Annals of Agri-Bio Research **29** (1): 1-7, 2024

Impact Score : 0.28 (Scopus)

Molecular Docking Studies of Triterpenoids from *Terminalia arjuna* Targeting VEGFR-2 Protein Involved in Cancers

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(Received: September 9, 2023; Accepted: October 27, 2023)

ABSTRACT

Vascular endothelial growth factor receptor-2 (VEGFR-2) plays a pivotal role in promoting the growth and progression of cancer cells, emphasizing its importance as therapeutic target as effectively addressing cancer. Triterpenoids from *Terminalia arjuna*, one of the popular medicinal plants in Indian subcontinent since ancient times, were screened for anticancer properties targeting VEGFR-2 protein. The 3-D structures and SMILES format of the six selected triterpenoids, and one standard drug (Dovitinib) were retrieved from PubChem, while the 3-D structure of the target protein (VEGFR-2) was downloaded from Protein Data Bank. Drug likeness assessments of the compounds based on ADME properties (absorption, distribution, metabolism and excretion), Lipinski's filters and radar plots for bioavailability were calculated by SwissADME. The selected four compounds were subjected to docking simulations with the target protein, to calculate the binding energies and to find the most stable interaction. The four ligand molecules viz., arjunic acid, arjungenin, terminic acid and arjunolic acid were found to significantly inhibit the target protein- VEGFR-2, with higher binding stabilities than the standard drug. It was concluded that these triterpenoids can be considered as promising candidates due to their low Gibbs free energy, safety, efficacy and selectivity as therapeutic agents for VEGFR-2 involved cancers, after experimental validation.

Key words: *Terminalia arjuna,* VEGFR-2, molecular docking, lipinski rule of five, anticancer drug

INTRODUCTION

Abnormal growth of cells due to multiple changes in the expression system of genes which disrupts the balance of cellular proliferation leads to cancer. It has been a persistent public health threat and the main cause of mortality all over the world (Choudhari *et al.,* 2020). By 2030, there could be an annual increment with 23.6 million new reported cases of cancer (Bray *et al.,* 2018). The global burden of mortality rates due to cancers underscores the pressing need for the scientific community and pharmaceutical industries to upgrade their methodologies for the identification of potential drug leads (Alam and Khan, 2018). Cancer cells possess unique abilities to survive characterized by uncontrolled replication, instability in genetic makeup, persistent formation of blood vessels, evasion of programmed cell death and independence from external growth signals (Abinaya *et al.,* 2021). Growth factor receptors (GFRs) are a group of proteins that are responsible for tumour growth and development and become active after binding with their corresponding ligands called growth factors. Many cancers involve over expressed receptors which lead to uncontrolled cell growth and proliferation (Mendie and Hemalatha, 2022). This over activity also triggers resistance development to cancer treatments. Vascular endothelial growth factor receptor (VEGFR) is often over expressed in many types of cancers such as colon, lung, brain, prostate, gastric, kidney, thyroid, ovarian and bladder cancers (Lutfiya *et al.,* 2019). Due to its important role in cancer progression, this receptor has become an important target for effective cancer treatment. At present, the treatment regime of cancer comprises the use of anticancer drugs, chemotherapy, radiotherapy, immunotherapy and surgery. Several limitations such as drug resistance,

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non-target cell effects, recurrence and many side effects and toxicities have been demonstrated by all of the above approaches (Choudhari *et al.,* 2020; Abinaya *et al.,* 2021). Traditional medicines have a rich heritage for curing numerous diseases. *Terminalia arjuna* is an important medicinal plant, found throughout the Indian peninsula and also distributed plentifully in the Sub-Himalayan tract. The stem bark of this plant is used as an effective medicine for treating tumors, ulcers, leucorrhoea, heart-related problems, diabetes, asthma and inflammation, etc. (Zhang *et al.,* 2019). Research has demonstrated that the stem bark of Arjuna plant comprises a significant value of polyphenols, containing approximately 60-70% of its composition. These compounds encompass tannins, triterpenoids and flavonoids which majorly contribute to the anti-tumor properties to the bark (Singh *et al.,* 2017). Several studies found the potency of phytochemicals as an effective anticancer agent due to their lower toxicity, abundant availability and ease of extraction. These phytochemicals can slow down cancer growth and progression due to their diverse mechanisms that include reduction in oxidative stress, blocking angiogenesis, arrest in cell cycle, inhibition of cell proliferation and programmed cell death induction (Qawoogha and Shahiwala, 2020). The development of anticancer drugs typically involves targeting one or more pathways or proteins involved in cancer development (Choudhari *et al.,* 2020). In the context of this study, triterpenoids from the *T. arjuna* against key receptor, VEGFR-2 were assessed to identify a potential lead compound for the development of novel anticancer drugs.

MATERIALS AND METHODS

Six triterpenoids; arjunin, arjunic acid, arjungenin, terminic acid, terminolitin and arjunolic acid present in the stem bark of *Terminalia arjuna* were selected for the present studies. SDF format of these compounds was retrieved from the publicly available database, PubChem (*https://pubchem.ncbi.nlm.nih.gov/*). For Terminolitin, the structure was drawn and predicted in 3D format with the help of ChemDraw, a molecule editor tool and then minimized, as its structure was not available in PubChem database (Table 1). SMILES format for all the compounds were also retrieved for the calculation of drug likeness properties.

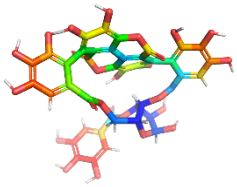
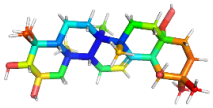
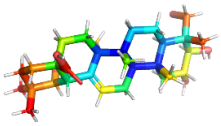
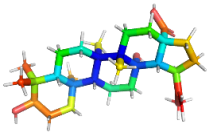
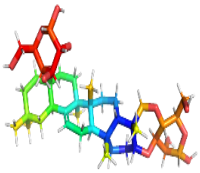
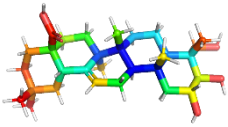
All these ligands were converted into PDB format using the Open Babel, open-source software (*http://openbabel.org/wiki/Main_Page*). SMILES format of Terminolitin was obtained with the conversion formula of Open Babel software by using SDF format obtained with ChemDraw tool.

3D structures of crucial human receptor VEGFR-2 (Vascular Endothelial Growth Factor Receptor-2) was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/) following PDB ID: 3VHE. One standard drug, namely, Dovitinib, identified by its Pubmed ID as CID_135398510 was acquired in 3D SDF format for the purpose of comparison with the ligand molecules.

Lipinski's rule of five was used as a criterion for the prediction of drug likeness property of a compound. Other pharmacokinetics features were determined in the ligand molecules such as absorption, distribution, metabolism and excretion by using SwissADME (*http://www.swissadme.ch/*). The same web server tool was used to determine oral bioavailability of the ligands with the help of radar plots.

The 3-D structures of the target protein were subjected to rigorous docking evaluations with the selected phytochemical compounds retrieved from PubChem and ChemDraw by utilizing one of the most powerful computational tools, Autodock Vina (Eberhardt *et al.,* 2021). Different methodologies of molecular docking on the screened collection of phytochemical compounds from *T. arjuna* to evaluate and assess binding affinities concerning target proteins involved in cancer were utilized. To facilitate the docking analysis, AutoDock 4.2.6 software was used to perform pre-processing on protein structure such as the removal of water molecules, addition of polar hydrogen atoms and addition of Kollman's charges. Molecular docking following the preparation of protein and ligands into PDBQT formats was conducted using AutoDock 4.2.6. Successful binding interactions were carried out by setting the dimensions in the x, y and z axes at 60x60x60. Visualization and analysis of binding interactions between ligands and protein was done with Pymol (*https://pymol.org/2/*).

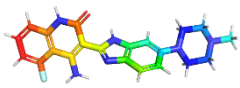
Table 1. List of compounds from *T. arjuna*; standard drug and receptor protein

Name of compound	Ligands			
	3D structure (PubChem/ ChemDraw)	SMILES format (PubChem database/ OpenBabel software)	Molecular formula	PubChemID
Arjunin		C1C2C(C(C(C(O2)O)O)OC(=O)C3=CC(=C(C(=C3)O)O)O)OC(=O)C4=CC(=C(C(=C4C5=C(C(=C6C7=C5C(=O)OC8=C(C(=C(C9=C(C(=C(C=C9C(=O)O1)O)O)O)C(=C78)C(=O)O6)O)O)O)O)O)O)O	$C_{41}H_{26}O_{26}$	102316370
Arjunic acid		C1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CC(C(C5(C)C)O)O)C)C)C2C1O)C)C(=O)O)C	$C_{30}H_{48}O_5$	15385516
Arjungenin		CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CC(C(C5(C)CO)O)O)C)C)C2C1O)C)C(=O)O)C	$C_{30}H_{48}O_6$	12444386
Terminic acid		CC(=C)C1CCC2(C1C3(CCC4C5(CCC(C(C5CCC4(C3(CC2)C)C)(C)C)O)C)O)C(=O)O	$C_{30}H_{48}O_4$	132568257
Terminoltin		C1[C@@H]2[C@@]([C@@H]3[C@](C1)([C@@H]1[C@@](CC3)([C@]3(C(=CC1)[C@@H]1[C@@](CC3)(CCC(C1)(C)C)O[C@@H]1[C@H](O[C@H](O)[C@H](O)[C@H]1O)CO)C)C)(C)CO[C@@H]1[C@H](O[C@H](O)[C@H](O)[C@H]1O2)CO		
Arjunolic acid		C1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CC(C(C5(C)CO)O)O)C)C)C2C1)C)C(=O)O)C	$C_{30}H_{48}O_5$	73641

Target protein

PDB ID	Structure	Name of protein	Chains	Sequence length
3VHE		Vascular endothelial growth factor receptor 2 (VEGFR)	A	359

Standard drug

Name	Structure	SMILES format	Molecular formula	PubChem CID
Dovitinib		CN1CCN(CC1)C2=CC3=C(C=C2)N=C(N3)C4=C(C5=C(C=CC=C5F)NC4=O)N	$C_{21}H_{21}FN_{6O}$	135398510

RESULTS AND DISCUSSION

Drug likeness predictions were carried out to scan the triterpenoids from the T. arjuna plant, whether these phytochemicals met the criteria for being considered as a drug-like compound. SwissADME (*http://www.swissadme.ch/*) is the free web tool that was used to assess Lipinski's filters that examined specific drug-likeness properties which included the number of hydrogen acceptors (<10 noN), number of hydrogen donors (<5 nOHNH), molecular weight (up to 500) and partition coefficient log P values (= 5) (Daina *et al.,* 2017). Evaluation of the Lipinski rule by considering the molecular weight, hydrogen donor count, lipophilicity and hydrogen acceptor count as key parameters was done. For the analysis of all the above calculations, the SMILES format of each phytochemical was provided as an input. Swiss ADME web server was accessed to evaluate the ADME (absorption, distribution, metabolism and excretion) values of ligands based on Lipinski's filters for all the compounds including the drug selected (Table 2). Selection criteria involved zero (0) and one (1) violations, good blood-brain barrier (BBB) penetration, Gastrointestinal (GI) absorption, the logarithm of the partition coefficient (log Kp) for skin permeability, P-glycoprotein substrate ability and better solubility. LogP values were used to determine lipophilicity which evaluates the absorption rate of a drug molecule. GI and BBB permeability were predicted by Boiled model which determined the high value of GI absorption for arjunic acid, arjungenin, terminic acid and arjunolic acid except arjunin and terminolitin (Diana and Zoete, 2016). All ligand molecules also satisfied Lipinski's rule of five with 3 or less than 3 violations assessed by SwissADME to predict drug-like properties. Only compounds having number of violations 0 and 1 were selected for docking studies and analysis by pymol software.

Rapid assessment of drug-likeness was achieved with bioavailability radar plots (Fig. 1). The pink area for each parameter in these plots showed their optimal range. For a phytochemical compound when considered as drug-like, a radar plot of a compound had to come in this pink area. That predicted the oral bioavailability of a ligand. Polarity and flexibility (Flex) were very important criteria to assess the bioavailability parameter for a compound. Compounds with more rotatable bonds (if there are more than 10) were least considered for oral bioavailability which determined its flexibility property. Some of the compounds like arjungenin, arjunolic acid, terminic acid and arjunic acid were considered to be orally bioavailable as these triterpenoids satisfied the radar plot, the same compounds were found to show 0/1 violations of the Lipinski rules as observed in previous section.

Vascular endothelial growth factor receptors (VEGFR) play a significant role in the growth and progression of cancer hence it is important to develop novel drug molecules that can inhibit growth factors with little or no effects. For this purpose, the four selected triterpenoids (Arjungenin: 1 violation, MW>500; Arjunic acid: 0 violation; Arjunolic acid: 0 violation: and Terminic acid: 1 violation, MLOGP>4.15) from *T. arjuna* were screened against VEGFR-2 (3VHE). Dovitinib, a commonly used anticancer drug having activities against target receptors was docked and binding parameters obtained were used for comparative studies with the binding energies of the six compounds

Table 2. Pharmacokinetic, chemical and physical properties of the drug (Dovitinib) and the triterpenoid compounds

Compounds	MW (g/mol)	HBD	HBA	GI ab	BBB	P-gp sb	log Kp (cm/s)	RB*	log P	Violations
Arjunin	934.63	15	26	low	No	Yes	-11.38	3	0.87	3
Arjunic acid	488.7	4	5	high	No	Yes	-5.61	1	5.84	0
Arjungenin	504.7	5	6	high	No	Yes	-6.18	2	4.5	1
Terminic acid	472.7	3	4	high	No	Yes	-4.5	2	6.6	1
Terminolitin	750.96	7	12	low	No	Yes	-9.28	4	2.26	3
Arjunolic acid	488.7	4	5	high	No	Yes	-5.13	2	5.84	0
Dovitinib (Std. drug)	392.43	3	4	high	No	Yes	-7.53	2	1.64	0

MW: Molecular weight, HBD: H-bond donor, HBA: H-bond acceptor: RB: Rotatable bond, XLogP3AA methodology for the calculation of LogP, GI ab: Gastrointestinal absorption, BBB: Blood Brain Barrier permeability: P-gp sb: P-glycoprotein substrate and Log Kp: Skin permeability.

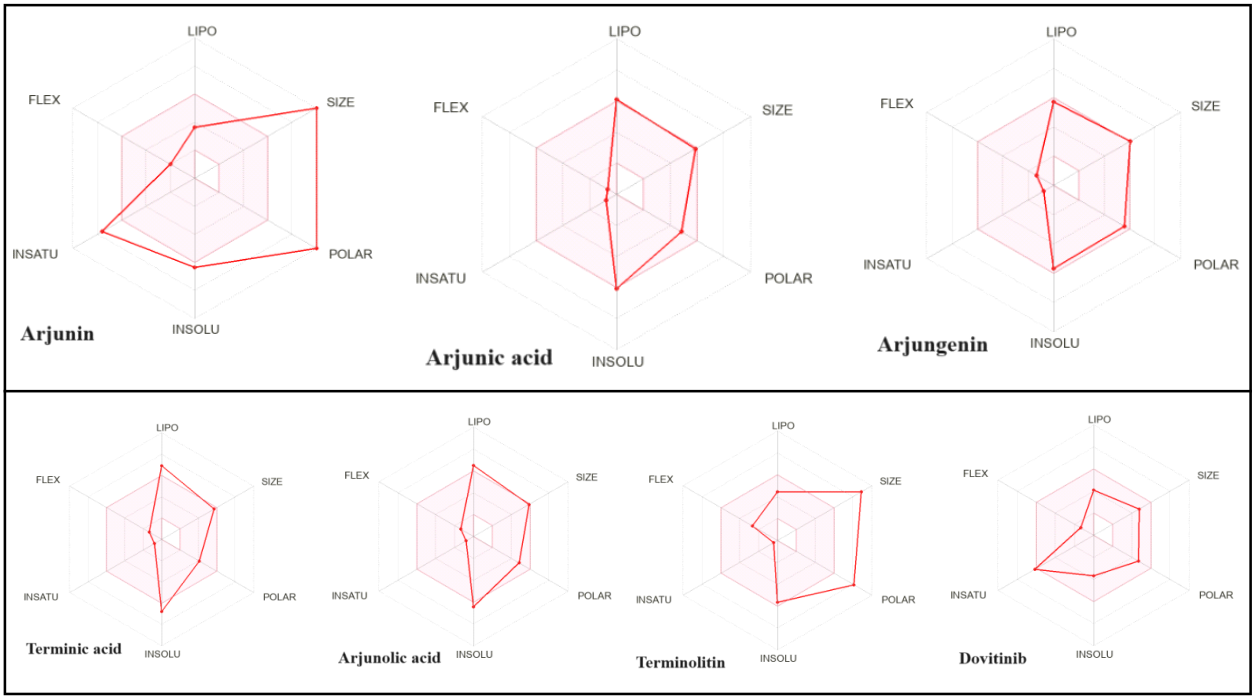


Fig. 1. Radar plots of triterpenoids from *T. arjuna* and dovitinib (drug).

selected. All four selected phytochemical compounds (arjungenin, arjunolic acid, terminic acid and arjunic acid) docked with the target protein i.e. 3VHE; showed a very good binding affinity with better modes compared with standard drug (Dovitinib) against the target receptor. After docking, the binding parameters in terms of binding energy (kcal/mol) and amino acids involved as interacting residues were noted. All these four compounds showed a strong binding affinity with receptor molecules. It was found that all these phytochemical compounds showed similar or higher binding energy than that of the drug Dovitinib (-8.1 kcal/mol). Arjungenin (-8.3kcal/mol), arjunic acid (-8.2 kcal/mol) and terminic acid (-8.9 kcal/mol) showed higher binding energy than Dovitinib (standard drug), whereas arjunolic acid (-8.1 kcal/mol) showed similar binding energy as Dovitinib against receptor molecule (3VHE). Residues of ligand molecules interacting with receptor molecules were visualized and analyzed through pymol (Table 3 and Fig. 2). These triterpenoids showed a powerful binding affinity with more stability than dovitinib (drug).

Computational methods are extremely valuable in pharmaceutical research, especially in the recognition of promising new compounds (Kumar *et al.*, 2023). In this study, arjunic acid, arjungenin, terminnic acid and arjunolic acid were identified as potential candidates for targeting VEGFR-2. These triterpenoids exhibit least toxicity or adverse effects and show safety, effectiveness and selectivity. However, additional research is needed to elucidate the pharmacodynamic and kinetic characteristics of these compounds. Further *in vitro* and *in vivo* research is essential to investigate the greatest potential for effectively targeting VEGFR-2 driven cancers.

Table 3. Binding parameters in terms of energy and interacting residues of selected triterpenoids from *T. arjuna* and standard drugs (Dovitinib) against targeted protein receptor (3VHE)

Compounds	Binding energy (Kcal/mol)	Interacting residues (amino acids involved in bonding)
Dovitinib (anticancer drug)	-8.1	Tyr1130, Ser1100
Arjungenin	-8.3	Arg842, Asp1052, Asp1058
Arjunic acid	-8.2	Tyr1082
Arjunolic acid	-8.1	Arg842, Asp1052
Terminic acid	-8.9	Asp1046, Leu1049, Lys868, Tyr1082

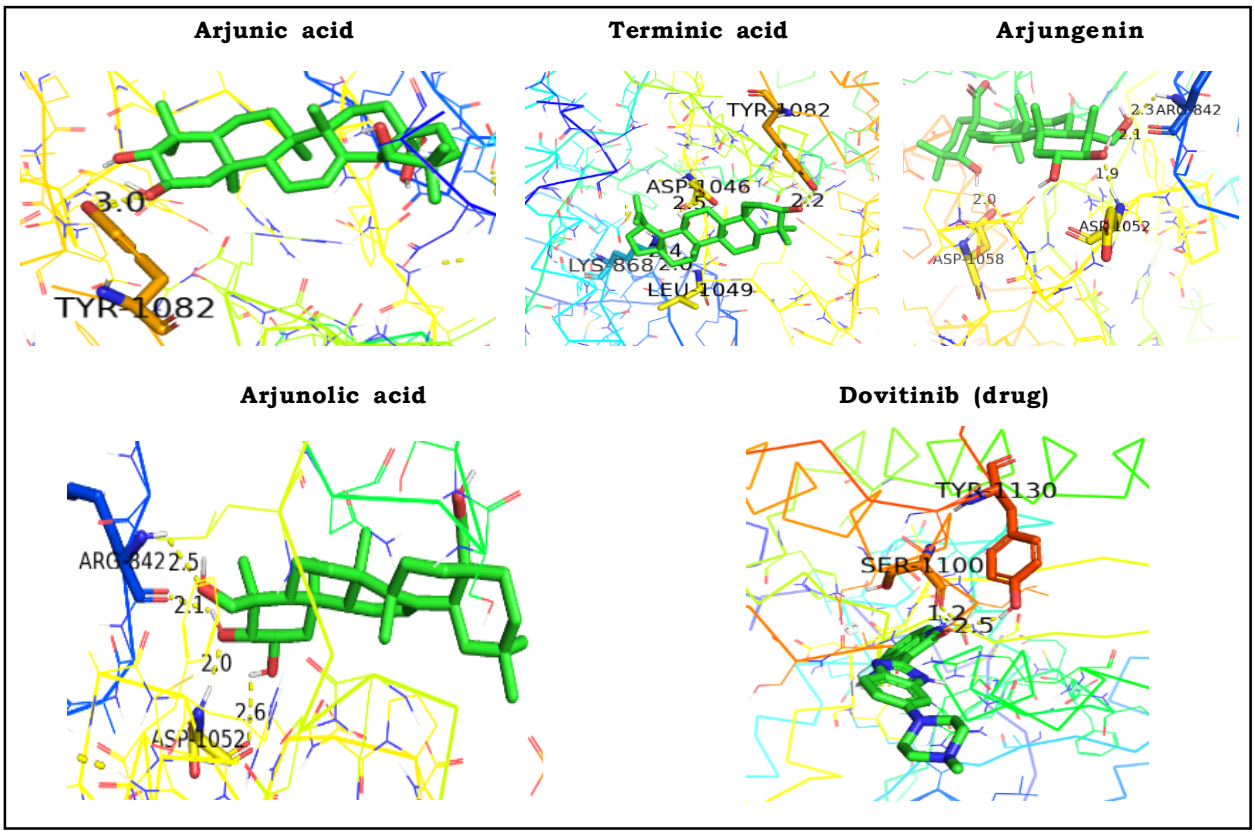


Fig. 2. Pymol structures showing best dock poses having interacting residues with the bond length between drug (dovitinib), and ligand molecules with the target protein (VEGFR-2).

CONCLUSION

The present study identified four triterpenoids (arjunic acid, arjungenin, terminic acid and arjunolic acid) of Arjuna plant which exhibited better drug-like properties comparable to standard anticancer drug (Dovitinib) against VEGFR2 (3VHE), the target protein for many types of cancers. Further, the molecular docking analysis revealed these compounds to form more stable interactions with the target protein compared to the standard drug. As the present day cancer treatments lack the fine efficacy and have so many side-effects, nature-based compounds are being looked upon as a better alternative. Subject to further experimental work, the present work recommends these compounds as promising candidate for drug molecules in cancer.

ACKNOWLEDGEMENT

The authors are thankful to Chaudhary Devi Lal University, Sirsa, Haryana for providing support for the research work.

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