IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Molecular Docking Studies, ADME Analysis and Toxicity Prediction of Phytochemicals of *Terminalia Arjuna* as Inhibitors of Proteins involved with Brain Tumor

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Abstract:

A brain tumor is an abnormal growth of brain cells. The skull is a bone protective cavity for the brain, thus any unexpected development may affect human functionality, depending on the area of the brain involved. Additionally, it could spread to other organs, potentially endangering human functions. The results of a computational analysis of phytochemicals of Terminalia arjuna physicochemical properties are presented in this study. Molecular docking were used to check ligand ability for binding within the active site. Additionally, using ADMET predictions, the druggability profile of the binding ligands was evaluated. Results shows that, the phytochemicals that may function as multitargeted inhibitors of brain tumor-associated proteins.

Keywords: Brain tumor, Terminalia arjuna, Molecular docking, ADMET analysis, Toxicity Prediction

INTRODUCTION

Cancer is a difficult disease to treat, and if it spreads to neighboring cells, there is a much lower chance of survival. There's little doubt that a great deal of lives could be saved if cancer was identified early on with accessible and rapid diagnostic techniques. There are invasive and noninvasive methods for diagnosing brain cancer. A biopsy involves making an incision to remove a sample of the lesion for examination. Brain tumors are the eighth most common type of cancer among Indians in 2018, according to studies. Headaches in the morning, seizures, blurred vision, loss of movement, vomiting, memory loss, increased sleep, behavioral changes, drooping eyelids, dizziness, loss of control over one's bowels or bladder, changes in hearing or smell, loss of consciousness, difficulties with reasoning, etc. are some of the symptoms that are indicative of a brain tumor[1].

Brain cancer is the most fetal type of cancer. Australia, North America, and Western Europe are the most vulnerable areas[2]. Tumor can be benign or malignant. Malignant tumors, on the other hand, grow out of control and

have the potential to spread to other sections of the body. Benign tumors, on the other hand, do not grow out of control or spread to other regions of the body. Scientists discovered 150 different forms of brain tumors. Gene mutations brought on by exposure to radiation, pesticides, industrial solvents, etc., can result in brain tumors. Environmental variables are also accountable for brain tumor development. It could be passed on from one generation to the next[3].

A brain tumor is an abnormal and uncontrollably growing tumor of brain cells. Our skulls have a limited amount of space, so this additional development increases internal pressure, which might damage the brain[4]. Most individuals prefer herbal medicines over conventional ones, even though medicinal plants are essential to health care and are utilized as a primary element in both conventional and traditional medicine formulations[4]. Arjuna is mentioned as an ayurvedic remedy in several ancient Indian medical texts, including Charaka Samhita, Sushruta Samhita, and Astang Hridayam[5].

At least seven species of Terminalia are traditionally used to treat cancer. The bark, stem, and leaves of T. arjuna, a native medicinal plant of Mauritius, are the main sources of the components that prevent the formation of cancer cells. It was noted that the key regulators of anticancer activity are flavones and tannins. Anti-dysentric, antipyretic, astringent, cardiotonic, anticoagulant, hypolipidemic, antibacterial, and antiuremic qualities can be found in T. arjuna bark. Its leaves have been shown to have analgesic and anti-inflammatory properties in studies conducted on mice[6-7].

MATERIALS AND METHODS

Molecular Docking

The atomic-level interaction of a tiny molecule (ligand) with a protein is known as molecular docking. Molecular docking can be used to study the behavior of tiny compounds at target protein binding sites and shed light on fundamental biological processes. The two main steps in the docking method are determining the orientation, position, and predicted ligand structure inside these sites, as well as the binding affinity[8].

Preparation of Protein

The 6l9O three-dimensional structure was obtained from the Protein Data Bank (PDB) and downloaded from RCBS (http://www.rcsb.org); the structure was then pre-processed. It entails removing the water molecules from the cavity, creating side chains, adding kolman charges to stabilize the charges, adding a H molecule to the bpolar region to fill in the residue gaps, and so on.

Preparation of ligand

2D structures of T. arjuna's several active ingredients were obtained from the PubChem database. Out of the ten ligands that were selected, only five were ultimately tested for docking.

Docking Setup

utilizing Pyrx software, which illustrates the binding energy analysis through grid and energy potential utilizing various search algorithms to identify precise binding features on the designated super molecule, molecular docking of proteins and ligands was carried out. Grid box numbers 126, 26,126 were used for docking along the X, Y, and Z axes.

ADMET Study

To develop a potent chemical into a drug, its absorption, distribution, metabolism, and excretion (ADME) must be evaluated. Additionally, it needs to be concentrated enough to reach its target in the body and stay there in a bioactive condition long enough for the expected biologic actions to occur[9]. Physicochemical properties of compound can be predicted by using Swiss ADMET tool.

Before beginning chemical synthesis, this online tool helps with hit selection by computing important ADME-Tox and drug properties. Early in the drug development process, when a large library of compounds is available for consideration but physical sample access is restricted, pharmacokinetic property assessment typically takes place[10].

SwissADME's strong points include its various input methods, its molecular computation capabilities, and its display, save, and exchange features. SwissADME is now considered a part of the Swiss Drug Design Workbench[11].

Methodology

The structures of the active ingredients in plant derivatives derived from arjuna were obtained from Pubchem. The SwissADMET tool was launched with the structure smiley. The SwissADME drug design project has produced verified results.

Toxicity Study

A negative impact on one's health resulting from drug use is referred to as toxicity. Medication side effects can be anything from minor to deadly. When developing new medications, the toxicological effects are evaluated. Toxicological chemicals are not prioritized during the drug discovery process and are found quickly[12]. Protox is a web tool that forecasts various end objectives for toxicity, such as hepatotoxicity, immunotoxicity, negative outcome pathways (Tox21), and acute toxicity, using pharmacophores, machine learning models, and chemical similarities[13].

In silico methods were applied to explore the immunotoxic, carcinogenic, mutagenic, and other possible toxic effect pathways of these chemicals by combining Tox21 information[14].

The de novo medication predicted was validated using ProTox3.0, an in silico Oral Toxicity Study software. According to Drwal et al., an in-silico analysis was carried out using parameters like nuclear receptor signaling stress response pathways, organ toxicity, specifically hepatotoxicity, immunotoxicity, and genetic toxicity, and rat oral acute toxicity, with a focus on median lethal dosage (LD_{50}) as mg/kg.

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Methodology

Using PubChem, three-dimensional structures for five active chemical components found in Termenalia arjuna bark were determined. Every chemical has the download of its canonical SMILE.

On ProTox-3.0, all canonical SMILEs were supplied. The median lethal dosage (LD_{50}) (mg/kg), toxicity class, cytotoxicity, carcinogenicity, hepatotoxicity, mutagenicity, and immunotoxicity were the endpoints employed in the ProTox-II toxicity prediction process[15].

RESULTS AND DISCUSSION

ADMET Study

The ADME study of the active components of the arjuna plant and its derivatives was done using the SwissADME web service. When taking drugs orally, gastrointestinal absorption becomes very important. The prediction that all analogs would be rapidly absorbed from the GI tract was confirmed by our investigation. These molecules cannot distinguish between malignant and normal cells, therefore unless they are modified suitably, they should only be given parenterally. The BBB's penetration determines whether a substance will affect the brain in a way that is favorable or unfavorable. When they cross the blood-brain barrier, conventional small anticancer medications have the potential to severely damage brain neurons, which could drastically impede brain function. Notably, none of the arjuna compounds—apart from arjunone—should ever cause neurotoxicity when taken because they cannot cross the blood-brain barrier. The degree of skin penetration can be ascertained using the Log Kp value. A higher Log Kp value indicates better skin permeability, and vice versa. The study's chemicals will have little to no effect on skin toxicity because none of them can easily penetrate the epidermis.

Table 2: Determination of physicochemical properties of active constituents of T. arjuna by using SwissADME webtool.

Sr. no.	**Phytochemicals**	**Physicochemical parameter**								
		Formula	**MW**	**HA**	**AHA**	**RB**	**HBA**	**HBD**	**MR**	**TPSA (Å2)**
1	Arjungenin	$C_{30}H_{48}O_6$	504	28	0	5	5	0	137.18	98.32A
2	Arjunone	$C_{19}H_{20}O_6$	3444.36	23	14	2	5	5	135.34	70.21
3	Arjunic Acid	$C_{30}H_{48}O_5$	488.70	34	12	2	6	4	120.78	84.12
4	Arjunolic Acid	$C_{30}H48O_5$	488.70	29	0	1	6	5	129.30	61.18
5	Arjunetin	$C_{36}H_{58}O_{10}$	650.84	46	0	4	10	7	170.95	177.14

Table 3: Prediction of Pharmacokinetic parameter of active constituents of T. arjuna by using SwissADMET webtool

Sr. no.	Phytochemicals	Pharmacokinetic parameter			
		GI absorption	BBB permeant	P-gp substrate	Skin permeation (logKp)
1	Arjungenin	High	No	Yes	-6.18cm/s
2	Arjunone	High	Yes	No	-6.33 cm/s
3	Arjunic Acid	High	No	Yes	-5.61 cm/s
4	Arjunolic Acid	High	No	Yes	-5.13 cm/s
5	Arjunetin	Low	No	Yes	-7.88 cm/s

All candidates, with the exception of arjunetin, passed Lipinski's filter, indicating that, in terms of bioavailability, they may potentially find use as oral medications. Martin et al. published a bioavailability score approach to predict the likelihood that a drug will exhibit measurable Caco-2 permeability or at least 10% oral bioavailability in rats. Each contender's oral bioavailability was determined to be moderate using this ranking system. The Synthetic Accessibility Score also indicated that the chemical synthesis of these substances was evaluated as "easy," suggesting that producing them in a lab won't be too tough.

Table 4: Prediction of Druglikeness properties of active constituents of T. arjuna by using SwissADMET webtool

Sr.no.	Phytochemicals	Druglikeness Properties		
		Lipinski	Bioavailability Score	Synthetic accessibility
1	Arjungenin	Yes	0.56	6.68
2	Arjunone	Yes	0.55	3.51
3	Arjunic acid	Yes	0.56	6.53
4	Arjunolic acid	Yes	0.56	6.45
5	Arjunetin	No	0.17	7.89

Prediction of oral acute toxicity

The globally harmonized system of categorization and labeling of substances (GHS) is used to define toxicity classes. The LD_{50} values are expressed in mg/kg.

Toxic doses and toxicity classes

Class I: if ingested, deadly ($LD_{50} \leq 5$)

If ingested, Class II is lethal ($5 < LD_{50} \leq 50$).

Class III: hazardous to ingestion ($50 < LD_{50} \leq 300$)

Class IV: dangerous to ingest ($300 < LD_{50} \leq 2000$).

Class V: if consumed, could be hazardous ($2000 < LD_{50} \leq 5000$).

Non-toxic (LD_{50} > 5000) is class VI.

Table 5: Prediction of oral acute toxicity, class and accuracy of active constituents of Terminalia arjuna.

Sr. no.	Phytochemicals	Oral LD_{50} value (mg/Kg)	Predicted toxicity class	Prediction accuracy (%)
1	Arjungenin	2000mg/kg	4	70.97%
2	Arjunone	2000mg/kg	4	69.26%
3	Arjunic Acid	2000mg/kg	4	70.97%
4	Arjunolic Acid	2000mg/kg	4	70.97%
5	Arjunetin	3220mg/kg	5	70.97%

Table 6: Various toxicity prediction of active constitunets of T. arjuna by using Protox 3.0

Sr. no	Compounds name	Hepatotoxicity	Neurotoxicity	Carcinogenicity	Immunotoxicity	Cytotoxicity
1	Arjungenin	Inactive	Inactive	Inactive	Inactive	Inactive
2	Arjunone	Inactive	Inactive	Inactive	Active	Inactive
3	Arjunic Acid	Inactive	Inactive	active	Active	Inactive
4	Arjunolic Acid	Inactive	Inactive	Inactive	Inactive	Inactive
5	Arjunetin	Inactive	Inactive	Inactive	Active	Inactive

Table 7: Toxicity target prediction for active constituents of T. arjuna by using Protox 3.0

Sr. no	Compounds name	Toxicity target				
		Androgen Receptor	Amine Oxidase A	Prostaglandin G/H Synthase 1	Glucocorticoid Receptor	
1	Arjungenin	5.01%	67.87%	69.43%	--	Avg Pharmacophore Fit
2	Arjunone	72.59%	0%	72.37%	-	Avg Similarity Known Ligands
3	Arjunic Acid	5.96%	34.59%	42.08%	-	Avg Pharmacophore Fit
4	Arjunolic Acid	79.15%	71.05%	87.66%	-	Avg Similarity Known Ligands
5	Arjunetin	5.1%	63.08%	66.25%	--	Avg Pharmacophore Fit

Here, we predict possible binding to toxicity targets-protein targets connected to toxic effects and unfavorable drug reactions-using a set of protein-ligand-based pharmacophores.

CONCLUSION

In-silico study molecular docking study is performed with phytochemicals of Terminalia arjuna with Pyrx software, and the SwissADMET webtool for the prediction of binding energy, physicochemical characteristics, drug-likeness, and toxicity of T. arjuna's active components, respectively. The herb arjungenin, which is extracted from Arjuna (Terminalia arjuna), has anti-inflammatory, anti-hypertrophic, anti-cancer, anti-oxidant, and anti-platelet properties.This paper examined arjungenin's structural inhibition of 6L9O via molecular docking. The outcomes of our molecular docking of 6L9O with arjungenin demonstrated that each docked conformation exhibited an effective 1H-bond binding between 6L9O and arjungenin, and that 6L9O had significant binding energies of -8.9 kcal/mol. Arjungenin exhibits the strongest binding affinity in molecular docking to protein 6L9O with a binding energy of -8.9, which is comparable to the standard medication Temozolomide, which is used to treat brain tumors. Using insilico approaches, the physicochemical properties and toxicity of arjungenin were successfully predicted. Using the SwissADMET webtool, the physicochemical parameters, drug similarity properties, and pharmacokinetic parameter of T. arjuna were successfully predicted. One measure of acute toxicity is LD_{50}. The dosage at which half of the studied animal population perishes is known as the lethal dose (LD_{50}) (4). Milligrams per kilogram of animal body weight (mg/kg BW) is a common way to express the LD_{50}. Oral LD_{50} values exceeding 2,000 mg/kg BW are considered to be mildly toxic, whereas values between 0 and 50 mg/kg BW are considered to be extremely harmful. Therefore, based on the results, we can infer that every T. arjuna chemical has mild toxicity, with a predicted oral toxicity of 2000 mg/kg.

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