**Docking studies in targeting proteins involved in cardiovascular disorders using phytochemicals from
*Terminalia arjuna***

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Abstract

Terminalia arjuna (Roxb.) Wight and Arnot (*T. arjuna*) commonly known as Arjuna has been known for its cardiogenic nature in heart failure, ischemic, cardiomyopathy, atherosclerosis, myocardium necrosis and also has been used in the treatment of different human disorders such as blood diseases, anaemia and viral diseases. Our focus has been on phytochemicals which do not exhibit any cytotoxicity and have significant cardioprotective activity. Since Protein-Ligand interactions play a key role in structure-based drug design, therefore with the help of molecular docking, we screened 19 phytochemicals present in *T. arjuna* and investigated their binding affinity against different cardiovascular target proteins. The three-dimensional (3D) structure of target cardiovascular proteins were retrieved from Protein Data Bank, and docked with 3D Pubchem structures of 19 phytochemicals using Autodock vina. Molecular docking and drug-likeness studies were made using ADMET properties while Lipinski's rule of five was performed for the phytochemicals to evaluate their cardio protective activity. Among all selected phytochemicals, arjunic acid, arjungenin, and terminic acid were found to fulfill all ADMET rules, drug likeness, and are less toxic in nature. Our studies, therefore revealed that these three phytochemicals from *T. arjuna* can be used as promising candidates for developing broad spectrum drugs against cardiovascular diseases.

Keywords: *Terminalia arjuna*; cardiovascular diseases; phytochemicals; Molecular docking.

Introduction

Cardiovascular diseases (CVD) are one of major cause of death in both developed and developing countries accounting approximately 31% of all deaths worldwide. Urbanization and changes in life style are main causes for increase of CVDs. It is expected that by the year 2030, more than 23.3 million people will die annually due to CVDs. It has been estimated that 17.7 million people died from CVDs in 2015, out of which, 7.4 million were due to coronary heart disease and 6.7 million were due to stroke. Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels including coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis and pulmonary embolism.

The renin-angiotensin system is an important regulatory system of cardiovascular and renal function. Any inhibition to this system, i.e. angiotensin converting enzyme inhibitors and angiotensin receptor has become a front-line treatments for hypertensive target organ damage and progressive renal disease [1]. High blood cholesterol level is one of the key factors for progression of Coronary artery disease (CAD) one of the key factors [2]. Inhibition to 3-hydroxy-3- methylglutaryl-coenzyme A reductase (HMGCR), a major cholesterol biosynthesis enzyme can result in decreasing the risk of coronary complications [3]. Braz *et al.* [4] reported that MAPK P38 plays a pivotal role in the signal transduction pathway mediating post-ischemic myocardial apoptosis and inhibition to MAPK P38 may attenuate reperfusion injury. Any stimulation of MAPK P38 induces intrinsic myocardial apoptosis activity and cellular signaling that results in reperfusion injury. β -Adrenergic receptors (β 1AR, and β 2AR) are G protein coupled receptors and are a promising tool that play regulatory function in the cardiovascular system (CVS) via signaling. It increases the $G\alpha$ level, which activates β AR kinase (β ARK) that affects and enhances the progression of heart failure (HF) through the activation of cardiomyocyte β -Adrenergic receptors [5]. C-reactive protein, among other systemic inflammatory mediators, has been widely accepted as a potent risk indicator, independently predicting future cardiovascular events. Evidence has cumulated to show that C-reactive protein levels are associated with different aspects of the cardiovascular risk spectrum [6]. An increased risk of cardiovascular events has been associated with the use of Non-steroidal anti-inflammatory drugs (NSAIDs), especially of COX-2 selective NSAIDs [7]. The synthetic drugs used for treatment of various cardiovascular diseases showed several side effects. In modern era, plants based medicinal system has become more popular because of low cost, easily availability, more effectiveness and lack of side effects.

Terminalia arjuna (Roxb) Wight and Arnot (*T. arjuna*) commonly known as ‘Arjuna’ has been used as cardiotonic in heart failure, ischemic, cardiomyopathy, atherosclerosis, myocardium necrosis [8-11] and also has been used in the treatment of different human disorders such as blood diseases, anaemia and viral diseases [12-15]. Several reports have studied the cardioprotective potential of *T. arjuna* stem bark using various models [16-21]; but mechanism of action of phytochemicals of *T. arjuna* against cardiovascular target proteins is still unclear. Mythili *et al.* [22] reported that arjunolic acid which is present in *T. arjuna* stem bark has protective effect against CSA-induced cardiotoxicity. To extend our research in cardiovascular disorders, we made a new approach to find the interaction between the phytochemical compounds from *T. arjuna* and synthetic drugs (Phosphocholine,

Simvastatin, Rofecoxib, Carazolol, Lisinopril, Losartan, Losmapimod) and cardiovascular targets using molecular docking tools. Therefore, we investigated *in silico* docking analysis of cardiovascular disease targets with phytocompounds from *T. arjuna* tree.

Methodology:

Ligand preparation

The 3D structures of selected phytocompounds and synthetic drugs were obtained from Pubchem in SDF format and converted to PDB format using the tool Open Babel [23]. The ligands were available in XML, SDF, JSON and ASN.1 format. Then the Gasteiger charges and rotatable bonds were assigned to the PDB ligands using Auto Dock Tool [24]. All rotatable bonds were allowed to move freely. The compound structures were energy minimized and considered for docking studies (Table 1).

Table-1: Major Phytocompounds present in various parts of *T. arjuna* for docking studies.

Sr. No	Compounds/Drugs	Formula	Compound ID
1	Arjunetin	C ₃₆ H ₅₈ O ₁₀	3052779
2	Arjunic acid	C ₃₀ H ₄₈ O ₅	15385516
3	Arjunolic acid	C ₃₀ H ₄₈ O ₅	73641
4	Arjunone	C ₁₉ H ₂₀ O ₆	14034821
5	Arjungenin	C ₃₀ H ₄₈ O ₆	12444386
6	β-sitosterol	C ₂₉ H ₅₀ O	222284
7	Casuarinin	C ₄₁ H ₂₈ O ₂₆	13834145
8	Ellagic acid	C ₁₄ H ₆ O ₈	5281855
9	Ethylgallate	C ₉ H ₁₀ O ₅	13250
10	Gallic acid	C ₇ H ₆ O ₅	370
11	Luteolin	C ₁₅ H ₁₀ O ₆	5280445
12	Quercetin	C ₁₅ H ₁₀ O ₇	5280343
13	Terminic acid	C ₃₀ H ₄₈ O ₄	355369274
14	Catechin	C ₁₅ H ₁₄ O ₆	73160
15	Rutin	C ₂₇ H ₃₀ O ₁₆	5280805
16	Kaempferol	C ₁₅ H ₁₀ O ₆	5280863
17	Leucocyanidin	C ₁₅ H ₁₄ O ₇	71629
18	3-O-Methylellagic acid 3'-rhamnoside	C ₂₁ H ₁₈ O ₁₂	73157208
19	Oleanolic acid	C ₃₀ H ₄₈ O ₃	10494
Drugs			
20	Phosphocholine (PC)	C ₅ H ₁₅ NO ₄ P ⁺	1014
21	Simvastatin	C ₂₅ H ₃₈ O ₅	54454
22	Rofecoxib	C ₁₇ H ₁₄ O ₄ S	5090
23	Carazolol	C ₁₈ H ₂₂ N ₂ O ₂	71739
24	Lisinopril	C ₂₁ H ₃₁ N ₃ O ₅	5362119
25	Losartan	C ₂₂ H ₂₃ ClN ₆ O	3961
26	Losmapimod	C ₂₂ H ₂₆ FN ₃ O ₂	11552706

Protein preparation

A total of seven proteins which were related to several cardiovascular diseases were selected on the basis of literature survey (Table 2). The 3D crystal structures of selected target proteins were obtained from the RCSB protein data bank (<http://www.pdb.org>). All the proteins had co-crystallized ligands (X-ray ligand) in the binding site. The ligands enclosed in each protein structure were removed from the binding site and saved to a new file. Automated molecular docking was performed to find out molecular interaction and optimized geometry by using docking software AutoDock vina. All heteroatoms including water molecules, and definitions for symmetry were excluded from the file. Polar hydrogens were added to each protein and it was minimized by applying Kollman's partial atomic charges. Minimized structure was saved in PDBQT file format that contains a protein structure with hydrogen in all polar residues. All docking parameters were set to default values on the basis of Lamarckian genetic algorithm principle [25].

Table 2: Targeted receptor proteins associated with cardiovascular disease along with structure

S. No.	Target Proteins	Disease	PDB ID	Structure
1	Angiotensin converting enzyme	(Atherosclerosis/ Coronary Artery Diseases)	1O8a
2	Human Angiotensin receptor	(Atherosclerosis/ Coronary Artery Diseases)	4YAY
3	P38 Mitogen activated protein kinase (MAPK)	Myocardial infarction	4DLI

4	HMG-Co A reductase	Coronary artery disease	1HW9
5	β -1-Adrenergic receptor	Chronic heart failure	2YCW
6	Human C-reactive Protein	Coronary heart disease	1BO9
7	Cox-2	Myocardial Infarction, Pain	1CX2

Grid box generation

The grid parameter file of each protein was generated using AutoDock Tool. A grid-box was created in such a way that it covers the entire protein binding site and accommodates all ligands to move freely in it. The center of the active binding site was estimated from the structure and taken as the center of the grid-box.

Molecular docking

The docking of selected ligands to the catalytic triad of protein was performed using AutoDock vina. Docking was performed to obtain a population of possible conformations and orientations for the ligand at the binding site. Polar hydrogen atoms were added to all the proteins and its non polar hydrogen atoms were merged by using the software.

All bonds of ligands were set to be rotatable. All calculations for ligand flexible protein-fixed docking were performed using the Lamarckian Genetic Algorithm (LGA) method. The best conformation was chosen with the lowest docked energy, after the completion of docking search. Standard docking settings were used and the 10 energetically most favorable binding poses are outputted. The interactions between selected phytocompounds and target receptor were visualized by using UCHF chimera 1.8.1 [26] and LigPlot [27].

ADMET and toxicity prediction

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) screening of ligands helps to determine their absorption properties, toxicity, and drug-likeness. Ligand molecules saved in .smiles format and selected drugs (Phosphocholine, Simvastatin, Rofecoxib, Carazolol, Lisinopril, Losartan, Losmapimod) were uploaded on SWISSADME (Molecular Modeling Group of the SIB (Swiss Institute of Bioinformatics), Lausanne, Switzerland), admetSAR (Laboratory of Molecular Modeling and Design, Shanghai, China), and PROTOX webserver (Charite University of Medicine, Institute for Physiology, Structural Bioinformatics Group, Berlin, Germany) for ADMET screening. SWISSADME is a web tool used for the prediction of ADME and pharmacokinetic properties of a molecule. The predicted result consists of lipophilicity, water solubility, physicochemical properties, pharmacokinetics, drug-likeness, medicinal chemistry, and Brain or Intestinal Estimated permeation method (blood-brain barrier and PGP $\pm$ prediction) [28]. AdmetSAR provides ADMET profiles for query molecules and can predict about fifty ADMET properties. Toxicity classes are Category I containing compounds with LD₅₀ values ≤ 50 mg/kg, Category II containing compounds with LD₅₀ values > 50 mg/kg; Category III (slightly toxic) containing compounds with $500 < LD_{50} \leq 5000$ mg/kg and safe chemicals (LD₅₀ > 5000 mg/kg) are included in Category IV [29, 30]. PROTOX is a Rodent oral toxicity server predicting LD₅₀ value and toxicity class of query molecule. The toxicity classes are as follows: (i) Class 1: fatal if swallowed (LD₅₀ ≤ 5), (ii) Class 2: fatal if swallowed (55000) [31].

Drug likeness calculations

Drugs scans were carried out to determine whether the phytochemicals fulfil the drug-likeness conditions. Lipinski's filters using Molinspiration (<http://www.molinspiration.com>) were applied for examining drug likeness attributes as including quantity of hydrogen acceptors (should not be more than 10), quantity of hydrogen donors (should not be more than 5), molecular weight (mass should be more than 500 daltons) and partition coefficient log P (should not be less than 5). The smiles format of each of the phytochemical was uploaded for the analysis

Results:

Molecular docking analysis

Docking results of selected phytocompounds with targeted proteins i.e. 1O8a, 4YAY, 4DLI, 1HW9, 2YCW, 1BO9 and 1CX2 showed that selected phytochemicals from *T. arjuna* had a good binding affinity and better binding modes than that of standard drugs against selected target receptors. All the selected 19 phytocompounds showed

strong or comparative binding with all selected receptors. The data of binding energy of selected phytocompounds with target proteins is presented in table 3.

It was found that all the phytocompounds showed strong binding with receptor 1B09 which is higher than that of drug PC (-4.2kcal/mol). Arjungenin (-16.1 kcal/mol), casuarinin (-15.4 kcal/mol), arjunic acid (-13.3 kcal/mol), terminic acid (-12.6 kcal/mol) showed higher binding energy with 1B09. With 1HW9 receptor, casuarinin (-12.0 kcal/mol), arjungenin (-8.4 kcal/mol), terminic acid (-7.5 kcal/mol), arjunic acid (-7.4 kcal/mol) showed higher binding energy as compared to drug simvastatin (-7.2 kcal/mol). Casuarinin (-19.4 kcal/mol), arjungenin (-17.3 kcal/mol), arjunic acid (-14.3 kcal/mol), terminic acid (-13.7 kcal/mol), showed higher binding energy with 1cx2 receptor, as compared to drug rofecoxib (-9.4 kcal/mol). Casuarinin (-24.0 kcal/mol), arjungenin (-19.3 kcal/mol), arjunic acid (-16.9 kcal/mol), arjunolic acid (-16.8 kcal/mol), showed higher binding energy with 4YCW receptor, as compared to drug Carazolol (-9.6 kcal/mol). Casuarinin (-14.7 kcal/mol), terminic acid (-9.0 kcal/mol), arjungenin (-8.4 kcal/mol), arjunic acid (-7.1 kcal/mol) showed higher binding energy with 1O8a receptor, as compared to drug lisinopril (-6.0 kcal/mol). Casuarinin (-21.0 kcal/mol), arjungenin (-16.5 kcal/mol), terminic acid (-14.6 kcal/mol), arjunetin (-10.3 kcal/mol) showed higher binding energy with 4YAY receptor, as compared to drug losartan (-9.7 kcal/mol). With 4dli receptor, arjungenin (-21.6 kcal/mol), casuarinin (-17.0 kcal/mol), terminic acid (-13.1 kcal/mol), ellagic acid (-11.8 kcal/mol) showed higher binding energy with 1B09 as compared to Losmapimod (-11.1 kcal/mol) (Table 3).

Table 3: Binding energy of 19 phytocompounds from *T. arjuna* against targeted protein receptors. Binding energy was expressed in terms of Kcal/mol.

Compounds	1B09	1HW9	1CX2	4YCW	1O8a	4YAY	4dli
Arjunetin	-6.6	-4.6	-7.8	-8.1	-6.6	-10.3	-7.1
Arjunic acid	-13.3	-7.2	-14.3	-16.9	-7.1	-8.8	-8.0
Arjunolic acid	-12.7	-7.4	-14.2	-16.8	-5.2	-10.1	-7.4
Arjunone	-8.1	-4.2	-7.0	-9.5	-5.1	-8.7	-7.1
Arjungenin	-16.1	-8.4	-17.3	-19.3	-8.4	-16.5	-21.6
β-sitosterol	-10.9	-5.7	-7.8	-13.2	-6.2	-13.0	-9.0
Casuarinin	-15.4	-12.0	-19.4	-24.0	-14.7	-21.0	-17.0
Ellagic acid	-7.9	-4.6	-9.3	-10.6	-6.7	-10.2	-11.8
Ethyl gallate	-6.5	-3.2	-6.5	-6.8	-4.1	-6.9	-7.3
Gallic acid	-6.3	-2.7	-6.0	-6.7	-4.2	-6.3	-6.8

Luteolin	-9.3	-4.6	-7.8	-10.3	-5.9	-9.0	-8.2
Quercetin	-9.9	-5.0	-10.6	-10.7	-6.4	-9.3	-11.6
Terminic acid	-12.6	-7.5	-13.7	-16.2	-9.0	-14.6	-13.1
Catechin	-8.6	-3.9	-8.9	-9.1	-5.6	-5.5	-10.0
Rutin	-7.2	-4.7	-8.3	-9.6	-7.0	-6.0	-7.7
Kaempferol	-8.5	-4.6	-8.5	-9.3	-5.6	-5.9	-7.5
Leucocyanidin	-8.8	-5.0	-7.9	-8.8	-5.8	-5.5	-7.1
3-O-Methylellagic acid 3'-rhamnoside	-7.5	-5.0	-9.0	-10.3	-6.1	-5.9	-7.4
Oleanolic acid	-6.6	-4.4	-7.1	-16.2	-6.2	-6.2	-7.2
Inhibitors	-4.2 (Phosphocholine)	-7.2 (Simvastatin)	-9.4 (Rofecoxib)	-9.6 (Carazolol)	-6.0 (Lisinopril)	-9.7 (Losartan)	-11.1 (Losmapimod)

Furthermore, Hydrogen bond interaction, hydrogen bond length and hydrophobic interactions of these phytochemicals with all receptors were analyzed through Ligplot and are summarized in table 4 and table 5.

Table 4: Intermolecular H-bonding and hydrophobic interaction between phytocompounds (Arjunic acid, arjungenin, casuarinin, terminic acid, standard drugs (inhibitor) and target proteins.

Compounds	1B09		1HW9		1CX2		2YCW		1O8a		4yay		4dli	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Arjunic acid	Ser44, Tyr49, Asp112	Thr41, Trp67, Tyr73, Val86, Thr90, Ala 92, Val94, Val111, Arg116,	Gln819	Glu550, Gln552, Met820	Lys468	Asn39, Cys41, Gln42, Asp43, Arg44, Leu152, Pro153, Glu456, Arg469, Ser471,	Asp121 Phe201, Ser215, Asn329, Tyr333	Trp117, Val122, Ser212, Ser215, Phe306, Phe307, Asn310, Phe325,	Ile264, Asn127, Arg253, Ala261, His610 Gly611,	Asn127, Arg253, His256, Leu266, Glu267	Tyr35, Thr88, Phe182	Trp84, Tyr87, Tyr92, Val108, Arg167, Ala181	Lys287, Leu291,	Gly243, Glu245, Lys267, Glu286, Val290, Asp292,
Arjungenin	Ser44, Tyr49	Thr41, Trp67, Tyr73, Val86, Thr90, Val94, Ala92, Val111, Asp112	-	Glu550, Met820, Gly822,	Gln461, Asn34	Cys36, Cys37, Asp39, Arg44, Gly45, Glu46, Leu152, Pro153, Val155, Ala156,	-	Val122, Phe201, Thr203, Ser211, Ser215, Phe307, Asn310, Phe325, Phe326,	His263	Tyr175, Asn265, Pro269, Cys496, Pro498	Tyr35	Trp84, Tyr87, Val108, Arg167, Cys180, Ala181	Ser251, Ser252, Ser293,	Glu192, Leu195, Trp197, Lys249, Ile250, Ala255, Asp292, Asp294, Leu346
Casuarinin	-	Trp67, Tyr73, Val86, Pro87, Thr90, Ala92, Val94, Arg116	-	Lys549, Gln550, Gln552, Met820,	-	Gln42, Asn43, Arg44, Lys468, Arg469, Ser471, Lys473,	Asn313, Asn310	Val122, Phe201, Thr203, Ala208, Ser211, Ser212, Phe325, Asn329, Phe306	Tyr175, Lys174, Glu176, His263,	Arg173, Lys174, His258, Tyr259, Gly260, Gln262	Tyr35, Tyr92, Arg167	Ile31, Trp84, Thr88, Ser105, Val108, Ala181, Pro285,	-	Arg70, Leu74, Ile146, His148, Arg149, Asp150, Phe169, Glu328, Asp468,

Terminic acid	-	Ser44, Tyr49, Trp67, Tyr73, Val86, Thr90, Ala92, Val94, Val111, Asp112	Glu548	Lys549, Glu550, Gln552, Glu819, Met820, Gly822	Lys468	Cys41, Gln42, Asp43, Arg44, Leu152, Pro153, Glu465, Arg469, Ser471	-	Asp121, Val122, Val125, Phe201, Ala208, Ser211, Ser212, Ser215, Phe306, Phe307, Asn310, Phe325, Asn329,	-	Gln262, Ile264, Asn265, Pro269, Cys496, Pro498,	Tyr35	Trp84, Tyr87, Tyr87, Tyr92, Ser105, Val108, Arg167, Cys180, Ala181	Leu291, Ser293	Glu192, Leu195, Trp197, Leu246, Lys249, Ile250, Asp292, Asp294
Inhibitors	-	Phe39, Ser44, Trp67, Tyr73, Val86, Glu88, Val89, Thr90, Val94	Asn750, Ser740, Gly739, Asn736	Lys735, Ala743, Ser745, Gly748, Ala751, His752, Ala754,	Arg44	Asn39, Cys41, Gln42, Asn43, Leu152, Pro153, Gln461, Glu465, Arg469, Lys468,	Asn329	Thr118, Val122, Val125, Phe201, Trp303, Phe306, Phe307, Asn310	Asn265, Gln618,	Ala261, Gln262, Pro269, Leu490, Cys496, Trp616, Pro617,	Tyr87	Tyr35, Tyr82, Trp84, Asp281, Pro285	Pro322, Asp329	His77, Ala144, Asp145, Ala320, Gln325

A-H -bond interaction residues; B- Vander waal's interacting residues

Table 5: LigPlot structure showing interactions between phytochemicals, drugs and target proteins.

Receptor protein	Arjunic acid	Arjungenin	Casuarinin	Terminic acid	Standard drug
1B09					
1HW9

Drug-likeness prediction

The Drug-likeness filters help in the early preclinical development by avoiding costly late step preclinical and clinical failure. The drug-likeness properties of molecules were analyzed based on Lipinski rule of 5 (Table 4). All the selected phytochemicals satisfied Lipinski's rule of five, except casuarinin.

Table 4: Drug-likeness prediction of selected phytochemicals from *T. arjuna*

Compounds	Log P	Polar Surface Area (Å ²)	Number of Atoms	MW	Number of Oxygen Nitrogen	Number of -OH and -NH ₂	Violations	Rotations	Lipinski rule
Arjunic acid	4.89	97.98	35	488.71	5	4	0	1	Yes
Arjungenin	3.72	118.21	36	504.71	6	5	1	2	Yes
Casuarinin	1.49	455.17	67	936.65	26	16	3	4	No
Terminic acid	6.1	-177.5	34	472.71	4	3	1	2	Yes
Phosphocholine	-4.66	66.76	11	184.15	5	2	0	4	Yes
Simvastatin	4.76	72.84	30	418.57	5	1	0	7	Yes
Rofecoxib	0.71	60.45	22	314.26	4	0	0	3	Yes
Carazolol	3.29	57.28	22	298.29	4	3	0	6	Yes
Lisinopril	-2.44	132.96	29	405.5	8	5	0	12	Yes
Losartan	4.87	92.52	30	422.92	7	2	0	8	Yes
Losmapimod	3.52	71.09	28	383.47	5	2	0	6	Yes

Toxicity and ADMET prediction

Toxicity of phytochemicals were analyzed through Protox II server. The server admetSAR generates pharmacokinetic properties of compounds under different criteria: Absorption, Distribution, Metabolism, and Excretion [32]. The results of admetSAR analysis and toxicity prediction have been shown in table 5.

All of the phytochemicals showed an acceptable range of ADMET profiles that reflect their efficiency as potent drug candidates. All the compounds showed good human intestinal solubility (HIA), and acute rat toxicity (LD₅₀) of selected compounds also belongs to same class (Class-II and Class III) to that of drugs except casuarinin (Class-I). None of the compounds are carcinogenic (Table-5). All the selected phytochemicals are inactive for cytotoxicity and hepatic toxicity. LD₅₀ value for all selected compounds was higher except casuarinin (1000 mg/kg), indicating

non toxic nature of these compounds. Therefore, based on ADMET and toxicity analysis, phytochemicals such as arjunic acid, arjungenin, terminic acid fulfill all the enlisted criteria and we can suggest that these are potential candidates for the development of a better drug against cardiovascular diseases.

Table 5: ADMET and Protox-II prediction of selected phytocompounds of *T. arjuna* and drugs used through Admet SAR and Protox-II software.

Compounds	Admet SAR			Protox-II		
	Human intestinal absorption	Carcinogens	Rate Acute toxicity (LD50) kg/mol	LD50, (mg/kg)	Hepatotoxicity	Cytotoxicity
Arjunic acid	Positive	non-carcinogen	2.71 (III)	2000 (Class 4)	Inactive	Inactive
Arjungenin	Positive	non-carcinogen	2.806 (III)	2000 (Class 4)	Inactive	Inactive
Casuarinin	Positive	non-carcinogen	1.828 (I)	1000 (Class 4)	Inactive	Inactive
Terminic acid	Positive	non-carcinogen	4.629 (III)	2500 (Class 5)	Inactive	Inactive
Simvastatin	Positive	non-carcinogen	2.301 Class III)	1000 (Class 4)	Inactive	Inactive
Rofecoxib	Positive	non-carcinogen	2.653 (Class III)	4500 (Class 5)	Inactive	Inactive
Carazolol	Positive	non-carcinogen	3.179 (Class II)	235 (Class 3)	Inactive	Inactive
Lisinopril	Negative	non-carcinogen	2.438 (Class III)	8500 (Class 6)	Inactive	Inactive
Losartan	Positive	non-carcinogen	3.322 (Class III)	300 (Class 3)	Inactive	Inactive
Losmapimod	Positive	non-carcinogen	1.611 (Class III)	200 (Class 3)	Inactive	Inactive
Phosphocholine	Positive	non-carcinogen	2.807 (Class III)	1640 (Class 4)	Inactive	Inactive

Discussions:

In pharmaceutical research, computational strategies are of great value as they help in the identification and development of novel promising compounds especially by molecular docking methods [33, 34]. Various research groups have applied these methods to screen potential novel compounds against a variety of diseases [35]. Angiotensin-converting enzyme (ACE) has a significant role in the regulation of blood pressure and ACE inhibition with inhibitory peptides is considered as a major target to prevent hypertension [35]. Several studies have used docking approach to inhibit the expression of ACE protein with natura compounds. Quercetin glycosides showed optimum binding affinity with angiotensin-converting-enzyme (-8.5 kcal/mol) as compared to enalapril (-7.0 kcal/mol), thereby indicating that Quercetin glycosides could be used as potential candidate to treat hypertension, myocardial infarction, and congestive heart failure [36]. Similarly, methyl gallate and quercetin 3-*O*- β -D-

glucopyranosyl-(1"-6")- α -rhamnoside are potential active compounds as ACE inhibitors from *P. niruri* herb [37]. Khan [38] studied the interactions between 4YAY (Angiotensin-I) receptor protein with phytochemicals from *Alangium salvifolium*. The compound alangium1 [Alangium1(4(benzoyloxy) methyl-2hydroxyphenoxy tetrahydroxy hexoxone 1,2,3,4,5, pentaum)] showed the best glide docking XP score -8.5 kcal/mol binding energy value with best fit simulation study. Impertonin from whole fruit extract of *Aegle marmelos* showed strong interaction with HMG-CoA reductase enzyme [39]. Secondary metabolites such as Dichloroacetic acid 2, 2- dimethylpropyl ester, 1, 6, 10- Dodecatriene-3-ol, 3, 7, 11-trimethyl-[S-(Z)]-, Isopropyl acrylate and 3, 3- Dimethylacryloyl chloride formed strong binding with active sites of HMGCoA reductase [40]. Study from Sellapan *et al.* [41] reported better docking of plant derived compound, guajavarin with HMG-CoA protein. Study on phytochemicals from *T. arjuna* with phosphodiesterase 5A, sodium-potassium pump and beta-adrenergic receptor showed that casuarinin showed multiple inhibitions on phosphodiesterase 5A and sodium-potassium pump, whereas Pelargonidin on phosphodiesterase 5A and beta-adrenergic protein targets [42]. Talapatra *et al.* [43] reported that phytochemicals from *Calotropis procera* such as methyl myristate (-3.0) and methyl behenate (-3.2), β -sitosterol (-5.6), uzarigenin (-5.5) and anthocyanins (-5.4) showed good binding with CRP receptors. Caffeic acid had remarkable interaction with proteins involved in inflammatory response (COX-2, COX-1, FXa and integrin α IIb β III), thereby, having the potential to be developed as cardiovascular-safe anti-inflammatory medicine [44]. In the present study, we have selected 19 compounds reported from stem bark of *T. arjuna* against protein targets of various cardiovascular disease. It was found that among all selected phytochemicals, arjunic acid, arjungenin, terminic acid satisfy all parameters of ADMET and toxicity, also showed good affinity with selected protein targets, therefore, they could be used as potential broad -spectrum candidate for treatment of different heart problems.

Conclusions

The present work was an attempt to computationally identify phytochemicals from *T. arjuna* which can bind to the various targets of cardiovascular disease. The docking scores, analysis of the interactions of the compounds suggest that most of the compounds have the ability to bind to multiple targets involved in cardiovascular disease. ADMET and toxicity prediction showed arjunic acid, arjungenin, terminic acid could be used as potential candidate against cardiovascular disease.

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Declaration of competing interest

The authors declare that there are no conflicts of interest.

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