 and -11.2 kcal/mol respectively. Silibinin has 4 conventional hydrogen bonds with Met430, Ala432, Glu438, and His465 amino acids along with one sigma interaction with His464 and alky interactions with Leu437 and Val439 amino acids. The binding energy is represented with -9.1 kcal/mol (Fig. 6). The overall results show the Isoorientin, Luteolin, Chryophanol have strong interaction with Dishevelled protein compared to reference drug structures. GSK-3β protein structure is docked with phytochemicals and the interaction energy is observed based on the number of hydrogen bonds and binding energy. The compound Luteolin has 5 hydrogen bonds in the 3D complex structure, it has 2 unfavorable acceptor-acceptor interactions with Asp133, Pi-Anion with Asp200, Alky interactions with Ala83, and Cys199 amino acids,

Table 6 Binding affinities of WNT signaling proteins with shortlisted phytochemicals

Ligands	APC		Axin		β-catenin		DSH		GSK3β	
	H-bonds	Binding energy	H-bonds	Binding energy	H-bonds	Binding energy	H-bonds	Binding energy	H-bonds	Binding energy
Casticin	2	−8.4	3	−6.9	0	−6.7	7	−7.3	1	−7.3
Isoorientin	5	−8.4	5	−6.9	1	−6.8	7	−8.4	2	−7.5
Luteolin	3	−8.0	3	−6.1	2	−7.5	7	−8.4	5	−6.8
4-hydroxy benzoic acid	2	−6.5	3	−4.2	0	−4.7	7	−5.9	1	−4.7
Chryophanol	3	−8.7	5	−6.3	2	−7.1	7	−8.4	1	−7.8
Silibinin	4	−8.2	4	−8.0	2	−8.8	7	−9.1	4	−9.1
Capecitabine	3	−7.8	2	−5.9	4	−6.6	6	−8.2	3	−7.1
Fluorouracil	0	−4.5	2	−4.7	1	−4.2	6	−5.2	2	−4.5
Irinotecan	1	−11.9	3	−8.4	2	−9.5	6	−11.2	3	−9.4

the overall interaction energy show -6.8 kcal/mol. The 4-hydroxy benzoic acid has 3 conventional hydrogen bonds with Pro325, Ser236, and Arg328 amino acids and unfavorable donor-donor sites in HIs173 amino acids show significant interaction with energy of -4.7 kcal/mol compared to reference compounds.

Silibinin has 4 conventional hydrogen bonds with Arg54, Arg330, and Arg517 amino acids. 3 Alkyl interaction with Val53, Ile221, and Leu576 amino acids and the overall interaction energy of -9.1 kcal/mol. The overall results conclude that the compound Luteolin and 4-hydroxy benzoic acid have a significant effect on GSK-3 β protein (Fig. 7).

Fig. 4 Binding affinities of AXIN protein with phytochemical structure using AutoDock Vina. The 2D structure of protein–ligand interactions is visualized using a Discovery studio visualizer and the interactions are predicted based on binding energy (kcal/mol) and hydrogen bonds

Discussion

Colorectal cancer is associated with WNT signaling pathways, several proteins help transcriptional regulation with β -catenin and GSK-3 β complexed along with AXIN and APC proteins degrade the proteolytic activity leads to protein phosphorylation. There are several chemotherapeutic drugs such as Silibinin, a flavonolignan isolated from milk thistle that is potentially used phytochemical drug for the chemotherapeutic effect to treat colorectal

cancer. Other compounds such as Capecitabine, 5-fluorouracil, and Irinotecan are also used for the treatment of colorectal cancer and the most common side effects include inflammation, loss of appetite, abdominal pain, vomiting, diarrhea, low blood cell counts, and hair loss. To reduce the toxic adverse side effect in chemotherapy treatment, there is a demand to identify the novel compounds which has less or no toxic effect and accuracy for the treatment of colorectal cancer.

Several researchers mentioned that *V. negundo* leaf extract has a critical role in apoptosis inhibition and results in valuable clinical applications in cancer therapy as a novel anticancer agent [28]. The methanolic extract of the *V. negundo* leaf sample has a strong inhibitory effect with HCT116 cell lines but still a further need to understand the phytochemicals which have a good

inhibitory effect with crude extracts. Arora et al., 2011 have performed GC–MS analysis of *V. negundo* leaf extract by methanol solvent and have identified several phytochemicals such as Castenin, Isoorientin, Luteolin, 4- hydroxybenzoic acid, and Chryophanol compounds [29]. These compounds have potential anticancer properties, but there is no in-silico prediction of the compound

activity with the WNT signaling proteins. In the present study, the phytochemicals identified from *V. negundo* leaf extract were screened as potential inhibitors against WNT signaling proteins. Here, we performed computational pharmacophore and drug likeness screening analysis to understand the effective functioning of phytochemicals in a biological system. We also performed molecular docking and virtual screening of the compounds with strong potential inhibitors against WNT signaling proteins in colorectal cancer.

In the current study, APC, AXIN, β -catenin, Dishevelled and GSK-3 β proteins were selected based on their pathogenesis and used for molecular docking. The protein 3D structures are predicted using homology modeling methods and the 3D conformations of stereochemical interaction of amino acids and Ramachandran plot are predicted to understand the complexity of protein structure. Active site amino acids are also predicted to understand the ligand-binding amino acids with phytochemical and reference compounds. The

overall results of target proteins, phytochemical interaction, binding energy, and number hydrogen bond formation are shown in Table 6. We have compared the phytochemicals interaction with reference compounds such as Silibinin, Capecitabine, 5-Fluorouracil, and Irinotecan. The overall results show the phytochemicals

such as Isoorientin, Luteolin and Chryophanol have a strong inhibitory effect with all WNT signaling proteins by forming 2–5 conventional hydrogen bonds and 2–3 Pi-alkyl interactions with target receptors compared with standard reference compounds and potential chemotherapeutic treatment for colorectal cancer.

Conclusion

The concept for chemotherapeutic targets in colorectal cancer is WNT signaling proteins which have become an attractive candidate to study drug design, although the structural and functional activity of the WNT signaling mechanism is well known and still a lot of work to be done to identify the protein 3D structures and potential drug inhibitors. Homology modeling is performed to construct 3D protein structures and validates the stereochemical activity of amino acid interaction in the complex structure, active sites are conserved and used for ligand interactions. Some of the chemotherapeutic drugs such as Silibinin, Capecitabine, 5-Fluorouracil, and Irinotecan are potential drugs for colorectal cancer, but there is a high risk of side effects in the several dosage levels. In the present study, we used *V. negundo* leaf extract that has flavonoid compounds such as Casticin, Isoorientin, Luteolin, 4-hydroxybenzoic acid, and Chryophanol are identified from extensive literature. We performed pharmacophore, pharmacokinetic, and molecular docking studies to confirm a tight binding of phytochemicals to 3D protein active site amino acids. The results show Isoorientin, Luteolin, and Chryophanol have tight interaction with active site amino acids. Further, these compounds can isolate individually and used invitro cytotoxicity study against colorectal cancer cell lines to predict the best toxic effect with no side effects with phytochemicals and used as a potential chemotherapeutic treatment for colorectal cancer.

Abbreviations

ADMET: Absorption, Distribution, Metabolism, Excretion, Toxicity; CRC: Colorectal cancer; SDF: Structure data format; PDB: Protein Data Bank; logP: Partial coefficient of Octanol and water solubility; TPSA: Total polar surface area; HIA: Human Intestinal absorption; BBB: Blood–brain barrier; NCBI: National center for biotechnology information.

Authors' contributions

VV have designed the experimental work, KG executed all the experiments. KG, VV and PCN are carried out all the data analysis. KG written the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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