

Mechanistic validation of an ergogenic agent Oxyjun® using a molecular docking approach

ABSTRACT

The bark of the tree *Terminalia arjuna* commonly referred as Arjuna is widely used in Ayurveda as a therapeutic agent for heart disease. More recently, a proprietary botanical extract of Arjuna with tradename, Oxyjun®, demonstrated cardiogenic and ergogenic benefits for the first time in a younger and healthier population. However, the mechanism of action and biological actives of this novel sports ingredient were not clear. A molecular docking approach was adopted to understand the protein-ligand interactions and establish the most probable mechanism(s) of cardio vascular actions of the phyto-constituents of Oxyjun®. Twenty-one phyto-chemicals (ligands) were chosen from Arjuna and their binding affinities against eight proteins serving cardiovascular functions (target proteins) were investigated. Autodock Vina was used to carry out the molecular docking studies. Drug likeliness was assessed on the basis of ADMET properties and Lipinski's Rule of 5. We found that arjunic acid, arjungenin, arjunetin, arjunoglucoside1, chrysin, kaempferol, luteolin, rhamnetin and taxifolin demonstrated good docking scores and bioactivity.

Keywords: *Terminalia arjuna, sports, ergogenic, cardiovascular, phytochemical standardization, molecular docking*

1.0 INTRODUCTION:

Identification and quantification of one or more biologically active constituents is desirable for maintaining quality of commercially supplied plant extracts for the dietary supplement and natural products industry. However, bioactivity-guided fractionation and characterization of most potent compounds is typically elaborate and expensive especially if the plant extract is newly developed or if the claimed biological activity is novel. Molecular docking or computer-aided (in silico) studies can provide an easier start to subsequent in vitro or in vivo definitive investigations. Preparations from the bark of the Indian tree Terminalia arjuna have been used in traditional medicine for heart disease. Further the cardio-protective role of Arjuna has been studied more recently by several groups [1,2,3,4&5]. Oxyjun®, a proprietary extract of T arjuna, was however developed by Enovate Biolife, Mumbai India, for use in sports nutrition mainly pre-workout formulas, for healthy adults of all age groups, not just cardiac patients. **Girandola and Srivastava, 2017** demonstrated an improvement in the left ventricular ejection fraction and other cardiac and ergogenic benefits in young active adults on a 2-month supplementation of Oxyjun® [6]. However, the precise mechanism of action and responsible biological actives of this novel extract remain unclear. LC-MS screening of Oxyjun® had earlier reported 21 possible glycosidic and polyphenolic compounds. Hence, we found it interesting to study the interactions between these Oxyjun® actives (ligands) and the target proteins (of cardiovascular importance) using molecular docking approach. Our work may provide some leads for further characterization of this promising heart health ingredient.

2.0 MATERIALS AND METHODS:

2.1 Ligand preparation

Ligands are the phytochemical moieties identified from the extract and shortlisted to study the docking properties, Twenty-one such moieties from arjuna (ligands) were shortlisted based on LCMS screen and also from literature search. The 3D structures of selected phyto-compounds were retrieved from PubChem database in SDF format (**Table 1**). Hydrogens were added to the ligands and minimized using MMFF94 force field to make them stable and ready for docking studies.

Table 1: Phyto-constituents(ligands)from Oxyjun® used for the docking study

Phyto-constituents (Ligands)	Molecular formula	PubChem ID (CID)
Arjunoglucoside I	C ₃₆ H ₅₈ O ₁₁	14658050
Arjugenin	C ₃₀ H ₄₈ O ₆	12444386
Arjunic acid	C ₃₀ H ₄₈ O ₅	15385516
Arjunetin	C ₃₆ H ₅₈ O ₁₀	21152828
Gallic acid	C ₇ H ₆ O ₅	370
Luteolin	C ₁₅ H ₁₀ O ₆	5280445
Kaempferol	C ₁₅ H ₁₀ O ₆	5280863
Rutin	C ₂₇ H ₃₀	5280805
Quercetin	C ₁₅ H ₁₀ O ₇	5280343
Catechin	C ₁₅ H ₁₄ O ₆	9064
Ellagic acid	C ₁₄ H ₆ O ₈	5281855
Epigallocatechin gallate	C ₂₂ H ₁₈ O ₁₁	65064
Corilagin	C ₂₇ H ₂₂ O ₁₈	73568
Butein	C ₁₅ H ₁₂ O ₅	5281222
Chrysin	C ₁₅ H ₁₀ O ₄	5281607
Epimedin A	C ₃₉ H ₅₀ O ₂₀	92043273
Epimedin B	C ₃₈ H ₄₈ O ₁₉	5748393
Epimedin C	C ₃₉ H ₅₀ O ₁₉	5748394
Esculetin	C ₉ H ₆ O ₄	5281416
Rhamenitin	C ₁₆ H ₁₂ O ₇	5281691
Taxifolin	C ₁₅ H ₁₂ O ₇	439533

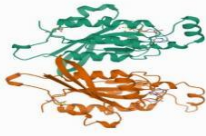
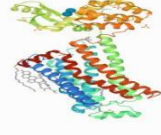

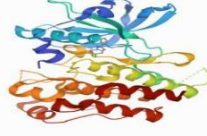
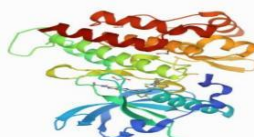
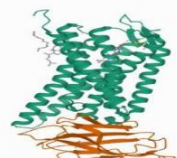
2.2 Retrieval of target proteins and its preparation

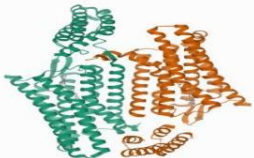
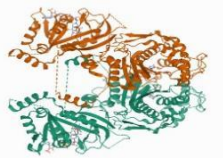
Several sets of protein targets which were known to play role in cardiovascular health or physical endurance were selected based on the basis of literature. The 3D crystal structures of these proteins were retrieved from RCSB protein data bank database (as enlisted in **table 2**). Heteroatoms as well as water molecules were removed from the protein structures. Polar hydrogens were added in the protein structures and were minimized by applying Kollman's charge. Molecular docking was performed by AutoDock Vina to check the interaction of minimized protein and ligand and to find its binding affinity or score. All the ligands were also checked for Lipinski's Rule of Five to assess whether the compounds have chemical and physical properties which make them biologically active as well as orally consumable by humans. This analysis was performed from Drulito as well as calculation of molecular properties using Molinspiration (www.molinspiration.com).

2.3 Docking using AutoDock Vina

Grid parameters for each protein were generated to create a grid-box, which would allow free movement of ligands. Docking was performed using AutoDockVina on all the ligands which passed the Lipinski's Rule of 5 and the bioactivity scores. AutoDockVina has been proven to outperform than AutoDock [7]. The results of the docked protein and ligand is resulted in the form of binding score. The lower the binding score (negative value) the stronger is the docking or binding affinity considered to be. Hence, these binding affinities or scores represents the accuracy of binding the ligand with the protein.

Table 2: Target proteins serving cardiovascular & cardio-tonic functions(<https://www.rcsb.org>)

SR. No.	Target protein / ID	Structure
1	CDC42/1A4-R	
2	β -adrenergic receptors / 2RH1	
3	MAPK1/2Y9Q	
4	VEGFR1/3HNG	
5	VEGFR2/3VNT	
6	Opioid receptors(κ)/6B73	

7	Opioid receptors(δ)/6PT3	
8	PGC1 α / 6KOT	

2.3.1 ADME Profiling:

ADME Profiling was performed to check the important properties such as Absorption, distribution, metabolism and excretion. Here we have used ORISIS Property Explorer to predict the logS value for all the compounds based on the SMILES notations. Bioactivity scores were predicted using the Molinspiration tool which calculates the Bioactivity score based on the structure and its functional groups present in the ligands.

2.3.2 Drug likeness calculations

Drugs scans were carried out to determine whether the phytochemicals fulfil the drug-likeness conditions. Lipinski's filters using Molinspiration were applied for examining drug likeness attributes as including quantity of hydrogen acceptors, quantity of hydrogen donors, molecular weight and partition coefficient log P. The smiles format of each of the phytochemical was uploaded for the analysis.

3.0 RESULTS AND DISCUSSION:

Autodock Vina was used to carry out the current docking studies. Autodock Vina is popular, user-friendly and vastly cited amongst the reputed publications [7]. CDC42/1A4R, β -adrenergic receptors/2RH1, MAPK1/2Y9Q, VEGFR1/3HNG, VEGFR2/3VNT, Opioid receptors(κ)/6PT3, Opioid receptors(δ)/6PT3 and PGC1 α / 6KOT) were the target proteins which were known to serve cardiovascular functions. Most of the phyto-constituents (out of 21 ligands) from *T. arjuna* demonstrated good binding affinities against selected target receptors. The data of binding energies of the selected ligands with target proteins is enlisted in **table 3**. Additionally, 14 ligands passed the Lipinski rule of five, proving them to be orally active.

Table 3: Summary of binding score (kcal/mol) of all the 21 ligands against the 8-target proteins

Phyto-constituents (Ligands)	Protein classes							
	1A4R	2RH1	2Y9Q	3HNG	3VNT	6B73	6PT3	6K0T
Arjunetin	-6	-7.7	-7.3	-1	-7.8	5.6	7.3	-9
Arjungenin	-6.8	-7	-6.9	-3.6	-6.4	-3.6	3.3	-8.6
Arjunglucoside_1	-7	-7	-7	2.5	-7.6	5.4	6.6	-8.6
Arjunic_Acid	-7.1	-7.7	-7.1	-5.3	-6.5	-3.9	3.7	-8.8
Butein	-7.4	-9.6	-8.4	-8.8	-8.8	-8.1	-8.2	-8.4
Catechin	-6.9	-9.2	-8.9	-8.8	-8.4	-8.2	-8.1	-7.7
Chrysin	-7.2	-9.7	-8.6	-9.2	-9	-9.3	-9	-8.4
Corilagin	-6.6	-6.8	-7	0.2	-7.5	4.8	9.3	-8.8
Ellagic_Acid	-7.3	-10.3	-9.8	-7.7	-7.9	-7.5	-8	-7.4
Epigallocatechin_	-7.9	-5.6	-9.2	-7.7	-9.6	-8	-7.2	-8.5

Gallate								
Epimedin_A1	-5.3	-5.9	-9.8	0	-8	-0.8	5.3	-8.1
Epimedin_B	-6.1	-6.6	-10	-3.9	-9	-2.6	1.8	-9.2
Epimedin_C	-6.1	-6.4	-10.5	-3.4	-9.2	-2.3	3	-9.2
Esculetin	-6.7	-7.7	-6.9	-7.3	-6.9	-7.7	-6.6	-7
Gallic_Acid	-5.8	-6.4	-6.2	-6.1	-6.6	-6.6	-5.5	-6.2
Kaempferol	-7.3	-9.7	-9.1	-8.4	-8.8	-8.5	-9	-8
Luteolin	-7.9	-9.9	-9	-9.1	-9.1	-8.9	-9.1	-8.6
Quercetin	-7.4	-9.8	-9.1	-8.4	-9	-8.8	-9	-8.4
Rhamnetin	-7.7	-9.3	-8.9	-8.6	-9	-8.9	-9.1	-8.4
Rutin	-7.5	-6.1	-10.1	-8.4	-9.2	-7.6	-6.3	-9.2
Taxifolin	-7.3	-9.7	-9	-8.2	-8.9	-8.9	-9.1	-8.4

In general, most of the ligands selected in the study have demonstrated good binding scores with decent bioactivities. Luteolin, kaempferol, catechin and chrysin, displayed higher bioactivities. The binding affinity values obtained by Autodock Vina for ligand Epigallocatechin Gallate and Luteolin for receptor CDC42 is -7.9, ligand Ellagic acid with β -adrenergic receptors is -10.3 and receptor MAPK1 is -9.8, ligand Chrysin with receptor VEGFR1 & PGC1 α is -9.2 & -9.3, ligand Luteolin with receptor VEGFR2 (3VHE & 3VNT) is -10.4 & -9.2, ligand Epigallocatechin_Gallate with receptor Opioid receptors(κ) is -9.6, ligand Luteolin, Rhamnetin, Taxifolin with receptor PGC1 α (6KOT) is -9.1, ligand Arjunic acid with receptor Opioid receptors(δ) is -8.8, respectively. Arjungenin, Arjunetin, Arjunglucoside are some of the signature bioactives of *T. arjuna* and in the current study they exhibited good docking scores with at least 5 of the 8 target proteins indicative of the mechanisms using which they bioactives impart the cardiovascular functions.

3.1 Molecular docking studies:

Twenty-one ligands were checked for the docking scores with respect to different classes of proteins playing role in cardiovascular functions. The results (Table 3) suggest that phytochemicals have higher binding scores with the target proteins of cardiovascular roles. Almost all the compounds of *Arjuna* have a very low (negative) binding score indicating good binding. All these compounds were the top scorers in almost 8 classes of protein known to play a role in cardiovascular functions.

3.2 Drug-likeness and bioactivity prediction:

Drug likeness calculations are based on qualitative parameters that help understand how drug like a substance is, with respect to factors like bioavailability. A traditional method to evaluate drug-likeness is to check compliance to Lipinski's rule of 5, which includes the numbers of hydrophilic groups, molecular weight, solubility and hydrophobicity to predict the oral bioavailability of a drug [8]. It evaluates the candidate molecules for the following parameters: (a) $\text{clogP} \leq 5$; (b) Molecular weight (MW) ≤ 500 g/mol; (c) Number of hydrogen bond acceptors (sum of N and O atoms) ≤ 10 and (d) Number of hydrogen bond donors sum of OH and NH groups) ≤ 5 [8]. Additionally, Number of rotatable bonds ($n\text{Rotb}$) ≤ 10 and Polar surface area (PSA) $< 140 \text{ \AA}^2$, are also assessed based on additions by Veber et al (2002). The simplicity of these criteria to remove outlier molecules made them very easy to implement with the use of specific software [9&10].

Total 21 ligands were considered for screening out of which 14 ligands passed the Lipinski rule of 5, proving them to be orally active (Table 4). However, it's noteworthy that only 51% of the drugs approved by FDA are compliant to Lipinski's rule of 5 and consumed orally. Further, the biologicals and natural or semi-synthetic natural drugs which do not comply to the rule have established therapeutic effects, which means that if certain phyto-constituents violate 1 or 2 rules of Lipinski's, but demonstrate biological activities, then they should still be considered for further evaluations [11].

Table 4: Drug likeliness evaluation of phyto-constituents (ligands) based on compliance with the Lipinski's rule of 5

Phytoconstituents (Ligands)	Parameters of Lipinski's Rule of 5 [#]						
	miLogP	TPSA	Natoms	MW	nON	nOHNH	nrotb
Arjunetin*	3.19	177.13	46	650.85	10	7	4
Arjungenin*	3.72	118.21	36	504.71	6	5	2
Arjunglucoside_I*	2.01	197.36	47	666.85	11	8	5
Arjunic_Acid	4.89	97.98	35	488.71	5	4	1
Butein	2.28	97.98	20	272.26	5	4	3
Catechin	1.37	110.37	21	290.27	6	5	1
Chrysin	2.94	70.67	19	254.24	4	2	1
Corilagin*	0.31	310.66	45	634.46	18	11	3
Ellagic_Acid	0.94	141.33	22	302.19	8	4	0
Epigallocatechin_Gallate	1.64	93.73	27	410.88	7	2	8
Epimedin_A1*	-0.26	317.36	59	838.81	20	11	12
Epimedin_B*	0.39	297.13	57	808.78	19	10	11
Epimedin_C*	0.97	297.13	58	822.81	19	10	11
Esculetin	1.02	70.67	13	178.14	4	2	0
Gallic_Acid	0.59	97.98	12	170.12	5	4	1
Kaempferol	2.17	111.12	21	286.24	6	4	1
Luteolin	1.97	111.12	21	286.24	6	4	1
Quercetin	1.68	131.35	22	302.24	7	5	1
Rhamnetin	2.22	120.36	23	316.26	7	4	2
Rutin*	-1.06	269.43	43	610.52	16	10	6
Taxifolin	0.71	127.44	22	304.25	7	5	1

miLogP- Octanol-water partition coefficient logP, **TPSA**-Topological polar surface area, **Natoms**-number of atoms, **MW**-molecular weight, **nON**-number of Oxygen Nitrogen, **nOHNH**-number of OH and NHn, **nrotb**-number of rotatable bonds and **violations**: number of rules violated; *: ligands that violated the Lipinski's Rule of 5.

The docking studies advocate that few functions are exhibited by all these 4 bioactives, which are true to arjuna extract. Mechanisms of the cardiotoxic functions include triggering the cascade of reactions that modulate the cardiac response to pressure overload and withstand stress or by functioning as the cardiac signaling effectors. This may be coupled with bronchodilation and smooth muscle relaxation too.

Molinspiration is a web-based tool used to predict the bioactivity scores of the shortlisted potential drug candidate compounds for activity with the human receptors such as GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors and enzyme inhibitors [12 & 13]. The bioactivity scores of all the ligands can be classified into three classes – Active (>0), Moderately Active (-5.0 – 0.0), Inactive (< -5.0). Ligands of Epimedin_A1, Epimedin_B and Epimedin_C were not found to be biologically active based on the properties of GPCR ligand, Ion channel modulator, Kinase inhibitor, Nuclear receptor ligand, Protease inhibitor and Enzyme inhibitor. Catechin, Arjunic Acid and Arjungenin showed highest bioactivity score followed by Luteolin, Quercetin, Chrysin, Kaempferol and Rhamnetin.

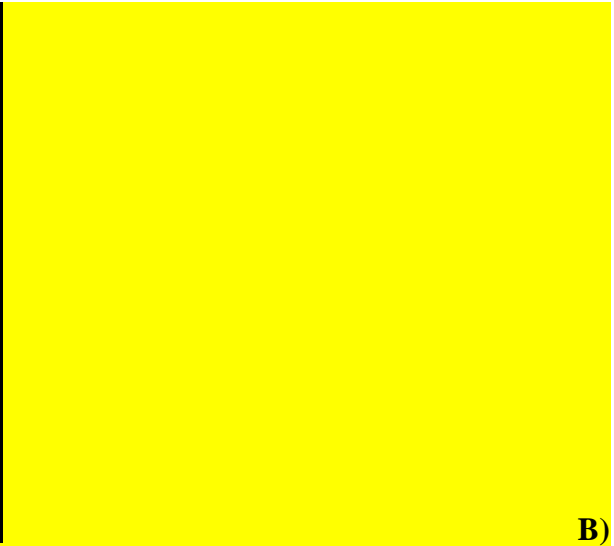
Table 5: Bioactivity scores of the selected phyto-constituents (ligands) from Arjuna

Phyto-constituents (Ligands)	Bioactivity Scores					
	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Arjunetin	-0.058	-0.917	-0.738	-0.022	0.086	0.095
Arjungenin	0.195	-0.229	-0.352	0.826	0.224	0.609
Arjunglucoside_	-0.175	-1.092	-0.846	-0.152	0.034	-0.012
Arjunic_Acid	0.237	-0.158	-0.384	0.839	0.221	0.6
Butein	-0.072	-0.108	-0.26	0.071	-0.273	0.114
Catechin	0.409	0.137	0.087	0.599	0.26	0.467
Chrysin	-0.106	-0.078	0.153	0.296	-0.303	0.262
Corilagin	-0.111	-0.707	-0.447	-0.441	-0.028	-0.15
Ellagic_Acid	-0.29	-0.266	-0.007	0.108	-0.178	0.165
Epigallocatechin_Gallate	-0.208	-0.529	-0.293	-0.364	-0.309	-0.281
Epimedin_A1	-2.129	-3.272	-2.872	-2.828	-1.661	-2.347
Epimedin_B	-1.703	-2.998	-2.493	-2.543	-1.342	-1.867
Epimedin_C	-1.91	-3.154	-2.7	-2.672	-1.481	-2.127
Esculetin	-1.046	-0.608	-1.06	-0.812	-1.167	-0.224
Gallic_Acid	-0.77	-0.255	-0.884	-0.519	-0.94	-0.173
Kaempferol	-0.1	-0.214	0.212	0.323	-0.272	0.264
Luteolin	-0.019	-0.067	0.259	0.388	-0.218	0.278
Quercetin	-0.06	-0.19	0.275	0.356	-0.248	0.28
Rhamnetin	-0.11	-0.272	0.214	0.274	-0.274	0.201
Rutin	-0.046	-0.518	-0.136	-0.233	-0.066	0.124
Taxifolin	0.086	0.025	-0.039	0.293	0.046	0.292

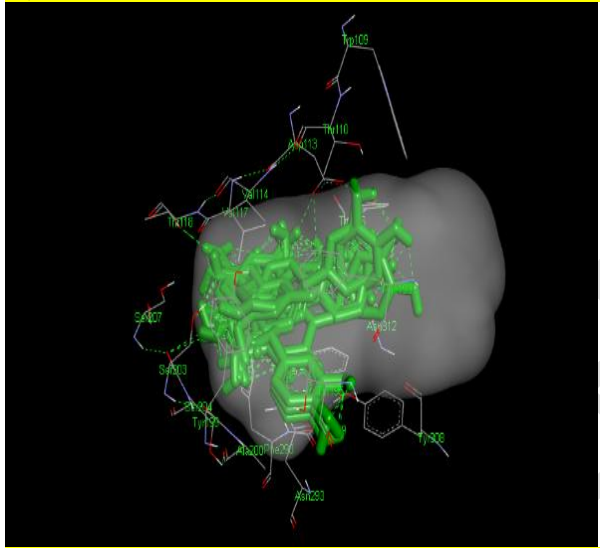
Quantitatively, Oxyjun® contains arjunetin, arjunglucoside-1, catechin, arjunic acid, arjungenin, luteolin, quercetin etc. arjunglucoside demonstrated good docking scores with the cell division control protein 42 (CDC42), β -adrenergic receptors, mitogen activated protein kinase-1 (MAPK1), vascular endothelial growth factor receptor-1 (VEGFR2) and peroxisome proliferator-activated receptor-gamma coactivator (PGC1 α) receptors. Thus arjunaglucoside 1 acts as a cardioprotective agent by its implications on the mechanisms involved with CDC42, which is involved in signaling effectors in the heart [14], or β -adrenergic receptors which mediate the physiologic responses such as smooth muscle relaxation and bronchodilation [15]. MAPK1 acts by initiating the cascade of signaling reactions that modulate the hypertrophic response of the heart to pressure overload [16], while VEGFR2 and PGC1 α are known to act by improving the epithelial function of the major vasculatures and enhancement of mitochondrial efficiency of cardiomyocytes, respectively [17]. Opioid receptors are known to be involved in enhancing the heart's ability to withstand stress. Arjungenin and Arjunetin also impart cardiovascular functions through multiple of the docking poses for top scoring compounds docked at the active site have been represented below (Figure 1).



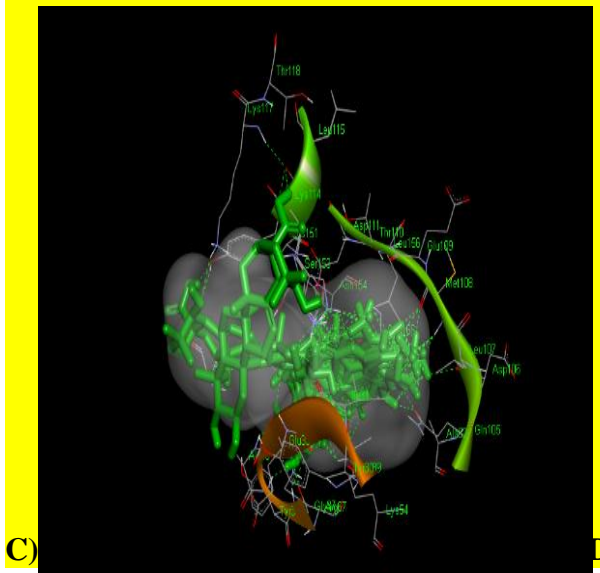
A)



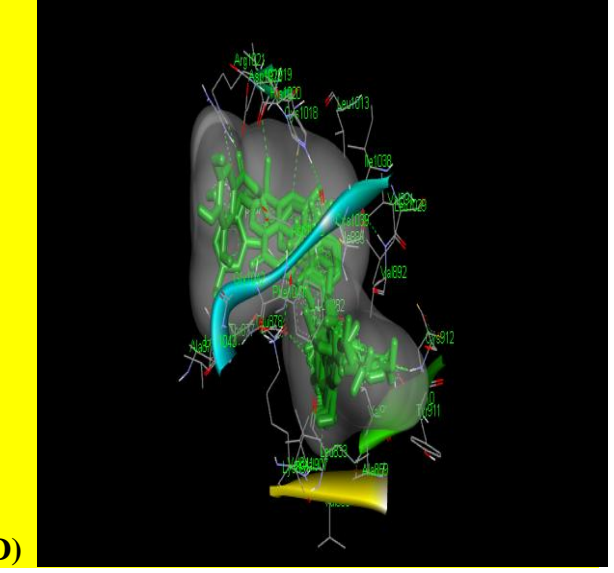
B)



C)



D)



4.0 CONCLUSION:

The docking scores, analysis of the interactions of the compounds suggest that most of the bioactives selected in this study, have the ability to bind to multiple targets involved in cardiovascular functions. Thus, this *in silico* study on the compounds of Oxyjun® (a standardized extract from *Terminalia arjuna* bark) indicates that compounds such as arjunic acid, arjungenin, arjunetin, arjunoglucoside¹, chrysin, kaempferol, luteolin, rhamnetin and taxifolin could be used as potential markers for biological activity. However, further *in vitro*, *in vivo* and quantification studies may be attempted to further establish the key bioactives in Oxyjun® that are claimed to benefit heart health and sports performance or their molecular and cellular roles.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References:

- 1 Oberoi, L., Akiyama, T., Lee, K. H., & Liu, S. J. (2011). The aqueous extract, not organic extracts, of *Terminalia arjuna* bark exerts cardiotoxic effect on adult ventricular myocytes. *Phytomedicine*, 18(4), 259-265.
- 2 Maulik, S. K., & Talwar, K. K. (2012). Therapeutic potential of *Terminalia arjuna* in cardiovascular disorders. *American Journal of Cardiovascular Drugs*, 12(3), 157-163.
- 3 Dwivedi, S., & Chopra, D. (2014). Revisiting *Terminalia arjuna*—an ancient cardiovascular drug. *Journal of traditional and complementary medicine*, 4(4), 224-231.
- 4 Kapoor, D., Vijayvergiya, R., & Dhawan, V. (2014). *Terminalia arjuna* in coronary artery disease: ethnopharmacology, pre-clinical, clinical & safety evaluation. *Journal of ethnopharmacology*, 155(2), 1029-1045.
- 5 Khaliq, F., & Fahim, M. (2018). Role of *terminalia arjuna* in improving cardiovascular functions: A review. *Indian Journal of Physiology and Pharmacology*, 62(1), 8-19.
- 6 Girandola, R. N., & Srivastava, S. (2017). Effect of E-OJ-01 on cardiac conditioning in young exercising adults: a randomized controlled trial. *American journal of therapeutics*, 24(3), e298-e307.
- 7 Gaillard, T., 2018. Evaluation of AutoDock and AutoDock Vina on the CASF-2013 benchmark. *Journal of chemical information and modeling*, 58(8), pp.1697-1706.
- 8 Lipinski, C. A. (2000). Drug-like properties and the causes of poor solubility and poor permeability. *Journal of pharmacological and toxicological methods*, 44(1), 235-249.
- 9 Veber, D. F., Johnson, S. R., Cheng, H. Y., Smith, B. R., Ward, K. W., & Kopple, K. D. (2002). Molecular properties that influence the oral bioavailability of drug candidates. *Journal of medicinal chemistry*, 45(12), 2615-2623.
- 10 Abad-Zapatero, C. (2007). A Sorcerer's apprentice and the rule of five: from rule-of-thumb to commandment and beyond. *Drug Discovery Today*, 12(23), 995-997.
- 11 Zhang, M.Q. and Wilkinson, B., 2007. Drug discovery beyond the 'rule-of-five'. *Current opinion in biotechnology*, 18(6), pp.478-488.
- 12 Proudfoot, J. R. (2002). Drugs, leads, and drug-likeness: an analysis of some recently launched drugs. *Bioorganic & medicinal chemistry letters*, 12(12), 1647-1650.

13 Jarrahpour, A., Motamedifar, M., Zarei, M., Youssoufi, M. H., Mimouni, M., Chohan, Z. H., & Hadda, T. B. (2010). Petra, osiris, and molinspiration together as a guide in drug design: predictions and correlation structure/antibacterial activity relationships of new N-Sulfonyl monocyclic β -lactams. *Phosphorus, Sulfur, and Silicon*, 185(2), 491-497.

14 Brown, J. H., Del Re, D. P., & Sussman, M. A. (2006). The Rac and Rho hall of fame: a decade of hypertrophic signaling hits. *Circulation research*, 98(6), 730-742.

15 Johnson, M. (2006). Molecular mechanisms of β 2-adrenergic receptor function, response, and regulation. *Journal of Allergy and Clinical Immunology*, 117(1), 18-24.

16 Muslin, A.J., 2008. MAPK signalling in cardiovascular health and disease: molecular mechanisms and therapeutic targets. *Clinical science*, 115(7), pp.203-218.

17 Rog-Zielinska, E.A., Craig, M.A., Manning, J.R., Richardson, R.V., Gowans, G.J., Dunbar, D.R., Gharbi, K., Kenyon, C.J., Holmes, M.C., Hardie, D.G. and Smith, G.L., 2015. Glucocorticoids promote structural and functional maturation of foetal cardiomyocytes: a role for PGC-1 α . *Cell Death & Differentiation*, 22(7), pp.1106-1116.

¹⁸ Mohanty, I.R., Borde, M. and Maheshwari, U., 2019. Dipeptidyl peptidase IV Inhibitory activity of Terminalia arjuna attributes to its cardioprotective effects in experimental diabetes: In silico, in vitro and in vivo analyses. *Phytomedicine*, 57, pp.158-165.

¹⁹ Khatkar, S., Nanda, A. and Ansari, S.H., 2016. Extraction, HPTLC Estimation, In silico and In vitro Evaluation of α -Amylase Inhibition Potential of Stem Bark of Terminalia arjuna. *Current Biomarkers (Formerly: Recent Patents on Biomarkers)*, 6(1), pp.47-53.

²⁰ Pugazhendhi, A., Shafreen, R.B., Devi, K.P. and Suganthi, N., 2018. Assessment of antioxidant, anticholinesterase and antiamyloidogenic effect of Terminalia chebula, Terminalia arjuna and its bioactive constituent 7-methyl gallic acid—an in vitro and in silico studies. *Journal of Molecular Liquids*, 257, pp.69-81.

²¹ Qaddir, I., Rasool, N., Hussain, W. and Mahmood, S., 2017. Computer-aided analysis of phytochemicals as potential dengue virus inhibitors based on molecular docking, ADMET and DFT studies. *Journal of vector borne diseases*, 54(3), p.255.

²² Ferdous, S., Mirza, M.U. and Saeed, U., 2013. Docking studies reveal phytochemicals as the long searched anticancer drugs for breast cancer. *Int. J. Comput. Appl*, 67, pp.1-5.

²³ Seal, A., Aykkal, R., Babu, R.O. and Ghosh, M., 2011. Docking study of HIV-1 reverse transcriptase with phytochemicals. *Bioinformation*, 5(10), p.430.

²⁴ Mekha Mohan, P.J., Valsalan, R. and Nazeem, P.A., 2015. Molecular docking studies of phytochemicals from Phyllanthus niruri against Hepatitis B DNA Polymerase. *Bioinformation*, 11(9), p.426.

²⁵ Ubani, A., Agwom, F., Morenikeji, O.R., Shehu, N.Y., Luka, P., Umera, E.A., Umar, U., Omale, S., Nnadi, E. and Aguiyi, J.C., 2020. Molecular docking analysis of some phytochemicals on two SARS-CoV-2 targets. *BioRxiv*.