



In silico analysis reveals α -amylase inhibitory potential of Taraxerol (*Coccinia indica*) and Epoxywithanolide-1 (*Withania coagulans*): a possible way to control postprandial hyperglycemia-induced endothelial dysfunction and cardiovascular events

Lokesh Ravi¹ · Venkatesh Sadhana² · Pratishtha Jain³ · Shree Kumari Godidhar Raghuram⁴ · Mohanasrinivasan Vaithilingam⁴ · Reji Manjunathan^{5,6} · Ajith Kumar Krishnan⁷ · Mookkandi Palsamy Kesavan⁸

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Abstract

Postprandial hyperglycemia (PPG) exacerbates endothelial dysfunction and impairs vascular function in diabetes as well in healthy people. Though synthetic drugs are available to regulate PPG, the severe gastrointestinal side effects of those medications have prompted the search for alternative treatments. Recently, some phytochemicals captured the attention because of their inhibitory effects on α -amylase to control diabetes. The aim of this study was to investigate and identify potential alpha-amylase inhibitors in *C. indica* and *W. coagulans*. This study also aims to understand one of the possible mechanisms of action of plants for their anti-diabetic activity. A total of 36 phytochemical ligands were subjected for protein–ligand docking analysis. Among the phytochemicals, Taraxerol and Epoxywithanolide-I demonstrated significant binding free energy of -10.2 kcal/mol and -11.9 kcal/mol respectively, which was higher than the reference acarbose with -8.6 kcal/mol. These molecules were subjected for molecular dynamics simulation (MDS) analysis with alpha-amylase protein for a duration of 150 ns. Among the three complexes, Taraxerol and Epoxywithanolide-I complexes demonstrates strong potential as inhibitors of the target protein. MDS results were analyzed via root mean square deviation (RMSD), fluctuation of residues, potential energy, radii of gyration and solvent access surface area analysis. Taraxerol demonstrated a significantly low potential energy of $-1,924,605.25$ kJ/mol, and Epoxywithanolide-I demonstrated $-1,964,113.3$ kJ/mol of potential energy. RMSD plot shows that Epoxywithanolide-I has much higher stability than the other MDS complexes. Drugability and toxicity studies show that the test ligands are demonstrating strong potential as drug like molecules. The results of the study conclude that, Taraxerol of *C. indica* and Epoxywithanolide-I of *W. coagulans* are strong inhibitors of alpha-amylase enzyme and that, this is one of the possible mechanisms of action of the plants for their reported anti-diabetic activities. Further *in-vitro* analysis is in demand to prove the observed results.

Keywords Alpha-amylase · Phytochemicals · *Coccinia indica* · *Withania coagulans* · GROMACS · PyRx · ADMET

✉ Lokesh Ravi
lokesh.ravi.fst@gmail.com

¹ Department of Food Technology, Faculty of Life and Allied Health Sciences, MS Ramaiah University of Applied Sciences, Bengaluru, Karnataka 560054, India

² Department of Chemistry, Atria Institute of Technology, Bangalore, Karnataka 560024, India

³ Department of Biotechnology, Faculty of Life and Allied Health Sciences, MS Ramaiah University of Applied Sciences, Bengaluru, Karnataka 560054, India

⁴ School of Biosciences and Technology, Vellore Institute of Technology, Vellore, Tamil Nadu 632014, India

⁵ Multi-Disciplinary Research Unit, Kottayam Government Medical College and Hospital, Kottayam, Kerala, India

⁶ Dr. ALM Postgraduate Institute of Basic Medical Science, University of Madras, Taramani Campus, Chennai, Tamil Nadu 600113, India

⁷ Department of Life Sciences, Kristu Jayanti College (Autonomous), Bengaluru, Karnataka 560077, India

⁸ Department of Chemistry, Hajee Karutha Rowther Howdia College, Uthamapalayam, Tamil Nadu 625533, India

Introduction

Indian subcontinent is a rich place of medicinally valuable plants that have been incorporated in the people's lifestyle and culture for several centuries. Medicinal plants such as 'Ivy Gourd' and 'Indian Rennet' have been part of Indian tradition for a very long time. *Coccinia indica* known as Ivy Gourd and *Withania coagulans* known as Indian Rennet are popular plants with several medicinal and pharmaceutical benefits ranging from antidiabetic, anticancer, and antibacterial activities (Ocvirk et al. 2013; Mahla et al. 2021). Traditionally people were using these plants as part of nutrition and as part of treating symptoms of diabetes and hyperglycemia. Recent literature evidences indicate that, these plants do indeed possess antidiabetic potential, as they have proven to regulate blood sugar level, and proven to inhibit several key enzymes involved in diabetes and blood sugar levels (Hemalatha et al 2004; Mallick et al. 2007; Doss and Rangasamy 2008; Maurya et al. 2010; Gunjan et al. 2010).

Antidiabetic effect of these two plants is reported via several animal model studies. A study by Mallick et al. (2007) proved that combined aqueous leaf extracts of *Musa paradisiaca* and *C. indica* contain antidiabetic activity via streptozotocin-induced diabetes rat model. Gunjan et al. (2010) examined the antidiabetic activity of the ethanolic extract of *C. indica*, which gradually decreased the blood glucose levels in streptozotocin induced diabetes rats (100/200 mg/kg). Results also suggest that chronic administration of *C. indica* fruit extract (200 mg/kg for 14 days) can reduce the glucose level in alloxan-induced diabetic rat. Study by Doss and Rangasamy (2008) reported that aqueous extract of *C. indica* can reduce the blood glucose, cholesterol, protein and urea in diabetes induced rats and it can also stimulate gluconeogenesis in liver of the study animals. A report by Maurya et al. (2010) stated that the coagulanolide (withanolide) obtained from *W. coagulans* fruit possess anti-hyper glycemc activity in rat model study. Study by Hemalatha et al. (2004) on *W. coagulans* also showed hypoglycemic (anti-hyperglycemic) activity using streptozotocin induced diabetes rats.

Majority of reports on *C. indica* and *W. coagulans* are only crude analysis of their antidiabetic role and does not narrow down on the mechanism of action of the effect. Crude plant extracts of such medicinal plants are a cocktail of bioactive phytochemicals that each have their own mechanism of action. The overall anti-hyperglycemic activity of the plant reported till date, could be due to the synergistic effect, that is a product of combined action of several bioactive phytochemicals present in these plants. Hence, this gives sparse understanding into, the key bioactive phytochemical and the mechanism of action that it

takes to induce such effect. This current study is an investigation to understand one of the possible mechanisms of action of these plants by inhibiting the alpha-amylase enzyme, that is mainly responsible for digestion of polysaccharides into monomeric carbohydrate molecules.

Elevated blood glucose levels after a meal, known as postprandial hyperglycemia, can cause endothelial dysfunction, which is a prelude to cardiovascular events. This disorder develops when elevated glucose levels cause inflammation and oxidative stress, which compromise the ability of endothelial cells to line blood vessels. As a result, there is a disturbance in the function of blood vessels, which lowers the availability of nitric oxide and increases the risk of thrombosis, inflammation, and vasoconstriction. As a result, people who experience frequent postprandial hyperglycemia are more likely to develop hypertension, atherosclerosis, and other cardiovascular diseases, which in turn increases the risk of adverse cardiovascular events like heart attacks and strokes (Ansar et al. 2011; Ceriello 2005; Eunice et al. 2011).

Although there are several possible mechanisms of actions that could induce the said anti-hyperglycemic activity, this study focuses on alpha-amylase as a drug-target molecule, since it is currently the major target in the field of diabetes management. Due to the presence multiple reference drugs that already are known to inhibit alpha-amylase enzyme, the work could be justified and can further be investigated for in-vitro and in-vivo applications without any harmful side effects (Demir et al. 2019; Papoutsis et al. 2021). Although several commercial synthetic drugs are available for anti-hyperglycemic activity, these synthetic drugs exert severe side effects in the patients, causing researchers to search for alternate approach in treatment options from natural sources without any severe side effects. Phytochemical sources such as medicinal plants are well known for their health benefits, without any severe impact on the physiological activities. This study was designed to result in identification of a lead molecule that can largely benefit the pharmaceutical industries which are turning towards phytochemistry for drug design and development. Natural products such as phytochemicals, possess increased bioactivity and bioavailability with reduced side-effects and high detoxification potential. Hence, pharma industries are currently focusing on developing new drugs from plant-based molecules.

A vital digestive enzyme called α -amylase helps the intestine to absorb complex carbohydrates like starch and glycogen by digesting them into simpler sugars like maltose and glucose. Maintaining normal blood glucose levels and general metabolic health depend heavily on its function. Normal functioning of α -amylase enzyme in a diabetic patient after food consumption could aggravate postprandial hyperglycemia, which is a major risk factor for cardiovascular and

type-2 diabetes. Hence, blocking α -amylase's activity in diabetes mellitus patients, offers scope for controlling postprandial hyperglycemia. For this reason, α -Amylase inhibitors are a prime target for investigations aimed at creating functional foods and therapeutic agents for improved glycemic control since they can help manage blood sugar spikes after meals by slowing down the digestion of carbohydrates. This lowers the risk of complications related to diabetes. Several natural products (phytochemicals) have already been established as α -amylase inhibitors, from various medicinal plant sources. Lectin-like inhibitors such as α -AI1 from *Phaseolus vulgaris*, Penta-O-Galloyl-B-Glucocopyranose from *Rhus coriaria* L., Glochidion from *Glochidion ferdinandi*, and Ellagic Acid from *Rubus subg. Rubus* and *Punica granatum* are some of the well established α -amylase inhibitors from plant sources via in-vitro experimentation (Ćorković et al. 2022; Hilda et al. 2015; Kalinovskii et al. 2023; Kashtoh and Baek 2023; Lijun et al. 2020; Lo Piparo et al. 2008).

Applications of computational methods are increasingly common and are becoming an essential component of drug discovery process. Protein–ligand molecular docking acts as a vital part in screening of large library of ligands and datasets against a given protein drug target, which upon narrowing down the number of hits, can further be investigated in detail for validation. Although protein–ligand molecular docking is one of the preliminary methods in computational drug discovery, it is not the finalizing technique. Molecular dynamics (MD) simulation is considered to be the current state of the art technique in computational drug discovery, where results of protein–ligand docking are further investigated in detail for validation and confirmation. Molecular dynamics (MD) is a simulation method that mimics the actual motions of atoms and molecules in a given system. It makes it possible to investigate the structural, dynamic, and thermodynamic characteristics of biomolecules by offering in-depth insights into molecular interactions and conformational changes over time. To comprehend a compound's binding mechanisms and atomic-level inhibitory potential, molecular dynamics simulations are indispensable. For these reasons, MD simulation based approach is considered to be the standard and required approach in confirmation of mechanism of action and inhibitory potential of drugs with their respective targets (Dutta and Ravi 2024; Ravi et al. 2023; Shukla and Timir 2021).

In this study phytochemical secondary metabolites reported to be present in *C. indica* and *W. coagulans*, were screened against the protein drug-target α -amylase enzyme, to identify potential drug molecules that can be further exploited for pharmaceutical application and also would provide understanding on the mechanism-of-action of *C. indica* and *W. coagulans* for their reported anti-diabetic ability. For this purpose, the study employs computational approaches such as protein–ligand docking and Molecular

Dynamic Simulation (MDS) to predict and propose potential phytochemical inhibitors from *C. indica* and *W. coagulans*.

Materials and methods

Curation of phytochemical

List of phytochemicals present in target plants i.e., *C. indica* and *W. coagulans* were retrieved from literature survey and database analysis. Chemical structure of the reported phytochemicals was retrieved from PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) in the form of.sdf (Structured Data File format, used for 2D and 3D chemical structures) file format and were stored for further analysis.

Ligand and protein preparation

Energy minimization and charges for all ligands were calculated using the in-built Open Babel tool present in the PyRx tool. Energy minimization was done using the ‘uff’ forcefield and ‘Conjugate Gradients’ algorithm. Processed ligand files were saved as.pdbqt format. The three-dimensional structure of the target protein (i.e., alpha-amylase) was downloaded from the Protein Data Bank website (<http://www.rcsb.org>). The downloaded protein structure was processed by removing co-crystallized molecules and solvents. The macromolecule structure was also subjected for energy minimization and was saved as.pdbqt format for further analysis. PyMOL visualization tool was used to investigate the 3D structure of ligand and protein. PyMOL was also used to visualize the binding pocket residues of the protein (Soudani et al. 2020; Egbuna et al. 2021).

Molecular docking analysis

Protein–ligand docking analysis were performed via AutoDock Vina using PyRx tool. Grid box for the docking was set to cover the active site/binding site residues identified from the interaction analysis of co-crystallized ligand. Results of the docking analysis were visualized using PyMOL and LigPlot+ tools, that were accessed via educational license obtained from the provider. Two-dimensional interaction analysis was performed using LigPlot+, while three-dimensional visualization and analysis was performed using PyMOL software (Soudani et al. 2020; Egbuna et al. 2021).

Molecular dynamic simulation

Molecular dynamic (MD) simulation study was performed using GROMACS, on UBUNTU operating-system (18.04 LTS). The phytochemical ligands (Taraxerol &

Epoxywithanolide-I) that exhibited most significant interaction with the receptor molecule (alpha-amylase) in docking were investigated further via MDS, in addition to acarbose as reference ligand. Simulation of the independent protein molecule was also performed to obtain a better understanding and base line of the stability of the independent protein. CHARMM-36 all atom force field was employed to carry out the simulation. TIP 3P solvent system was used for this study.

The receptor was converted to GROMACS recognizable structure file and a topology was generated via GROMACS commands. Structure file of the ligands were obtained using the CGENFF server used along with a python script to generate the GROMACS structure file of the ligand as well as for the generation of ligand topology and parameters. The ligand topology and parameters were manually added to the topology of the protein through a text editor and the complex file was generated by combining the co-ordinates of both the protein and the ligand structure file. The receptor-ligand complex was enclosed in a dodecahedron system of solvent molecules and the system was solvated. An appropriate number of positively charged sodium ions were added to stabilize the charge on the system. Energy minimization was carried out and equilibration was achieved through the NVT and NPT ensemble. Finally, the simulation was set up for a period of 150 ns (100,000 ps). The results of the simulation were analyzed by extracting the data for RMSD, RMSF, H-bond, potential energy, SASA, and radii of gyration of all the MDS complexes and by plotting graph to compare and contrast the variation (Nayeem et al. 2021).

ADMET analysis of ligands

Absorption, distribution, metabolism, excretion and toxicity (ADMET) and drugability analysis of the test ligands and reference ligand were predicted using online webtools, i.e., molinspiration (<https://molinspiration.com/>) and SwissADME (<http://www.swissadme.ch/>). The predicted physicochemical properties were retrieved and tabulated for comparison and analysis (Ben et al., 2021).

Results

Target protein and binding site

Pancreatic alpha-amylase protein of human, co-crystallized with acarbose (PDB ID: 2QV4) was chosen as the protein drug target to perform this in-silico screening. Initial investigation of PDB crystal structure of the protein along with its known inhibitor, revealed the binding site/active site of the protein, that was used as the docking site to perform this investigation. The site where the acarbose was bound to, is composed of Ile-051, Trp-058, Trp-059, Glu-060, Tyr-062,

Gln-063, His-101, Gly-104, Asn-105, Ala-106, Val-107, Tyr-151, Leu-162, Thr-163, Gly-164, Leu-165, Arg-195, Asp-197, Ala-198, Lys-200, His-201, Glu-233, Ile-235, Asn-298, His-299, Asp-300 and His-305. The position of acarbose molecule and conformation of the active site and its residues are shown in Fig. 1. The site where acarbose was bound to in the protein crystal structure (PDB ID: 2QV4) was adopted as binding site for the current docking study.

Protein–ligand docking

Protein–ligand docking analysis was performed using AutoDock Vina algorithm, inbuilt within PyRx tool. A total of 37 ligands (Acarbose as reference ligand, 14 ligands from *C. indica* & 22 ligands from *W. coagulans*) were docked with the targeted protein within the highlighted binding site. Results of the protein–ligand docking analysis are tabulated in Table 1. Three-dimensional and two-dimensional analysis of protein–ligand interactions of the most significant ligands are represented in Figs. 2 and 3 respectively. The reference ligand Acarbose (41,774) demonstrated a strong binding interaction with the target protein with a binding free energy of -8.6 kcal/mol and forming 7 hydrogen bonds with Gln-063, Asn-105, Thr-163, Asp-197, Glu-233, His-299 and Asp-300. Three-dimensional representation of acarbose-amylase interaction is shown in Fig. 2 and two-dimensional representation of their interaction is shown in Fig. 3A. Among the 14 ligands of *C. indica* most significant interaction was demonstrated by Taraxerol (Pubchem ID: 92097) with a binding free energy of -10.2 kcal/mol without the formation of any hydrogen bonds. Taraxerol demonstrated 11 hydrophobic interaction with Trp-58, Trp-59, Tyr-62, Gln-63, Leu-162, Thr-163, Leu-165, Asp-197, Ala-198, Glu-233, and Asp-300, making significant non-polar interactions with the protein target. Three-dimensional representation of Taraxerol-Amylase interaction is shown in Fig. 2 and two-dimensional representation of their interactions is shown in Fig. 3B. Among the 22 ligands of *W. coagulans* most significant interaction was demonstrated by Epoxywithanolide-I (Pubchem ID: 10,790,456) with an exceptional binding free energy of -11.9 kcal/mol and formation of 3 hydrogen bonds with Thr-163 (2.61\AA), Asp-197 (3.09\AA) and Glu-233 (2.88\AA) and formation of 11 hydrophobic interactions with Trp-59, Tyr-62, Gln-63, Tyr-151, Leu-162, Leu-165, Ala-198, Lys-200, His-201, Ile-235, and Asp-300, making a strong polar and non-polar interactions with the protein target. Three-dimensional representation of Epoxywithanolide-I-Amylase interaction is shown in Fig. 2 and two-dimensional representation of their interactions are shown in Fig. 3C. Based on the docking analysis, Taraxerol from *C. indica* and Epoxywithanolide-I of *W. coagulans* were

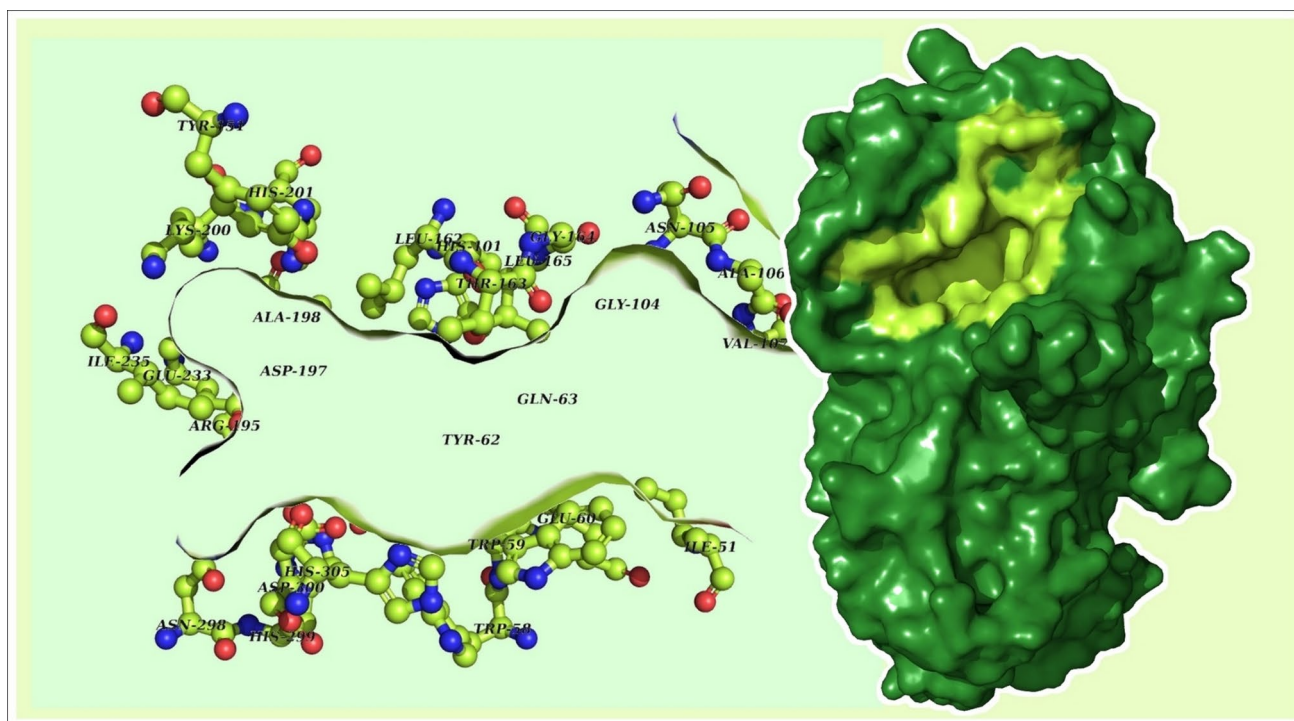


Fig. 1 Binding site analysis of pancreatic alpha-amylase enzyme (2QV4). The allosteric sites of the protein are represented in forest green color, while the active site is highlighted in limon green color

identified as the most significant ligands and were further subjected for detailed analysis as potential alpha-amylase inhibitors in their respective plants.

Molecular dynamics simulation

Molecular dynamics simulation (MDS) analysis for a time period of 150 ns was performed to study the stability of the protein–ligand complex and to understand the ability of the selected phytochemicals to be a potential inhibitor of alpha-amylase. A total of three different MDS (150 ns each) were performed i.e., 1. Alpha-Amylase with Acarbose; 2. Alpha-Amylase with Taraxerol; 3. Alpha-Amylase with Epoxywithanolide-I. The results of all the MDS were compared to understand the ability of the tested phytochemicals to inhibit the alpha-amylase protein when in comparison to known inhibitor acarbose. The results of the MDS were analyzed by means of RMSD, RMSF, SASA, potential energy, hydrogen bonds and protein gyration.

Root means square deviation (RMSD)

RMSD of the protein backbone structure for the 3 MDS combinations are graphically represented in Fig. 4A. Initial observation strongly suggests that, among the 3 combinations of MDS, the most stabilized protein structure with

least and lowest fluctuation of backbone RMSD was demonstrated by alpha-amylase enzyme when in combination with Epoxywithanolide-I ligand. Taraxerol demonstrated good complex stability up to 70 ns of the MDS, after which the structure demonstrated very high fluctuation in the RMSD ranging up to 3.4 Å, which was highest among the 3 MDS complex. However, the stability of the complex increased over time and reached 2.2 Å at the end of 150 ns simulation. Epoxywithanolide-I demonstrated much significant complex stabilization with lowest RMSD of 1.8 Å towards the end of the 150 ns MDS, while, the reference molecule also demonstrated a lowest RMSD of 1.8 Å towards the end of 150 ns MDS. RMSD graph in Fig. 3A strongly suggests that, among the 2 test ligands, Epoxywithanolide-I shows promising inhibition potential, on par with the reference molecule acarbose.

Root mean square fluctuation (RMSF) of residues

RMSF analysis of individual residues in the protein structure for all 3 MDS complexes are represented in Fig. 4B. It is evident that, Epoxywithanolide-I strongly reduces the RMSF of interacting residues (i.e., Asn-100 to Lys-200) than the reference ligand acarbose itself. This strongly suggests that the test ligand Epoxywithanolide-I is imposing great restraint on the flexibility of the interacting residues at the active site of the protein, thereby conferring a much

Table 1 Protein–ligand docking analysis of phytochemicals against alpha-amylase protein

Chemical	PubChem ID	Docking binding free energy (kcal/mol)
Acarbose	41,774	– 8.6
<i>Coccinia indica</i>		
Taraxerol	92,097	– 10.2
Daucosterol	5,742,590	– 10.2
Beta-carotene	5,280,489	– 10
Beta-amyrin	73,145	– 9.8
Karounidiol	159,490	– 9.8
Taraxerone	92,785	– 9.7
Lupeol	259,846	– 9.2
Delta-7-stigmastene-(3)	5,748,344	– 9.2
Erythrodiol	101,761	– 9.1
Betulin	72,326	– 9
29-Hydroxy lupeol	489,919	– 8.9
Caffeic-acid	689,043	– 6.7
Citrulline	9750	– 5.3
5-Methylcytosine	65,040	– 5.2
<i>Withania coagulans</i>		
14,15beta-Epoxywithanolide I	10,790,456	– 11.9
20beta-Hydroxy-1-oxo-(22R)-I-2,5,24-trienolide	637,266	– 11.2
17beta-Hydroxywithanolide K	44,562,998	– 11.1
Coagulin C	44,562,952	– 11
Withacoagulin	12,115,994	– 10.9
24,25-Dihydrowithanolide D	23,266,167	– 10.9
Withacoagin G	102,190,729	– 10.8
Withacoagin I	102,190,731	– 10.5
Coagulin F	24,941,991	– 10.4
Coagulin B	101,936,450	– 10.3
3beta-Hydroxy-2,3-dihydrowithanolide F	135,887	– 10
Coagulin E	15,341,418	– 10
Coagulanolide	44,562,997	– 10
Sitosterol glucoside	70,699,351	– 10
Withacoagulin H	71,524,298	– 9.9
Withacoagin H	102,190,730	– 9.9
Coagulin D	101,936,452	– 9.8
Coagulin R	15,977,628	– 9.6
Coagulin M	100,920,595	– 9.6
Coagulin J	15,968,792	– 9.2
Coagulin I	15,969,311	– 9.2
Coagulin G	24,941,992	– 9.1

stronger protein–ligand complex stabilization than the reference ligand. The most significant restraint was imposed on Asn-152 residue, which demonstrated 2.8 Å with Acarbose, 3.2 Å with Taraxerol and 2.4 Å with Epoxywithanolide-I. However, the key active residue of this protein structure is annotated to be Asp-197, for which a RMSF 0.7 Å with Acarbose, 0.6 Å with Taraxerol and 0.7 Å with Epoxywithanolide-I. Highest fluctuation in this study was shown by

residue 146 in when combination with Taraxerol, with a maximum of 6.7 Å. This RMSF analysis shows that, both the test ligands, Taraxerol and Epoxywithanolide-I instills rigidity to the protein structure, conferring a stronger protein–ligand complex and stability, when compared to the reference ligand Acarbose.

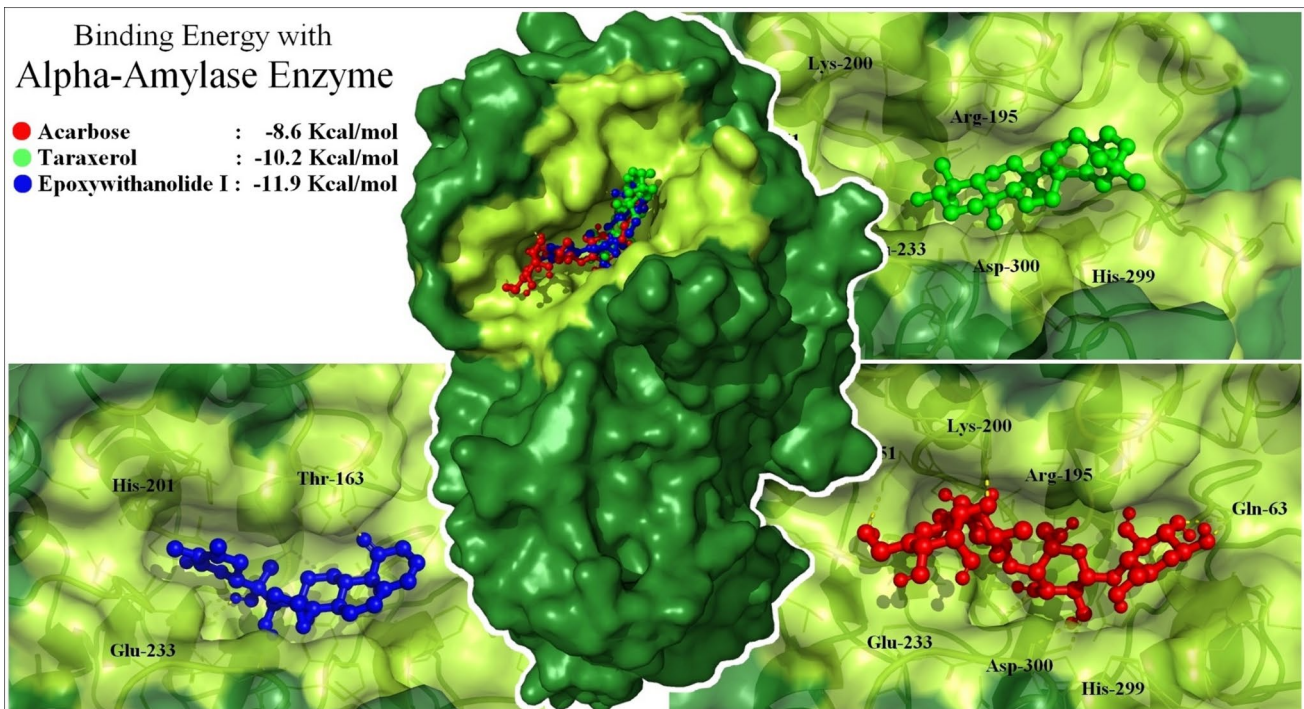


Fig. 2 Protein–ligand docking analysis with alpha-amylase protein; interaction with Acarbose, Taraxerol & Epoxywithanolide I

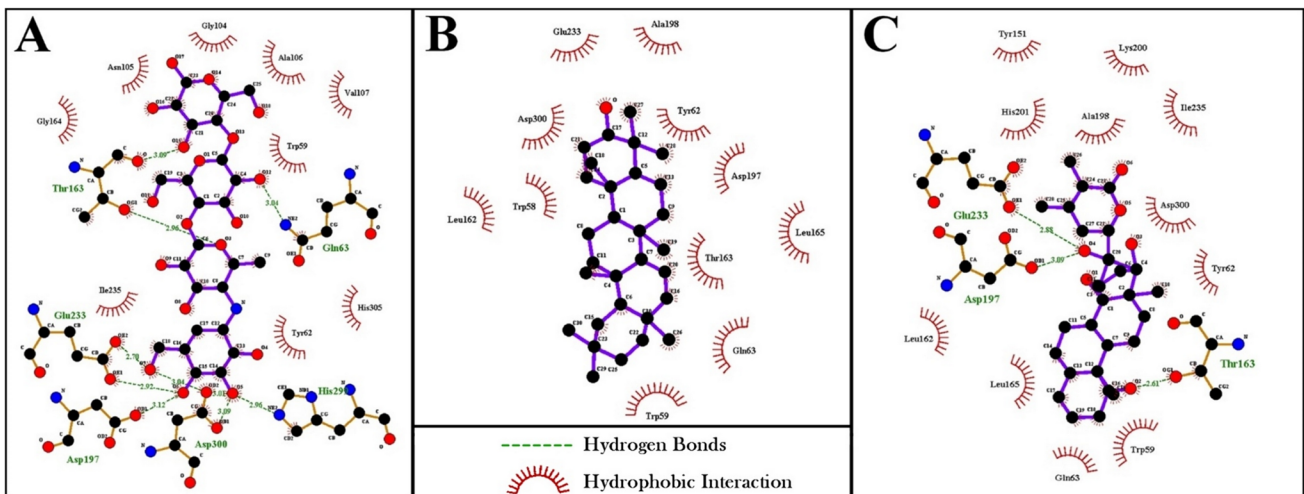


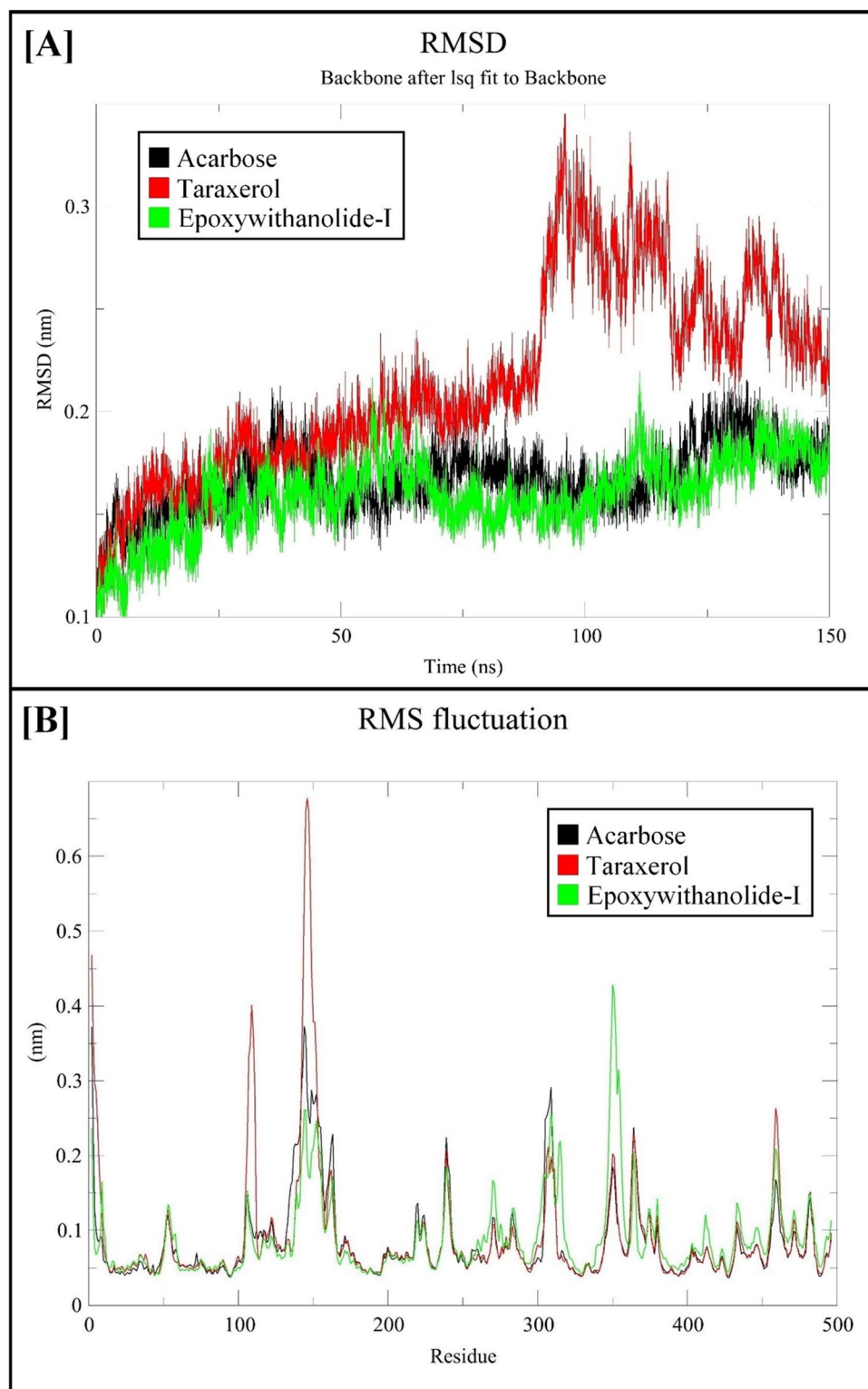
Fig. 3 Two-dimensional analysis of protein–ligand interactions with alpha-amylase enzyme; **A** Acarbose; **B** Taraxerol; **C** Epoxywithanolide-I

Solvent accessible surface area (SASA)

SASA of a protein denotes the expansion of the protein surface which denotes functionality of the protein. Reduction in the SASA denotes restriction in the functionality of the protein, there by suggesting the inhibition of the protein. SASA analysis of the 3 MDS combinations are represented in Fig. 5A. Overall comparison shows that Taraxerol shows least SASA value ranging between 200 and 210 nm² only

upto 80 ns after which its increased to 210–220 nm². Reference ligand Acarbose demonstrated highest SASA value, averaging between 210 and 220 nm² throughout the 150 ns MDS. Epoxywithanolide-I demonstrated a SASA value, averaging between 200 and 210 nm² throughout the entire 150 ns MDS. Overall, the hydrophobicity of Taraxerol contributed towards reduced SASA values of the complex, but was not constant throughout the MDS. It is evident that, Epoxywithanolide-I demonstrated a stronger and much

Fig. 4 MDS analysis; **A** root mean square deviation; **B** root mean square fluctuation

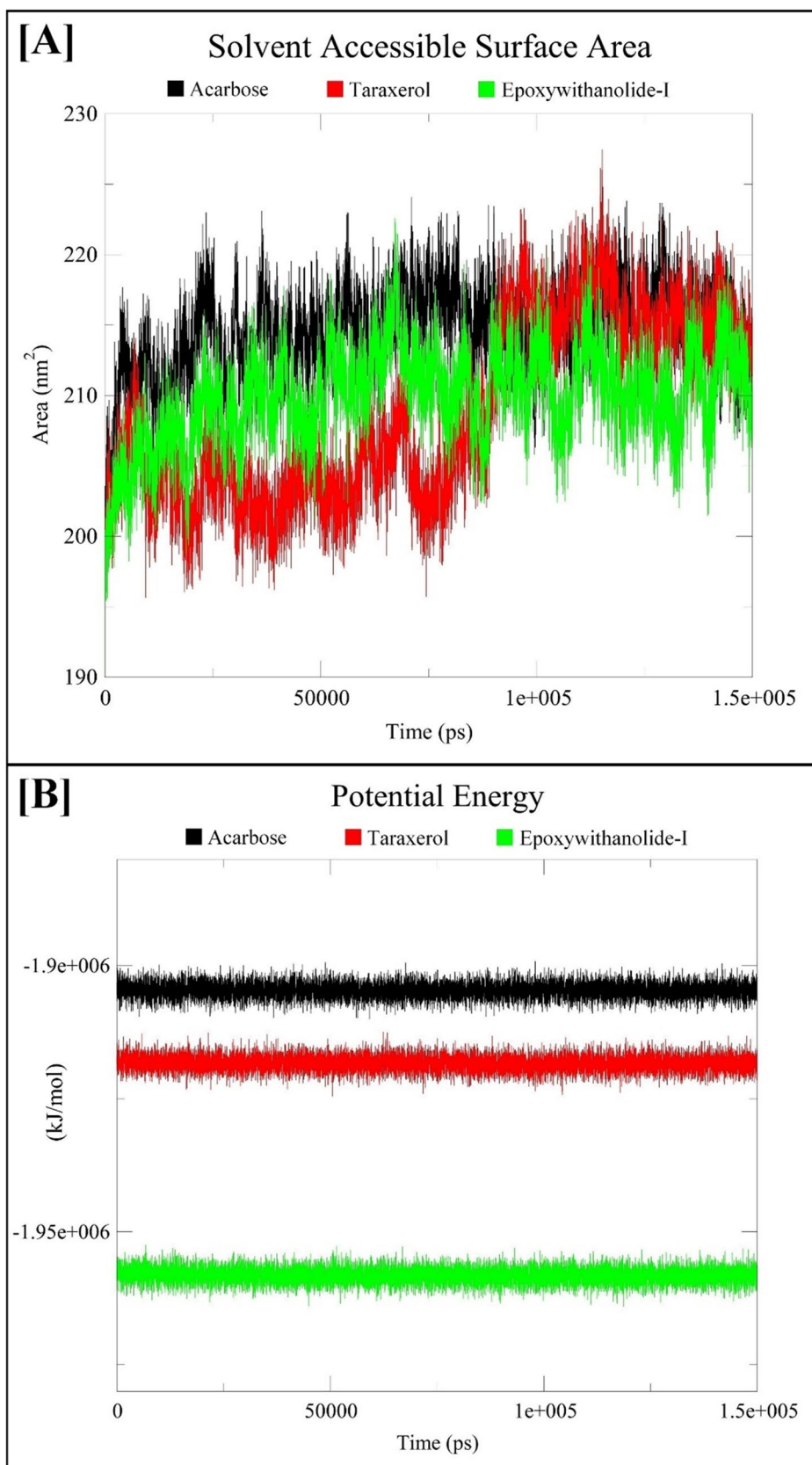


stable complex, as the SASA value ranged in a steady average and wide fluctuations were not observed. The SASA value demonstrated by Epoxywithanolide-I and Taraxerol were highly significant than the reference ligand Acarbose, suggesting them to be a potential inhibitor.

Potential energy of the complexes

Total potential energy of the protein–ligand complex determines the inhibition potential and its complex stability. Potential energy plot of the 3 MDS complexes are

Fig. 5 MDS analysis; **A** solvent accessible surface area; **B** potential energy analysis



graphically represented in Fig. 5B. Among the 3 MDS complexes, the lowest potential energy was demonstrated by Epoxywithanolide-I, suggesting it to be the most potential inhibitor in this comparison. The reference ligand Acarbose complex demonstrated a lowest potential energy of $-1,910,036$ kJ/mol at 59.2 ns. Both the test ligands demonstrated a much significant potential energy, lower than the reference molecule, suggesting an increased inhibition potential. Taraxerol complex with protein demonstrated a lowest potential energy of $-1,924,605.2$ kJ/mol at 50.4 ns, while Epoxywithanolide-I demonstrated a lowest potential energy of $-1,964,113.3$ kJ/mol at 118.7 ns. This plot clearly indicates that, the test ligands Epoxywithanolide-I and Taraxerol demonstrated high potential as an inhibitor of alpha-amylase, that is better than the reference ligand Acarbose.

Hydrogen bond analysis

Total number of hydrogen bonds determines the strength of the polar interactions between protein–ligand complexes. Hydrogen Bond analysis plot of the 3 protein–ligand complexes is represented in Fig. 6 (i.e., A: Acarbose; B: Taraxerol; C: Epoxywithanolide-I). It is evident from the graph that, Acarbose exhibited strong polar interactions with the amylase protein, with a maximum of 4 hydrogen bonds with a significant frequency throughout the 150 ns MDS. However, the 2 test ligands, (Taraxerol & Epoxywithanolide-I) demonstrated weak polar interactions with the amylase protein, where Taraxerol formed an average of one hydrogen bond with high frequency throughout the MDS, while Epoxywithanolide-I demonstrated very weak polar interactions, with formation of one hydrogen bond occasionally during

the entire MDS. This provides insight into the nature of the test ligands, that, both Taraxerol & Epoxywithanolide-I are hydrophobic molecules, that do not involve formation of hydrogen bonds with the target amylase protein. However, these hydrophobic molecules are demonstrating a higher potential to inhibit the target protein, when compared to the contrasting reference ligand Acarbose.

Radius of gyration (Rg)

Radii of Gyration (Rg) determines the rigidity and compactness of a system and hence indicates the inhibition of the target protein. Rg analysis of the 3 MDS complexes are represented in Fig. 7. Reduced Rg values and fluctuation determines the rigidity of the protein and there by inhibition of the catalytic function. All 3 MDS complexes, exhibited an average Rg value of 2.3 nm with mild fluctuations. Among the 3 ligand complexes, Taraxerol demonstrated greater stability in regards to fluctuation of Rg values. Acarbose & Epoxywithanolide-I complexes however, demonstrated significant fluctuations throughout the MDS likewise. The test ligand Epoxywithanolide-I behavior was comparative to that of Acarbose and hence the Rg values could be justified to be significant. This plot also confirms that the 3 ligand complexes instill stability and rigidity to the target protein structure there by inhibiting the target protein from its catalytic function.

Potential inhibitors of *alpha*-amylase

The MDS analysis of the test ligands confirms that, both Taraxerol of *C. indica* and Epoxywithanolide-I of *W. coagulans* are demonstrating significant potential as inhibitors of

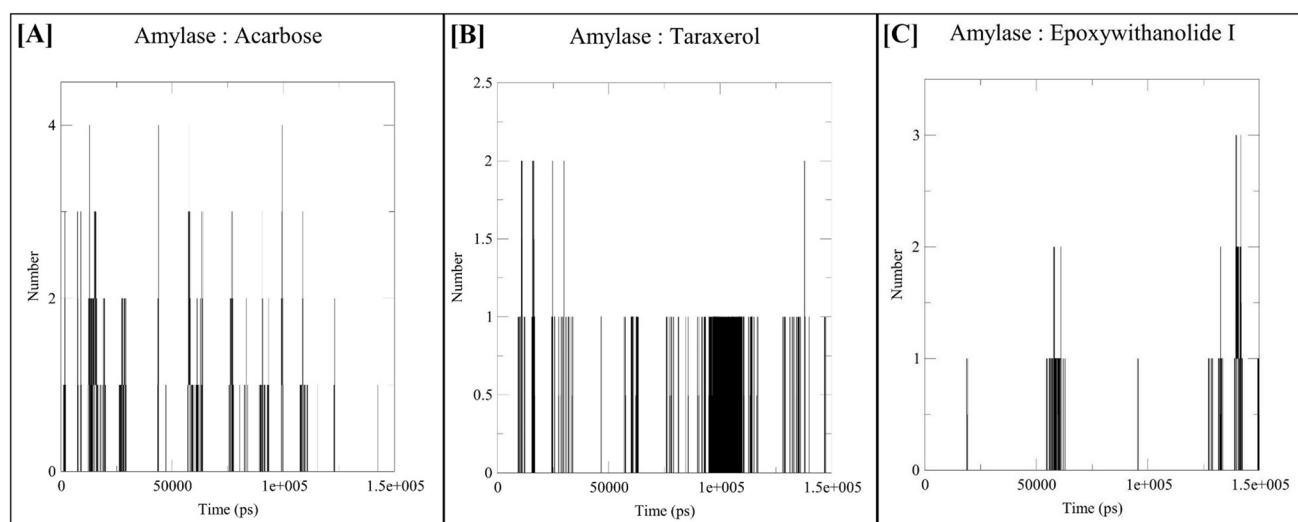


Fig. 6 Hydrogen bond analysis of MDS for amylase with **A** Acarbose; **B** Taraxerol; **C** Epoxywithanolide-I

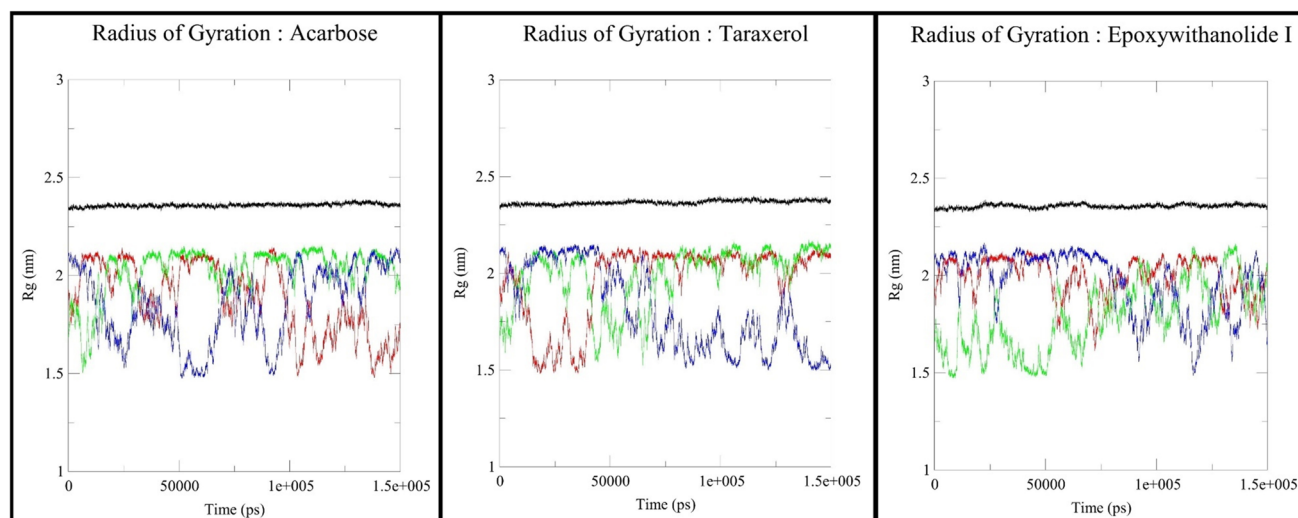


Fig. 7 Radius of gyration of protein in 3 combinations of MDS

the target Alpha-Amylase protein. When compared to the known reference inhibitor, results of Epoxywithanolide-I are more promising than the Taraxerol, based on the RMSD and RMSF analysis. The results of MDS suggests that both the phytochemical test ligands are potential inhibitors of Alpha-Amylase enzyme, aiding to the anti-diabetic properties of their respective plant sources. Suggestive conclusion of this MDS analysis would however strongly support that, Epoxywithanolide-I has much stronger inhibition potential than the reference Acarbose molecule and could further be investigated in-vitro for further validation of this in-silico study.

ADMET and drugability analysis

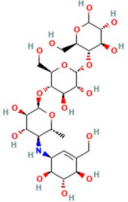
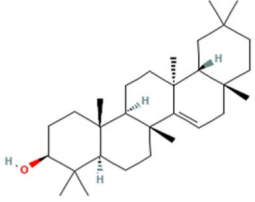
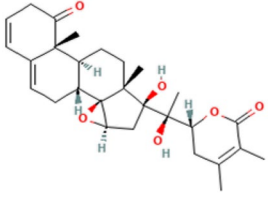
Adsorption, distribution, metabolism, excretion and toxicity (ADMET) and drugability of the two phytochemical test ligands were investigated using in-silico tools, by comparing these to the properties of reference molecule Acarbose. The results of the in-silico ADMET and Drugability analysis are summarized in Table 2. Molinspiration analysis of the ligands strongly suggest that, the 2 test ligands are more prominent enzyme inhibitors than that of the reference acarbose. The 2 test ligands show higher LogP values than the reference acarbose, aiding to their hydrophobic nature and poor solubility in water. However, all other physicochemical properties of Taraxerol and Epoxywithanolide-I are significantly better than the reference Acarbose ligand. Epoxywithanolide-I did not show any violation of Rule-of-Five (RoF), while Taraxerol showed 1 violation of the RoF, however this is a significant improvement compared to Acarbose that demonstrated 3 violations of RoF. The ADMET properties of Taraxerol and Epoxywithanolide-I are significantly better

than Acarbose, in regards to their cellular metabolization, excretion and being non-toxic to host cells. The test ligands were observed to be substrates of Cytochrome P450 enzyme, suggesting their ability to be metabolized and detoxified in the liver. The test ligands also demonstrated low risk for hERG inhibition, when compared to the reference which demonstrated an uncertain potential for hERG inhibition. This suggests that there is very low potential for cardiotoxicity by the test ligands, however being natural products as part of the edible plant sources, the low risk can be neglected. Based on the data presented in Table 2, it is evident that, Taraxerol and Epoxywithanolide-I are promising drug like molecules that demonstrate significant physicochemical properties with great potential to inhibit the target enzyme Alpha-Amylase.

Discussion

The two target plants *C. indica* and *W. coagulans* are popular ethnobotanical sources of antidiabetic phytochemicals (Ocvirk et al. 2013; Mahla et al. 2021). These plants are predominantly used in Indian culture to manage diabetes and hyper blood sugar levels. Reports on in-vitro and in-vivo investigations on these plant extracts, strongly support their ethnobotanical claims and applications as antidiabetic agents (Hemalatha et al. 2004; Mallick et al. 2007; Doss and Rangasamy 2008; Maurya et al. 2010; Gunjan et al. 2010). Although several studies were conducted to report the cumulative anti-diabetic effect of these two plants, very little information is available regarding the pinpoint mechanism of action of their anti-diabetic effect.

Table 2 ADMET/drugability analysis of target ligand molecules

Parameter	Acarbose	Taraxerol	Epoxywithanolide I
<i>Structure</i>			
Chemical structure			
<i>Molinspiration (bioactivity)</i>			
GPCR ligand	- 0.02	0.21	0.02
Ion channel modulator	- 0.49	0.02	0.23
Kinase inhibitor	- 0.33	- 0.2	- 0.43
Nuclear receptor ligand	- 0.29	0.54	0.67
Protease inhibitor	0.21	0	0.01
Enzyme inhibitor	0.21	0.49	0.77
<i>Molinspiration (chemical properties)</i>			
miLogP	- 5.51	8.02	4.26
TPSA	321.16	20.23	96.36
natoms	44	31	34
Molecular weight	645.61	426.73	474.64
nON	19	1	6
nOHNH	14	1	2
Nviolations	3	1	0
nrotb	9	0	2
Volume	544.93	460.7	453.89
<i>Druglikeness</i>			
Lead like rule	Violated	Violated	Violated
Lead like rule violations	2	2	2
Rule of five	Violated	Suitable	Suitable
Rule of five violations	3	1	0
<i>ADMET</i>			
BBB	0.0271005*	21.1287	0.335261
Buffer solubility mg/L	5793.5	18.0234	95.9579
CYP_2D6_substrate	Weakly	Non	Non
CYP_3A4_substrate	Substrate	Substrate	Substrate
Pgp_inhibition	Non	Inhibitor	Non
Plasma_Protein_Binding	0	100	89.939331
Pure water solubility mg/L	9.25	0.0022879	1.70931
Ames_test	Non-mutagen	Non-mutagen	Non-mutagen
hERG_inhibition	Ambiguous	Low_risk	low_risk

Inhibition of alpha-amylase enzyme has proven to be one of the most effective mode of diabetes management without any side effects and hence is being exploited in pharmaceutical industries (Bashary et al. 2020). Acarbose is a well-known drug that is used to control type 2 diabetes mellitus by inhibiting alpha-amylase protein. This known drug acarbose exhibits a significant in-vitro alpha-amylase inhibition potential with an IC_{50} value of 0.044–0.058 mg/ml (Laoufi

et al. 2017; Vyas et al. 2019). In the current investigation the antidiabetic activity of these plants (*C. indica* and *W. coagulans*) were analyzed by focusing on the ability of their phytochemicals to inhibit alpha-amylase enzyme. The study was aimed to identify molecules that are efficient inhibitors of alpha-amylase, on power with or better than the reference molecule acarbose. In-silico computational screening of the phytochemicals present in the two target plants suggested

that, Taraxerol and Epoxywithanolide-I from their respective plants demonstrates significant potential as alpha-amylase inhibitors, when compared to the reference Acarbose. Further detailed analysis using MDS also suggests that, Taraxerol and Epoxywithanolide-I are promising alpha-amylase inhibitors.

Taraxerol has been reported in few other in-silico investigations as potential anti-diabetic compounds from *C. indica* and other plant sources (Trinh and Le 2014; Vo et al. 2016). Epoxywithanolide-I belong to a class of molecules named withanolides that are common in the members of Solanaceae family. These withanolides are reported to be responsible for numerous pharmaceutical properties (such as, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetic, neuroprotective, etc.,) that the Solanaceae members are widely known for (Hemalatha et al. 2008; Verma et al. 2010). These supporting evidences confirm that, Taraxerol and Epoxywithanolide-I could be the bioactive molecules that play significant role in the anti-diabetic properties of their respective plants. Among the list of phytochemicals reported to be present in *C. indica* and *W. coagulans*, the test molecules, Taraxerol and Epoxywithanolide-I demonstrates strong potential to inhibit alpha-amylase enzyme. This could be one of the multiple synergistic mechanisms that are triggered by the phytochemicals present in the investigated plants.

Conclusion

Investigation of the phytochemicals present in the ethnobotanical plants *C. indica* and *W. coagulans* resulted in identification of Taraxerol and Epoxywithanolide-I as potential inhibitors of alpha-amylase enzyme from the respective plants. The results of this computational study provides insight to the anti-diabetic potential of the two target plants, by preventing the enzymatic digestion of starch via inhibition of alpha-amylase protein. Although both the investigated plants (*C. indica* and *W. coagulans*) are popularly known for their anti-diabetic activity, the key bioactive molecules responsible for this activity was not reported. Based on the results of this study, it is concluded that, among the several synergistic effects potentially exhibited by the phytochemicals present in these plants, inhibition of alpha-amylase enzyme by Taraxerol (*C. indica*) and Epoxywithanolide-I (*W. coagulans*) is possibly one mechanism-of-action that adds to the anti-diabetic property of these plants. Findings of this study provides phytochemical insights of *C. indica* and *W. coagulans* that could help in development of nutraceuticals for management of hyperglycemia, thereby preventing endothelial dysfunction and other possible cardiovascular events. However, further in-vitro and in-vivo investigations are needed to validate the proposed hypothesis.

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Author contributions L.R: conceptualized, executed experiment, wrote draft manuscript, reviewed manuscript V.S: conceptualized, wrote draft manuscript, reviewed manuscript P.J: interpretation, reviewed manuscript S.K: executed experiments, wrote draft manuscript M.V: supervised, reviewed manuscript R.M: reviewed manuscript A.K: executed experiments, wrote draft manuscript M.K: supervised, reviewed manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors confirm that there are no known conflicts of interest for this study.

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