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IN SILICO ANALYSIS ON PHYTOESTROGENS FROM DRIED FRUITS AS BETA-CATENIN INHIBITORS IN LIVER CANCER

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ABSTRACT: Hepatocellular Carcinoma (HCC), the primary liver cancer, is the third leading cause of most cancer related mortality worldwide. The canonical Wnt/β-catenin signaling pathway is found deregulated in most HCC and makes an attractive therapeutic target. β-catenin is the key effector of Wnt signaling pathway. In the present study, an attempt was made to evaluate the binding mode of some phytoestrogens found in dried fruits such as raisin and dates, to β-catenin in the wnt signaling pathway. The structural coordinates for the compounds used as ligands were retrieved from the PubChem chemical structure database. Based on Lipinski rule of five for druglikeness, the compounds were filtered and used as ligands in the docking analysis using CDocker. Results show that Secoisolariciresinol formed an interaction with β catenin with a least binding energy value of -35.1622Kcal/mol. The study provides a molecular insight into the binding mode of some phytoestrogens found in dried fruits that can be a potential drug candidate to β-catenin while targeting the Wnt signaling for HCC therapy.

KEYWORDS: Hepatocellular Carcinoma, Wnt signaling, β-catenin, Phytoestrogens, Lignans, CDocker, ADMET

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1. INTRODUCTION

Malignant Hepatoma or Hepatocellular Carcinoma is the sixth most common type of cancer occurring in the liver cells and is the third leading cause of cancer related mortality world-wide [1]. The risk factors include the Hepatitis C viral infection, alcoholic cirrhosis and there is a higher prevalence of HCC in patients with chronic liver diseases [2]. Mostly, HCC is diagnosed at an advanced stage which limits the efficacy of most potential curative therapeutic options. Due to the resistance to conventional chemotherapy and a very poor prognosis of the disease, there is a need for developing more effective therapeutic strategies [3]. Understanding the underlying cellular and molecular mechanism involved in the HCC can help in developing more specific and targeted therapies [4]. Several major cell signaling pathways such as the Wnt/b-catenin, HGF/c-MET, PI3K/AKT/mTOR, Ras/Raf/MAPK, IGF, VEGF and PDGF pathways are found to be deregulated in HCC [5]. Among these signaling pathways, the wnt/β-catenin signaling pathway plays a major role in the hepatic carcinogenesis [6]. The β-catenin is involved in the various stages of cell development and in maintaining the homeostasis of the cell in adults [7]. Mostly β-catenin is found in the cell membrane bound to E-cadherin in the absence of Wnt proteins [8]. The concentration of β-catenin in the cytoplasm is kept low through phosphorylation by kinases glycogen synthase kinase-3β (GSK-3β) and casein kinase I (CK1) found in the enzymatic complex that include the translated tumor suppressor genes for Adenomatous Polyposis Coli (APC) and Axins. They facilitate the ubiquitination of the phosphorylated β-catenin resulting in its degradation through proteolysis [9]. The wnt signaling is activated when the secreted growth factors of the wnt family binds to the frizzled receptors at the cell surface which in turn activates the dishevelled protein. This facilitates the dissociation of the cytoplasmic destructive complex and inhibition of GSK-3β which results in the accumulation and stabilization of cytosolic β-catenin. The β-catenin then enters the nucleus and binds to the TCF/LEF proteins. In the absence of β-catenin, the TCF/LEF proteins are bound to groucho co-repressors along with their cognate DNA recognition elements that ensure the transcriptional silencing of β-catenin target genes that includes Cyclin D1, c-myc and Survivin [10]. Activation of wnt/β-catenin signaling leads to binding of β-catenin to TCF/LEF proteins that result in the subsequent dissociation of groucho co-repressors,and activation of β-catenintarget genes including Cyclin D1, c-myc and Survivin, all of which promote cell cycle progression and inhibit apoptosis [11, 12]. Mutation in the β-catenin gene appears to be the most frequent genetic event in many cancers including human HCC and Colorectal cancer. The inhibition of β-catenin signaling observed in the HCC cell lines shown to have anti-tumoral effects thus making the β-catenin as an attractive molecular target for cancer therapies. Mostly the chemical agents that targets Wnt/β-catenin pathway areat the membrane, cytosol

and transcription factor levels. Currently, there are no small molecule inhibitors that target the wnt/β-catenin is under clinical trials [13]. Although, the small molecular agent FH535 which is a dual inhibitor of peroxisomeproliferator-activated receptor (PPAR) and β-catenin/TCF/LEF, has shown to inhibit proliferation of HCC and its specificity on inhibition of β-catenin/TCF/LEF activity was shown in hepatoblastoma cellline HepG2 [14]. The aim of our current was to identify some phytoestrogens found in dried fruits such as dates and raisins as small molecular inihibitors of the β-catenin via *in silico* molecular docking methods. Recently there is an increasing interest in studying the phytochemicals as anti-cancer agents [15-18]. Dietary phytoestrogens present in dried fruits have attracted much interest due to their potential protective effects against various disease conditions such as cancer, cardiovascular disease (CVD), osteoporosis, and menopausal symptoms. They comprise three major classes: isoflavones, lignans, and coumestan. Some dried fruits, such as apricots, currants, dates, prunes, and raisins, contain phytoestrogens [e.g., isoflavones (formononetin, genistein and glycitein), lignans (e.g., matairesinol, lariresinol, pinoresinol, and secoisolariciresinol)]. Various studies on the phytoestrogens present in dried fruits have shown lower risk of diabetes, cancer and CVD in individual consuming them [19,20]. In order to identify these phytochemicals as inhibitor of beta-catenin the current study was initiated.

2. MATERIALS AND METHODS

2.1 ADME-T Properties

The structural coordinates for the compounds identified through literature survey were retrieved from PubChem chemical database (pubchem.ncbi.nlm.nih.gov). The pharmacokinetic properties such as Absorption, Distribution, Metabolism, Excretion and Toxicity of the compounds were predicted using the ADMET predictor under the Calculate Molecules Properties tool in Accelrys Discovery Studio (DS) v4.1.

2.2 Ligand preparation

The prepare ligand protocol of DS is used for standardizing charges for common groups, adding hydrogens, enumerating ionization states, ionizing functional groups, to generate tautomers and isomers, remove duplicates and to fix bad valencies in the 3D structural coordinates of the compounds. For formononetin, three ligands were generated based on the 2 ionization state and 1 stereoisomer and for glycitein, 3 ioinization state and 1 stereoisomer were generated making four ligands. For the lignans matairesinol, lariciresinol, pinoresinol and secoisolariciresinol, only one ligand state was generated for each based on their ionization and the stereoisomeric form. The generated isomers were used as ligands in a receptor-ligand interaction analysis.

2.3 Protein preparation

The X-ray crystallographic structure of the protein β-catenin used as receptor was retrieved from the Protein Data Bank (PDB) (www.rcsb.org/pdb) with the pdb id: 1JPW. The prepare protein protocol in DS is used for protein preparation. The parameters in the protocol include steps for standardizing atom names, to insert missing atoms in residues and to remove alternate conformations, also to remove water and ligand molecules, to insert missing loop regions based on either SEQRES data or user specified loop definitions, for optimizing short and medium size loop regions with the LOOPER algorithm, to minimize the remaining loop regions and to calculate the pK and protonate the structure.

2.4 Active site prediction

The active site regions in target proteins were predicted based on the receptor cavities on their surface using the binding site definition tool in DS. For β-catenin, 9 sites were generated based on the cavities. Site 1 was chosen for the current docking analysis as it was covered in the groove region where TCF4 binds and activates the target genes responsible for cell proliferation in carcinogenesis [21].

2.5 Molecular Docking

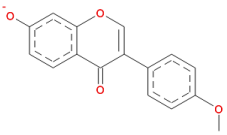
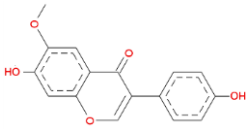
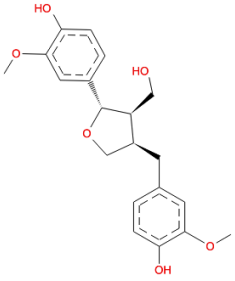
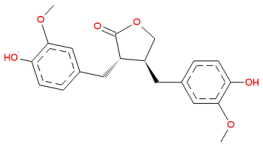
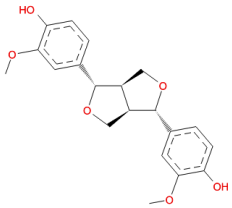
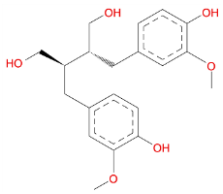
The molecular docking analysis of the compounds against the protein β-catenin was carried out using CDocker module of Accelrys Discovery Studio v4.1. CDocker is a grid based molecular docking algorithm that employs CHARMmforcefield for carrying out molecular dynamics. Initially random ligand conformations are generated from ligand structure through high temperature molecular dynamics, followed by random rotations. The random conformations are later refined by Grid-based (GRID1) simulated annealing and a final grid-based or full forcefield minimization. The using of forcefield for the docking in this algorithm enables more reliability of the final docking results.

3. RESULTS AND DISCUSSION

Dietary phytoestrogens in dried fruits play valuable roles in bone health, diabetes, breast cancer, and in other metabolic disorder. Lignans from dates act through various mechanisms for modulating the pancreatic insulin secretion. They also act through estrogen receptor-mediated mechanisms. Hence, it may be presumed that high amounts of phytoesterogens contained in dried fruits may potentially help to maintain normal glucose and lipid metabolism in both healthy populations as well as in obese/diabetic patients. Various scientific evidence suggest that individuals who consume dried fruits regularly have a lower risk of CVD, obesity, certain types of cancer, type II diabetes, metabolic syndrome, inflammatory bowel disease, and osteoporosis as well as other NCDs [22-24]. β-catenin encoded by the gene CTNNB1 plays a central role in the wnt signalling pathway. The cytoplasmic level of β-catenin is controlled by the destructive complex comprising the APC/GSK3/Axin1 and other protein. Upon activation by wnt signaling, the β-catenin enters nucleus and binds to TCF4 which

leads to the activation of genes responsible for cell growth and proliferation. So this makes β-catenin an attractive target in cancer therapy. In our present study, we investigated the potential of the isoflavones and lignans found as phytoestrogens in dried fruits as inhibitor of β-catenin via *in silico* molecular docking method. The structural coordinates of the compounds identified were retrieved from PubChem chemical database and the calculated molecular properties along with their chemical structures are shown in table 1.

Table 1 – Molecular properties of the phytoestrogens found in dried fruits.

Compounds name	Chemical Structure	Mol.Wt	Hbond donors	Hbond Acceptors	Rotatable Bonds
Formononetin		284.391	2	4	2
Glycitein		300.391	3	5	2
Lariciresinol		372.496	3	6	6
Matairesinol		372.496	3	6	6
Pinoresinol		370.48	2	6	4
Secoisolariciresinol		374.512	4	6	9

The *in silico* pharmacokinetics prediction was carried out for the compounds using the ADMET predictors in Small molecule tool of DS. The function uses six mathematical models based on the available drug information. These models include algorithms for predicting the Human Intestinal Absorption (HIA), Aqueous Solubility, Blood-Brain Barrier (BBB), CYP2D6, Hepatotoxicity and Plasma Protein Binding (PPB) for the compounds based on their chemical structure. Optimizing these properties during early drug discovery is crucial for reducing ADMET problems later in the development process [25,26]. The result is represented as a biplot containing a 2D chart of ADMET_PSA_2D versus ADMET_ALogP98 and two sets of ellipses for prediction confidence space (95% and 99%) for the Blood Brain Barrier (BBB) penetration and Human Intestinal Absorption (HIA) models (Fig.2).

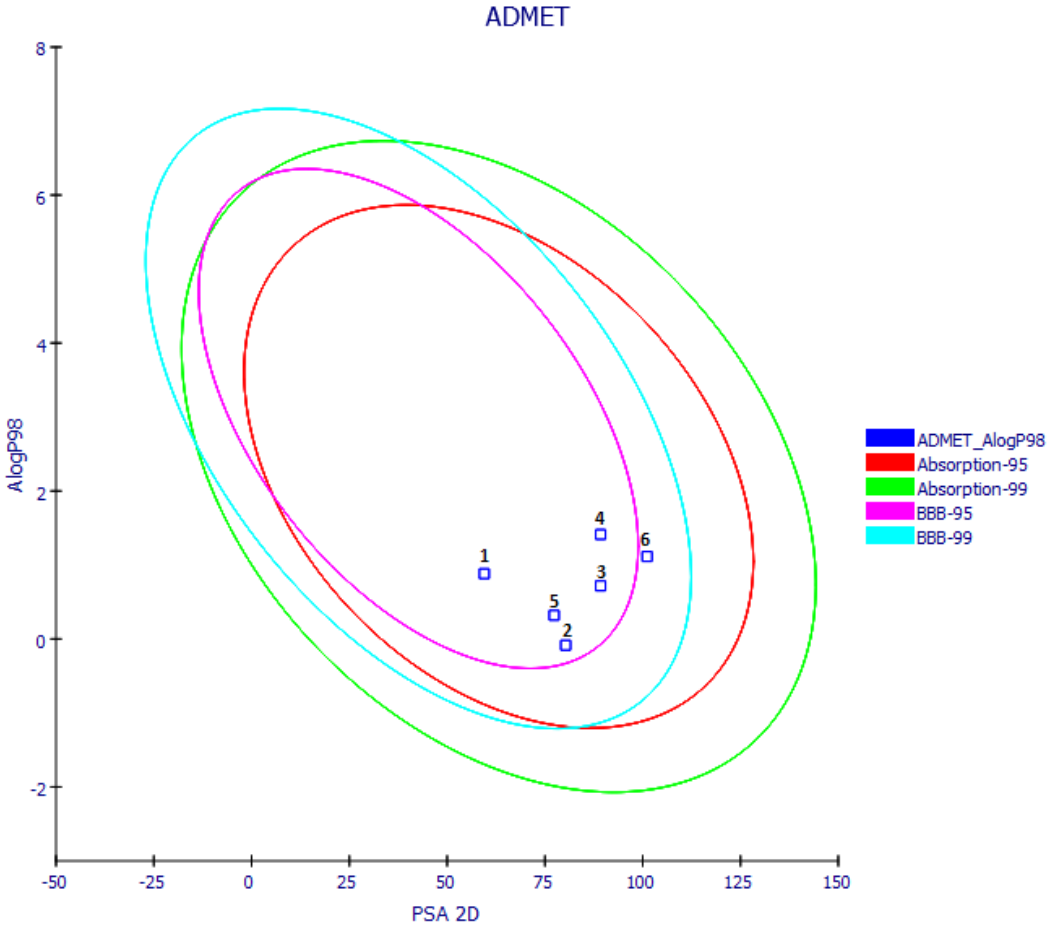


Fig. 1- Biplot of ADMET prediction for the phytoestrogens used in the study. The point plot of predicted polar surface area versus the AlogP98 (Log of the octanol-water partition coefficient using Ghose and Crippen's method) for the compounds 1.Formononetin, 2.Glycitein, 3. Lariciresinol, 4. Matairesinol, 5.Pinoresinol and 6. Secoisolariciresinol.

The ADMET prediction for the phytoestrogens shows that five of them were within the 95% confidence level of BBB and HIA whereas only one secoisolariciresinol was within the 99% confidence level of BBB and none of them were outside the 99% ellipse of BBB and HIA. So these compounds naturally passed the ADMET test and were used in the further docking analysis using CDocker. CDocker uses molecular dynamics-based prediction for ligand-receptor interaction. Using the simulated annealing molecular dynamics method the conformational search of the ligands was carried out. The ligands were heated to a temperature of 700K in 2000 steps and then cooled to 300K in 5000 steps. The pose cluster radius was set to 0.5Å and ten random conformations were generated from equilibration and minimization of the ligand structure. CDocker uses the CHARMM forcefield and combining the molecular dynamics with docking provides better accuracy than other methods [28-30]. The active site in the groove region of the β-catenin where TCF4 binds was chosen for the study and is shown in Fig.2.

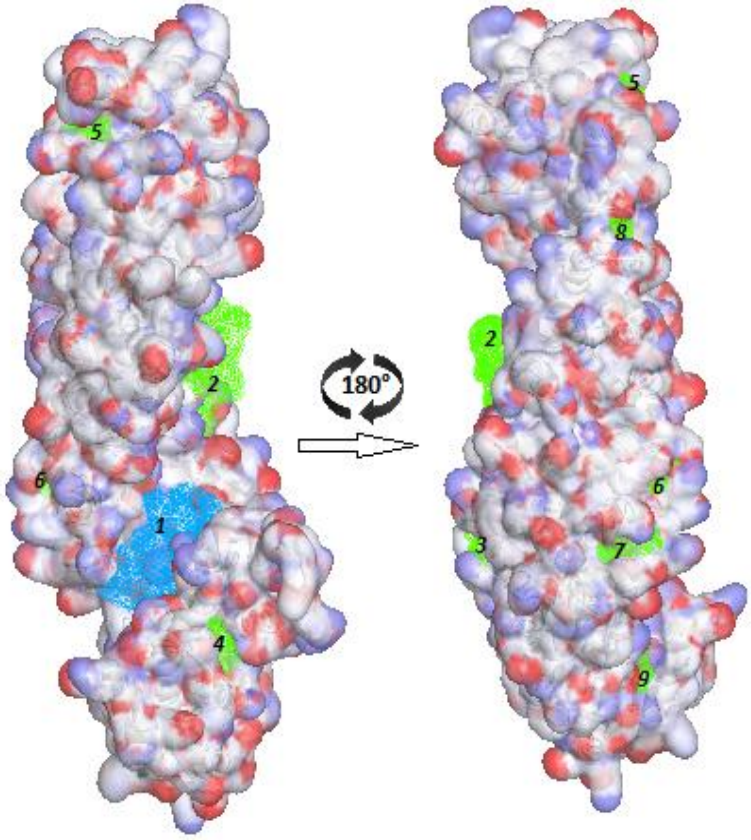


Fig. 2 – Active site predicted based on the receptor cavities in the surface of β-catenin. Site 1 shown in blue was used for the study.

Results show that the compound Secoisolariciresinol formed an interaction in the active site region of β-catenin with least binding energy value. The scoring of the docking is obtained as CDocker energy and CDocker interaction energy, shown in Table 2.

Table 2 – CDocker results obtained for the compounds against Beta catenin

Compounds	CDocker Energy Kcal/mol	CDocker Interaction Energy Kcal/mol
Formononetin	-32.7743	-39.946
Glycitein	-17.8859	-26.4948
Lariciresinol	-8.30293	-34.7446
Matairesinol	-30.787	-41.0508
Pinoresinol	1.49761	-34.199
Secoisolariciresinol	**-35.1622**	**-53.111**

The analysis of the receptor-ligand interaction shows that the compound Secoisolariciresinol formed 3 conventional hydrogen bonds with the residues ARG469 and ARG515 shown in fig.3.

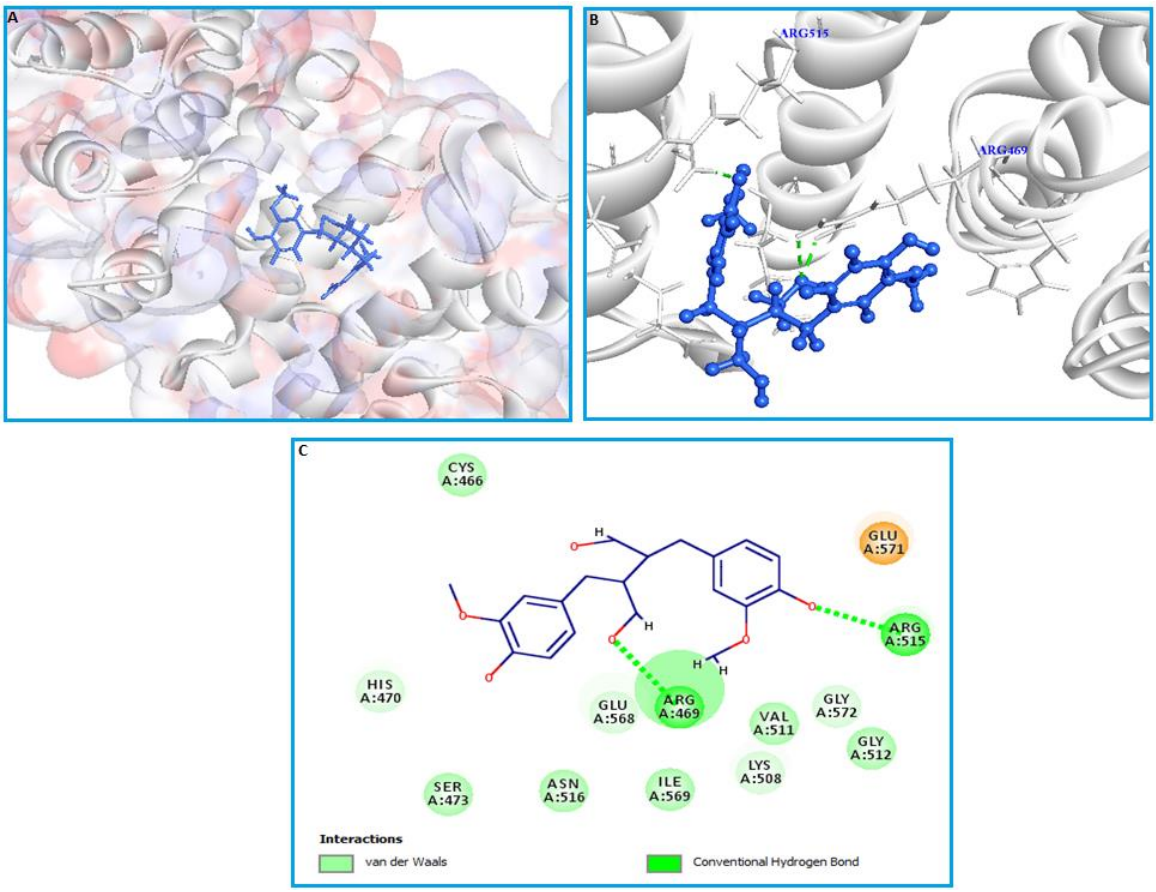


Fig. 3 – Receptor-Ligand interaction between Secoisolariciresinol and β-catenin. A –Secoisolariciresinol (Stick-blue model) docked into the groove of β-catenin (Ribbon with soft atomic charge surface model) where the active site is found. B – three dimensional representation of the ligand-receptor interaction between Secoisolariciresinol (Ball and Stick –Blue model) and β-catenin (Ribbon –white model with the interacting residues in stick). C- 2D interaction representation of Secoisolariciresinol in the active site of β-catenin.

Table 3 – Non-Bond Interaction (HBond) Obtained for the compounds against Beta-Catenin

Interacting Atoms (HDonor – HAcceptor)	Distance Å
Formononetin	
A:ASN609:HD22 - Formononetin:O2	2.87
Glycitein	
Glycitein:H32 - A:SER473:OG	2.39
Glycitein:H33 - A:GLU568:OE1	2.17
Lariciresinol	
A:HIS470:HE2 - Lariciresinol:O5	2.00
Matairesinol	
A:ARG469:HH12 - Matairesinol:O2	2.06
A:ARG469:HH22 - Matairesinol:O2	1.92
Pinoresinol	
A:HIS470:HE2 - Pinoresinol:O6	2.05
A:LYS508:HZ3 - Pinoresinol:O2	2.59
Secoisolariciresinol	
A:ARG469:HH12 - Secoisolariciresinol:O2	2.06
A:ARG469:HH22 - Secoisolariciresinol:O2	2.66
A:ARG515:HH11 - Secoisolariciresinol:O5	2.02

Identifying the non-bond interaction such as hydrophobic counts (pi-pi stacking, alkyl, pi-sigma), hydrogen bond (conventional, non classical) and electrostatic interaction (lone pair cation, anion) plays a crucial role in determining the binding affinity of a drug to its receptor molecule [27]. The non-bond interactions obtained for the compounds against β-catenin is shown in Table.3 The interacting receptor residues are mostly identified with hotspots for known inhibitors of β-catenin such as Quercetin and BC21 [31,32]. Study shows that the accuracy of the CDocker also depends on the total number of rotatable bond. For compounds having the total number of rotatable bonds less than 8, the predictions were more accurate [28]. For all the compounds except secoisolariciresinal used as ligands in the molecular docking analysis the number of rotatable bonds was below 8 and for secoisolariciresinol it is 9 which is close to 8 (refer table 1). The compounds studied all shown good interaction energy. There is enough evidence that these phytoestrogens can be inhibitors of β-catenin from the docking analysis.

4. CONCLUSION

The current study was initiated in order to identify the phytoestrogens found in dried fruits as potential β-catenin inhibitors for targeting the Wnt signaling pathway in Hepatocellular Carcinoma. B-catenin plays a key mediator in the Wnt Signaling pathway and has been gaining much interest as potential target for HCC therapy. Binding of Wnt to the FZD receptors activates the Wnt signaling pathways resulting in the disassociation of the β-catenin destructive complex increasing the cytosolic concentration of β-catenin which then enters the nucleus and activates transcriptor factors responsible for cell growth and proliferation resulting in tumorigenesis. *In silico* molecular docking analysis helps in lead optimization in the drug discovery. From the present docking analysis, we conclude that the phytoestrogens used as ligands can be considered as potential drug candidate while targeting β-catenin in the Wnt signaling.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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REFERENCES

1. El-Serag HB, Rudolph KL. Hepatocellular Carcinoma: Epidemiology and Molecular Carcinogenesis, Gastroentero. 2007;132:2557–76.
2. Kanwal F, Hoang T, Kramer JR, Asch SM, Goetz MB, Zeringue A, et al. Increasing prevalence of HCC and cirrhosis in patients with chronic hepatitis C virus infection. Gastroenterol. 2011;140:1182–8.
3. Faloppi L, Scartozzi M, Maccaroni E, Di Pietro Paolo M, Berardi R, Del Prete M, et al. Evolving strategies for the treatment of hepatocellular carcinoma: from clinical-guided to molecularly-tailored therapeutic options. Cancer Treat Rev. 2011;37:169–77.
4. Villanueva A, Llovet JM. Targeted therapies for hepatocellular carcinoma. Gastroenterol. 2011;140:1410–26.
5. Whittaker S, Marais R, Zhu A. The role of signaling pathways in the development and treatment of hepatocellular carcinoma. Oncogene. 2010;29:4989–5005.
6. Dahmani R, Just P-A, Perret C. The Wnt/β-catenin pathway as a therapeutic target in human hepatocellular carcinoma. Clin Res Hepatol Gastroenterol. 2011;35:709–13.

7. Berg T, Delanghe S, Al Alam D, Utley S, Estrada J, Wang KS. β-Catenin regulates mesenchymal progenitor cell differentiation during hepatogenesis. J Surg Res. 2010;164:276–85.
8. Pokutta S, Choi H-J, Ahlsen G, Hansen SD, Weis WI. Structural and Thermodynamic Characterization of Cadherin-β-catenin-α-catenin Complex Formation. J Biol Chem. 2014;289:13589–601.
9. Xing Y, Clements WK, Kimelman D, Xu W. Crystal structure of a beta-catenin/axin complex suggests a mechanism for the beta-catenin destruction complex. Genes Dev. 2003;17(22):2753–64.
10. MacDonald BT, Tamai K, He X. Wnt/β-Catenin Signaling: Components, Mechanisms, and Diseases. Developmental Cell. 2009;17:9–26.
11. Daniels DL, Weis WI. Beta-catenin directly displaces Groucho/TLE repressors from Tcf/Lef in Wnt-mediated transcription activation. Nat Struct Mol Biol. 2005;12:364–71.
12. Liao DJ, Thakur A, Wu J, Biliran H, Sarkar FH. Perspectives on c-Myc, Cyclin D1, and their interaction in cancer formation, progression, and response to chemotherapy. Crit Rev Oncog. 2007;13:93–158.
13. Yao H, Ashihara E, Maekawa T. Targeting the Wnt/β-catenin signaling pathway in human cancers. Expert Opin Ther Targets. 2011;15:873–87.
14. Liu J, Li G, Liu D, Liu J. FH535 inhibits the proliferation of HepG2 cells via downregulation of the Wnt/β-catenin signaling pathway. Mol Med Rep. 2014;9:1289–92.
15. Surh Y-J. Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer. 2003;3:768–80.
16. Øverby A, Zhao CM, Chen D. Plant phytochemicals: Potential anticancer agents against gastric cancer. Curr Opin Pharmacol. 2014;19:6–10.
17. Turan N, Kennedy JF. Phytochemicals as Bioactive Agents. Carbohydr Polym. 2002;47:394.
18. Lee KW, Bode AM, Dong Z. Molecular targets of phytochemicals for cancer prevention. Nat Rev Cancer. 2011;11:211–8.
19. Awad AB, Fink CS. Phytosterols as Anticancer Dietary Components: Evidence and Mechanism of Action. J Nutr. 2000;130:2127–30.

20. Jones PJH, AbuMweis SS. Phytosterols as functional food ingredients: linkages to cardiovascular disease and cancer. Curr Opin Clin Nutr Metab Care. 2009;12:147–51.
21. Poy F, Lepourcelet M, Shivdasani R a, Eck MJ. Structure of a human Tcf4-beta-catenin complex. Nat Struct Biol. 2001;8:1053–7.
22. Patel MD, Thompson PD. Phytosterols and vascular disease. Atherosclerosis. 2006;186:12–9.
23. Ostlund RE. Phytosterols and cholesterol metabolism. Curr Opin Lipidol. 2004;15:37–41.
24. Ostlund RE. Phytosterols, cholesterol absorption and healthy diets. Lipids. 2007;42: 41–5.
25. Wang J, Urban L. The impact of early ADME profiling on drug discovery and development strategy. Drug Discov World. 2004;Fall:73–86.
26. Wang J, Skolnik S. Recent advances in physicochemical and ADMET profiling in drug discovery. Chemistry and Biodiversity. 2009;6:1887–99.
27. Shan Y, Kim ET, Eastwood MP, Dror RO, Seeliger MA, Shaw DE. How does a drug molecule find its target binding site? J Am Chem Soc. 2011;133:9181–3.
28. Bohari MH, Sastry GN. FDA approved drugs complexed to their targets: Evaluating pose prediction accuracy of docking protocols. J Mol Model. 2012;18:4263–74.
29. Gagnon JK, Law SM, Brooks CL. Flexible CDOCKER: Development and application of a pseudo-explicit structure-based docking method within CHARMM. J Comput Chem. 2016;37:753–62.
30. Wu G, Robertson DH, Brooks III CL, Vieth M. Detailed analysis of grid-based molecular docking: A case study of CDOCKER - A CHARMm based MD docking program. J Comput Chem. 2003;24:1549–62.
31. Amado NG, Fonseca BF, Cerqueira DM, Neto VM, Abreu JG. Flavonoids: Potential Wnt/beta-catenin signaling modulators in cancer. Life Sciences. 2011;89:545–54.
32. Tian W, Han X, Yan M, Xu Y, Duggineni S, Lin N, et al. Structure-based discovery of a novel inhibitor targeting the beta-catenin/Tcf4 interaction. Biochemistry. 2012;51:724–31.