RESEARCH ARTICLE

Promising Discovery of Alpha Amylase Enzyme Inhibitors from *Terminalia arjuna* for Antidiabetic Potential

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ABSTRACT

The aim of this work was to discover alpha amylase inhibitors from selected phytoconstituents of *Terminalia arjuna* by utilizing computational and *in-silico* methodology. Identified 37 phytoconstituents of *T. arjuna* were docked as test molecules. The complex structure of alpha-amylase in associated with Acarviostatin I03 was retrieved from protein data bank. Molecular screening by GA-based was performed to generate the cavity-enabled complex prototype using VLife MDS 4.4 software. Docking was used to identify the potent alpha-amylase inhibitor. Ligand-enzyme poses of the selected *T. arjuna* phytoconstituents identified seven potential biomolecules: baicalein, quercetin, gallic acid, kaempferol, pelargonidin, pyrocatechol and epicatechin with prominent interactions and showed better results than standard Acarviostatin I03 to the residues of alpha-amylase targets. interestingly, gallic acid showed multiple interactions as hydrogen bond, pi stacking and charge to the alpha-amylase target. This study reveals that phytoconstituents of *T. arjuna* possess promising antidiabetic potential. However, this research gives an appropriate platform for detecting newer inhibitors from plant sources for diabetic disease.

Keywords: Alpha-amylase, Antidiabetic potential, Molecular docking.

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INTRODUCTION

Diabetes mellitus (DM) is not a single disease but a disorder of metabolic consequences in humans and is seen over the entire world, including Asia, Europe, and America regions, where it has recently been noted as impacting 9.7% of the population. Nervous system deformities in diabetic patients are commonly observed. It has two forms: Type 1 insulin-dependent, mostly associated at onset in childhood, and severe require insulin therapy for survival. Type 2 is non-insulin dependent, associated with obesity, modern lifestyle, family history of disease and aging. It may be controlled by food and oral hypoglycaemic medicines.[1]

Alpha amylase is α-1, 4 glucan-4-glucanohydrolase fell under the hydrolases class which may be observed in plants, animals, and microorganisms. The alpha-amylase normally present in salivary secretions and pancreatic liquid hydrolyses alpha bonds of polysaccharides, especially starch and glycogen, which permit rapid glucose and maltose entry into systemic circulation. It is the most important amylase form seen in mammals including humans. Alpha amylase inhibition prolongs the consumption activity by obstructing the dissociation of starch in the gastrointestinal region, which is probably a prominent tactic for controlling high glycaemic situations.[2]

T. arjuna is a renowned medicinal plant with synonym 'Arjuna' in the ethnic applicability. *T. arjuna* may be widely used by the local tributes as an herbal medicine and holds several pharmacological actions, including antihypertensive, anti-inflammatory, anti-cancer, antiviral and antioxidants effects. *T. arjuna* comprises valuable phytochemicals in the type of secondary biproducts such as tannins, triterpenoids, minerals, flavonoids, glycosides and lipid molecules.[3]

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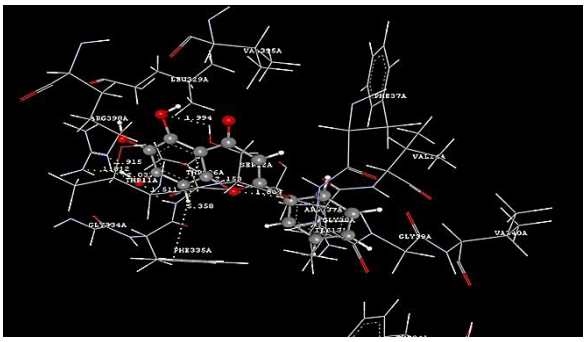

Figure 1: Baicalein binding interactions with alpha-amylase.

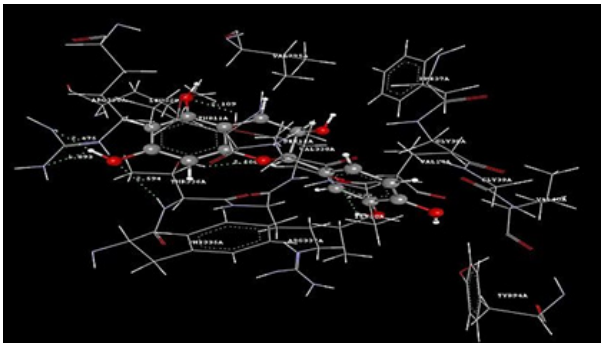

Figure 2: L-epicatechin binding interactions with alpha-amylase.

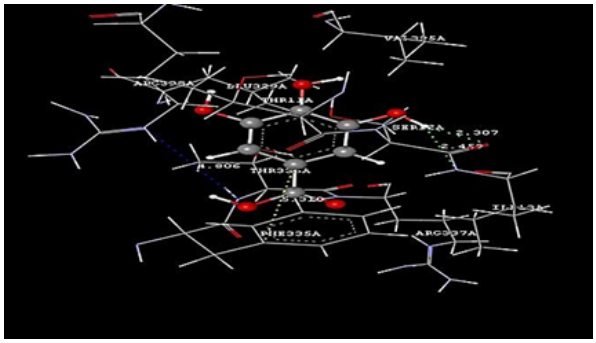

Figure 3: Gallic acid binding interactions with alpha-amylase.

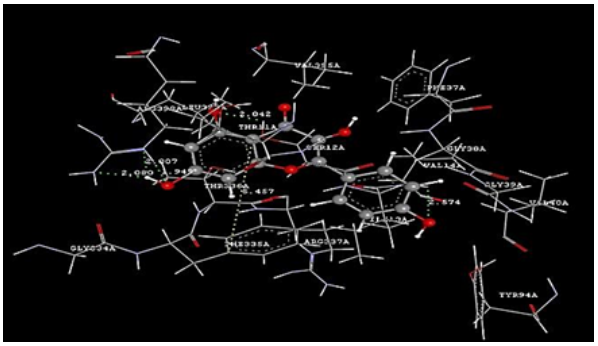

Figure 4: Kaempferol binding interactions with alpha amylase.

Molecular docking is a very advanced technology and performs crucial role in the identification of newer constituents and assists in detecting active targets. Computational screening is primarily applied for studying ligand-enzyme interactions and predict the affinity site of chemical with respective enzyme cavities to determine the binding and potency. Molecular docking method is more efficient and cost-effective compared to *in-vitro* and *in-vivo* experimental methods which provide prime binding modes of the ligand with known protein.[4,5]

In view of the above, present study was attempted to investigate the antidiabetic potential phytochemicals from *T. arjuna* which can interact and block the activity of alpha amylase enzyme by computational approach.

MATERIALS AND METHODS

Virtual Screening

Protein Refinement/Preparation

The complex structure of alpha-amylase in associated with Acarviostatin I03 was taken from RCSB (https://www.rcsb.org/) data depository with protein data bank (PDB) id 3OLD. The prominent resolution by XRD technique of complex structure was the criteria for enzyme selection. 3OLD is configuration of alpha amylase enzyme with standard acarviostatin I03 as target enzyme inhibitor. The files were retrived as pdb. For docking studies with selected phytochemical, the standard Acarviostatin I03 associated with active sites was separated from the complex enzyme structure. Water moieties were separated, and valency was adjusted by addition of polar hydrogens to the geometric structures for enzyme preparation employing software vLife MDS 4.4. The generated file was brought to mol^2 nature for further screening.

Ligands Criteria and Refinement

T. arjuna is very versatile and medicinally valued plant. It contains several important secondary metabolites which were selected as ligands. Reported 37 phytoconstituents of *T. arjuna* tree were detected from reported data.[6] Two dimensional chemical structures were procured from the PubChem databank as sdf file. These selected chemicals were changed to three dimensional mol^2 file and energy minimized employing the Merck Molecular Force Field option through software. Index of chosen phytochemicals of *T. arjuna* is shown in Table 1.

Parameters for Docking

A genetic algorithm (GA) methodology was utilized to relocate the one enzyme surface to the further enzyme surface towards the best surface suitability between each other.[7] GA type of docking was performed where thirty placements taken into consideration along with ten-degree rotation angle and selected best ten alignments by keeping non-flexible test chemicals.

Molecular Docking Analysis

To identify the most suitable alpha-amylase enzyme inhibitors, computational screening was used. The computational screening was performed by taking selected 37 phytochemicals of *T. arjuna* together with alpha-amylase structure. Inhibitory effects of the selected phytomolecules were studied. GA-based docking study was performed through biopredicta tool of software. After ligand enzyme docking, the cavity enabled

Table 1: Index of phytochemicals of *T. arjuna.*

Source	*Class*	*Phytochemicals*
Terminalia arjuna	Secondary metabolites	Arjunic acid[a]
		Arjungenin[a]
		Arjunin[a]
		Arjunolic acid[a]
		TermiarjunosideI[b]
		TermiarjunosideII[b]
		QuadranosideVIII[b]
		Arjunolone[b]
		Arjunetin[b]
		ArjunglucosideII[b]
		(l)-epicatechin[c]
		Arjunone[c]
		Luteolin[c]
		Kaempferol[c]
		Baicalein[c]
		Ethyl gallate[c]
		Oligomeric proanthocyanidins[c]
		Gallic acid[c]
		Ellagic acid[c]
		Pelargonidin[c]
		Quercetin[c]
		Pyrocatechols[d]
		TerflavinC[d]
		Punicallin[d]
		Castalagin[d]
		Punicalagin[d]
		Casuariin[d]
		Terchebulin[d]
		Casuarinin[d]
		Terminolic acid[e]

a-triterpenoids, b-ursane triterpenoids and glycosides, c-flavonoids and phenolics, d-tannins, e-other compounds

complex prototype was generated based on various aspects, including interactions *via* pi-stacking, intermolecular hydrogen bonding, and charge interactions, and best-fitted poses inside the target site, including binding score.[8-11]

RESULTS AND DISCUSSION

Virtual Screening

Alpha amylase residues THR11A, SER12A, ILE13A, ARG195A, TYR231A, THR336A and MET394A were observed to be crucial areas for binding sites and associated towards ligand-enzyme interactions. All residues participated through hydrogen bonding. A total output of all types of interactions were believed to be excellent for virtual selection.

Molecular Docking Analysis

Out of total 37 phytoconstituents of *T. arjuna*, molecular docking resulted in seven potential biomolecules: baicalein, quercetin, gallic acid, kaempferol, pelargonidin, pyrocatechol and epicatechin with prominent associations and docking score than standard acarviostatin I03 to the residues of alpha-amylase targets. The obtained geometrical poses of the test molecules have mainly shown ionic, aromatic and hydrogen bonding interactions with the enzyme counterparts. Baicalein has shown interactions *via* hydrogen bonding to SER12A, ILE13A, THR336A, ARG398A and ARG398A and pi-stacking with PHE335A residues of the target enzyme. The binding interactions of baicalein with alpha-amylase was shown in Figure 1.

L-epicatechin has showed interactions *via* only hydrogen bonding to SER12A, ILE13A, THR336A and ARG398A residues of the target enzyme. The binding interactions of epicatechin with alpha-amylase was shown in Figure 2.

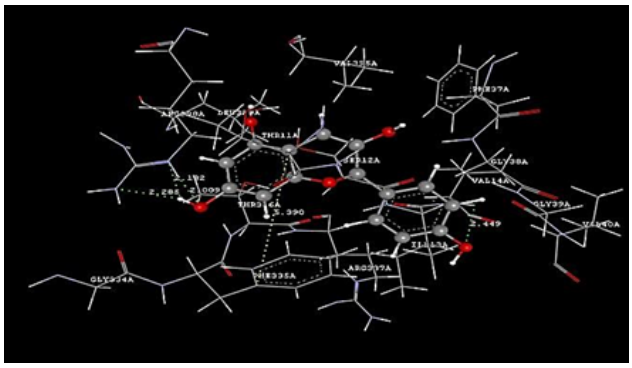

Figure 5: Pelargonidin binding interactions with alpha-amylase.

Figure 6: Pyrocatechol binding interactions with alpha-amylase.

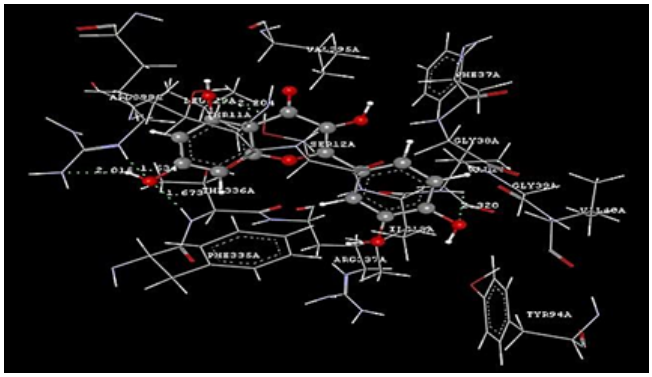

Figure 7: Quercetin binding interactions with alpha-amylase.

Table 2: Binding energy and various interactions with residue in alpha-amylase.

S no	*Phytomolecule*	*BE in kcal/mol*	*H -Bond distance in Å*	*Pi-Stick distance in Å*	*Charge distance in Å*
1	Baicalein	-4.331978	1.994 1.804 1.511 2.153 1.915 1.812 2.037	5.358	-
2	Epicatechin	-4.408502	2.109 2.222 2.594 2.508 2.475 1.893	-	-
3	Gallic Acid	-4.974751	2.307 2.459	5.310	4.806
4	Kaempferol	-4.468944	2.042 2.574 2.007 2.080 1.949	5.457	-
5	Pelargonidin	-4.739519	2.449 2.182 2.285 2.009	5.390	-
6	Pyrocatechol	-4.698799	-	4.271	-
7	Quercetin	-4.901004	2.204 2.320 1.673 2.015 1.634	-	-
8	Standard-Acarvivostatin	-4.222003	1.977 1.514 2.284 2.209 1.594 2.559 2.537 1.775	-	-

Gallic acid has associated by multiple interactions viz. hydrogen bonding to SER12A and ILE13A, pi-stacking by PHE335A and ionic interactions with ARG398A residues of the target enzyme. Binding interactions of Gallic Acid with alpha-amylase was shown in Figure 3.

Kaempferol has showed interactions *via* hydrogen bonding to PHE335A, SER12A, VAL14A and ARG398A with pi-stacking to PHE335A residues of the target enzyme. Binding interactions of kaempferol with alpha-amylase was shown in Figure 4.

Pelargonidin has shown interactions *via* hydrogen bonding to VAL14A and ARG398A with pi-stacking to PHE335A residues of the target enzyme. Binding interactions of pelargonidin with alpha-amylase was shown in Figure 5.

Pyrocatechol has been associated by only pi-stacking to PHE335A residues of the target enzyme. Binding interactions of pyrocatechol with alpha-amylase was shown in Figure 6.

Quercetin has showed interactions *via* only hydrogen bonding to SER12A, VAL14A, THR336A and ARG398A residues of the target enzyme. Binding interactions of quercetin with alpha-amylase was shown in Figure 7.

Binding energy (BE) and various interactions with residue in alpha-amylase were shown in Table 2.

Interestingly, gallic acid showed multiple interactions as hydrogen bonds, pi-stacking and charge to the alpha-amylase target. This research emerges as an option to the expensive laboratory methodologies and has offered a cost-effective seven lead compounds baicalein, L-epicatechin, gallic acid, kaempferol, pelargonidin, pyrocatechol and quercetin for the treatment of a diabetic disorder.

CONCLUSION

The *T. arjuna* tree consists of versatile medicinal properties mentioned in the alternative medicine system. The computational approach aiming to deliberate the intermolecular

associations of *T. arjuna* phytochemicals on diabetic enzyme target is still under study. Hence, current research was carried out for identified phytochemicals from *T. arjuna* towards alpha-amylase enzyme with docking. This research resulted in important highlights on the screening of antidiabetic inhibitors. A computational study revealed seven prominent phytochemicals baicalein, L-epicatechin, gallic acid, kaempferol, pelargonidin, pyrocatechol and quercetin with antidiabetic potential. Overall, this research gives an appropriate platform for detecting newer inhibitors from plant sources for diabetic disease.

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