In silico Investigation of the Antidiabetic Potential of *Putranjiva roxburghii* Phytoconstituents

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ABSTRACT
Background: Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by impaired insulin secretion and insulin resistance, leading to persistent hyperglycemia and associated complications. Inhibition of carbohydrate-hydrolyzing enzymes such as α-amylase represents an effective strategy for controlling postprandial blood glucose levels. *Putranjiva roxburghii*, a medicinal plant widely used in traditional systems of medicine, contains numerous bioactive phytoconstituents with potential therapeutic applications.
Objective: The present study aimed to investigate the antidiabetic potential of phytoconstituents from *Putranjiva roxburghii* through molecular docking against α-amylase (PDB ID: 2QV4).
Methods: Thirty phytoconstituents reported from *P. roxburghii* were selected for in silico analysis. The three-dimensional structures of the ligands were prepared using ChemDraw and Chem3D, while molecular docking studies were performed using AutoDock Vina. Binding affinities and protein–ligand interactions were evaluated within the active site of α-amylase, and interactions with key catalytic residues were analyzed.
Results: Docking scores ranged from –5.8 to –12.3 kcal/mol. Among the screened compounds, Amentoflavone and Putraflavone exhibited the highest binding affinity (–12.3 kcal/mol), followed by 4'''-O-methylamentoflavone (–9.9 kcal/mol), 3-O-methyl ellagic acid-4-O-α-rhamnopyranoside (–9.5 kcal/mol), Putrol (–9.4 kcal/mol), Putron (–9.4 kcal/mol), Catechin (–9.1 kcal/mol), and Gallocatechin (–9.0 kcal/mol). These compounds formed multiple hydrogen-bond, hydrophobic, π–π stacking, and electrostatic interactions with crucial active-site residues, including TRP59, TYR62, GLN63, ARG195, GLU233, HIS299, and ASP300. Several phytoconstituents demonstrated interaction patterns comparable to those of the co-crystallized ligand, indicating strong binding within the catalytic pocket of α-amylase.
Conclusion: The findings suggest that *Putranjiva roxburghii* is a promising source of antidiabetic phytochemicals. Amentoflavone and Putraflavone emerged as the most potent α-amylase inhibitors among the screened compounds and may serve as lead molecules for the development of novel antidiabetic agents. Further in vitro, in vivo, and pharmacokinetic studies are warranted to validate their therapeutic potential.
Keywords: Type 2 diabetes mellitus; α-Amylase; Molecular docking; Amentoflavone; Putraflavone; Phytoconstituents; *In silico*
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1.INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a common metabolic disease caused by tissue resistance to insulin and impaired insulin production [1-3]. Currently classified as the eighth most common cause of death and disability, diabetes is a serious worldwide health concern. Diabetes afflicted 830 million people in 2022, a substantial increase from the 200 million cases

recorded in 1990. The prevalence of diabetes was 6.1% worldwide in 2021, up 90.5% from 3.2% in 1990. It is predicted to reach 9.8% in 2050, impacting 13.1 billion people [4-6]. With a male-to-female ratio of 1.14 in 2021, diabetes was more common in men than in women worldwide, while regional variations were noted [7]. Researchers have looked into both pharmaceutical and non-pharmacological methods of treating diabetes, but no drug has been found to be totally safe [8–10]. Among non-pharmacological methods, regular exercise is frequently advised for improving insulin sensitivity [11,12]. The primary classes of pharmaceuticals used to treat diabetes include biguanides, which slow the hepatic production of glucose, sulfonylureas, which increase the release of insulin from pancreatic islets, peroxisome proliferator-activated receptor-γ (PPARγ) agonists, which increase the action of insulin, and α-glucosidase inhibitors, which delay the absorption of glucose in the intestine [13–15].These drug classes have disadvantages such weight gain, severe hypoglycemia, metabolic side effects, and some other problems with target selectivity, permeability, and solubility. They can be taken alone or in combination with other hypoglycemic medications [16,17]. Conventional therapies may result in long-term toxicity, adverse effects, and reliance [18–20]. Investigating complementary therapies, such as natural cures, can help reduce these dangers. Antioxidants are essential for lowering oxidative stress and scavenging dangerous free radicals [21]. Numerous studies have linked oxidative stress to problems associated with diabetes [22–23]. High blood sugar levels decrease the activity of antioxidant enzymes while increasing the generation of reactive oxygen and nitrogen species. Critical biomolecules like proteins, lipids, and DNA are harmed by this imbalance, which upsets cellular homeostasis and eventually produces toxic byproducts that accelerate the course of disease [24, 25]. Stress pathways are triggered when the body's antioxidant system is unable to combat this, which results in cellular damage and the advancement of diabetes. Antioxidant therapy protects β cells, maintains their function, and lessens problems, according to research. Because of their therapeutic advantages, safety, accessibility, and affordability, natural antioxidants are being employed more frequently [26]. Natural antioxidants can help prevent or reduce type 2 diabetes by lowering oxidative stress, protecting against lipid peroxidation, and boosting antioxidant enzyme activity. Natural antioxidant products should also be assessed in light of the numerous physiological mechanisms that influence Type 2 diabetes oxidative stress, including glycemic management, postprandial oxidative stress [27], the polyol pathway, high-calorie, high-fat diets, exercise, and sleep. Minimizing processes that cause chronic oxidative stress and increasing intake of natural antioxidants may help to prevent or reduce the evolution of Type 2 diabetes [28-29]. For thousands of years, people have used different parts of *Putranjiva roxburghii*, a member of the Putranijvaceae family, as a traditional herbal remedy in Ayurvedic and Unani medicine. [30] This plant's natural habitats include Southeast Asia, the Indian subcontinent, Japan, southern China, and New Guinea. It is widely grown in Asia, particularly in Bangladesh, India, Nepal, Thailand, Indochina, Myanmar, and Sri Lanka. [31] It is known as "Putranjiv" in Bangladesh, "child life tree" in English, Jivanputra, Putranjiva, Kumarajiva, Mava, Pavitra, and Putrajiva, and Karupali or Irukolli in India's Siddha medical system. "Pootranjeeva" is a combination of the terms "pootra" (son) and "jeeva" (life). *Putranjiva roxburghii* also goes by the names Kudrajuvi, Patravanti, Jivputrak, and Nageia. According to reports, it is effective against liver disorders, fever, and infertility [32]. *Putranjiva roxburghii* contains anthelmintic, anticancer, anti-inflammatory, antioxidant, aphrodisiac, diuretic, and laxative effects. [33-38] Leaves and seed paste are used to alleviate burning feelings, filariasis, inflammation, and eye problems. [39] Many of these synthetic drugs are either directly or indirectly derived from plants, which are widely recognized as one of the most reliable sources of disease-curing molecules. According to new research, plants and plant-based products may have promising antidiabetic properties. Plant sources of antidiabetic chemicals have been utilized for centuries since they are safer and less expensive than manmade medications. They are also mentioned in numerous traditional remedies, including those used in Chinese, Korean, and Indian cultures. [40-41] Furthermore, in silico research provides several significant advantages, including the capacity to conduct high-throughput virtual screening of huge phytochemical libraries swiftly and economically. It facilitates the development of hypotheses on atomic-level mechanisms of action, which can then be tested experimentally. It streamlines

efforts in phytochemical separation and extract standardization by focusing the discovery of key chemicals. It reduces the need for early-stage clinical research and animal testing, which helps to alleviate ethical concerns while saving costs. It enables the rational design and optimization of natural analogues by assisting in the formation of SAR (structure-activity relationships). It encourages the study of polypharmacological interactions, which is congruent with herbal treatments' multitarget activity in the treatment of complicated disorders like diabetes. When coupled, computational tools act as a powerful addition to laboratory research, hastening the identification and verification of plant-derived antidiabetic drugs and allowing for the logical conversion of traditional cures into cutting-edge, evidence-based treatments. Compared to the traditional time-consuming procedure (from selecting the plant to separating chemicals in accordance with bioassay standards), the CADD methodology is faster and more effective. [42]

The targeted protein in this study was previously examined as potential drug target for Type 2 diabetes mellitus, α-Amylase(2QV4) [43] Alpha (α)-amylase is a calcium-dependent enzyme that breaks down polysaccharides. However, it can promote postprandial hyperglycemia. α-Amylase inhibitors such as acarbose, miglitol, and voglibose can efficiently regulate blood sugar levels. Acarbose, in particular, aids in weight management, minimizes cardiovascular risks, and delays diabetes onset by beneficially regulating blood pressure, lipids, platelet aggregation, and vascular health.[44]

The goal of this study was to investigate a wide range of bioactive substances (phytoconstituents) from *Putranjiva roxburghii* leaves and see how they interacted with target proteins in order to possibly shed light on the underlying molecular mechanisms required for enzyme inhibition.

2.Materials and methods

2.1 Dataset

Table 1 shows a list of 30 bioactive phytoconstituents chosen for docking studies, including 1. 3β-Acetoxyadiane-5-ene 2. 3β-Acetoxy-Cycloart-24-en-23-one 3. 3-O-methyl ellagic acid-4-O-α-rhamnopyranoside 4. Adian-5-ene-3β,29-diol 5. Amentoflavone 6. β-Amyrin 7. Caffeic Acid 8. Catechin 9. Chlorogenic Acid 10. Epicatechin 11. Erythrodiol 12. Ferulic Acid 13. Friedlein 14. Gallocatechin 15. Genistic Acid 16. Laminitol 17. 4"'-O-methylamentoflavone 18. Methyl Putranjivate 19. Oleanolic Acid 20. Putraflavone 21. Putrajivadion 22. Putralone 23. Putranjivic Acid 24. Putrol 25. Putrone 26. Roxburghonic Acid 27. Syringic Acid 28. Syringin Methyl Ether 29. Umbelliferone 30. Vanillic Acid.

2.2 Preparation of ligands

The dataset's ligands and molecules were drawn with ChemDraw. Chem3D was used to import and transform identical two-dimensional structures to three dimensions. As part of the final preparation, the structures were decreased with the default parameters. The structures were stored in mol format and then imported into the Autodock 4.2 application to detect torsions. The structures were eventually saved in the pdbqt format to allow for additional docking studies.

2.3 Preparation of protein

The target protein with the pdb id 6C9X was obtained from the protein data bank for docking research. The 6C9X protein, a hydrolase, represents alpha-glucosidase in its interaction with voglibose at a resolution of 1.46 Å. Autodock 4.2 was used to reduce the downloaded target protein. The co-crystallized ligands in each protein show that the polar hydrogens were provided after the excess water molecules were removed. The Kollmann charges were inserted alongside the missing residues. The generated proteins were kept in pdbqt format for future docking study.

2.4 Preparation of the grid box

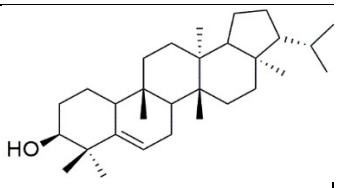
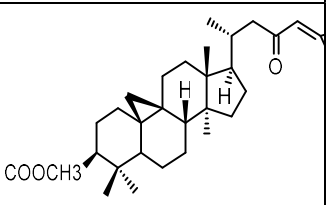
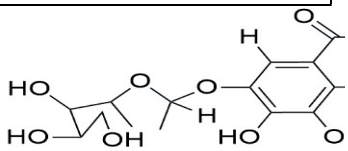
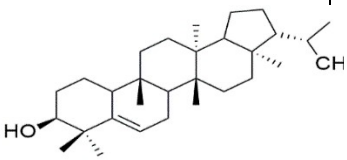
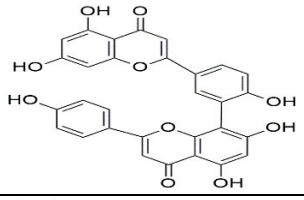
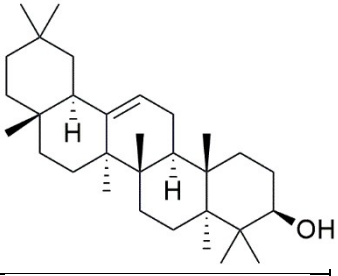
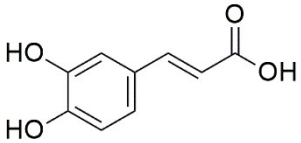
The docking grid box represents the specific area of the protein structure where the ligands bind. This is mostly determined by the co-crystallized ligand. The grid box was defined using the XYZ coordinates of the co-crystallized ligands from the downloaded targets. The coordinates for the proteins 2QV4 were 12.384, 48.136, 26.209.

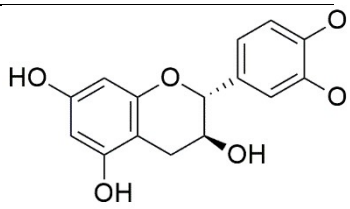
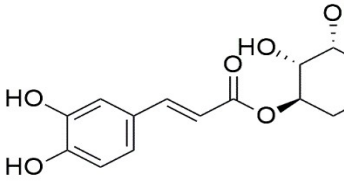
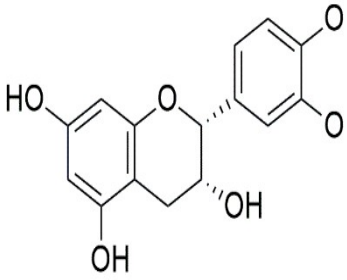
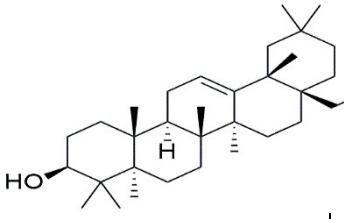
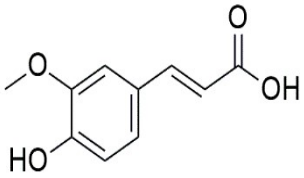
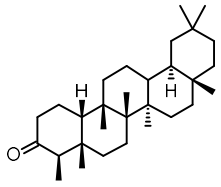
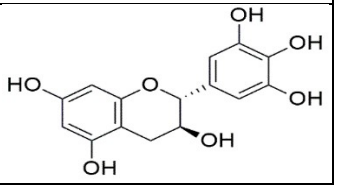
2.5 Docking studies

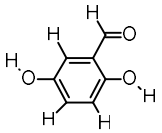
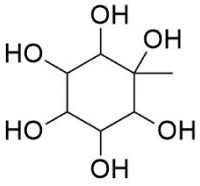
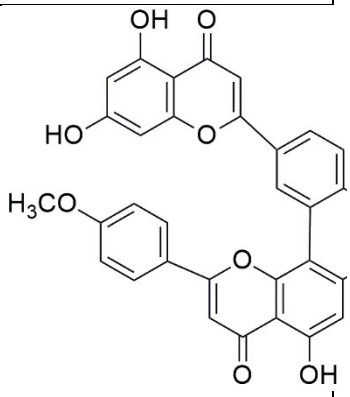
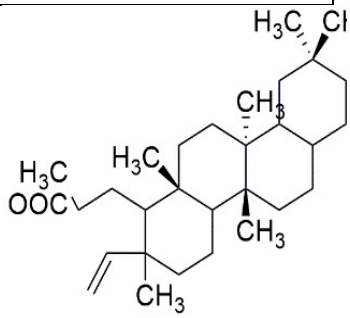
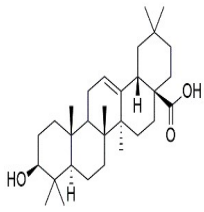
The ligands were docked with Autodock Vina. This software generates configuration files that principally include the protein and ligands in pdbqt format, as well as the grid box's XYZ coordinates.

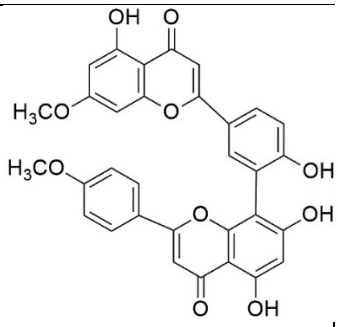
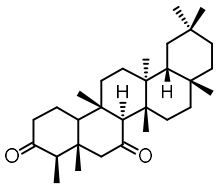
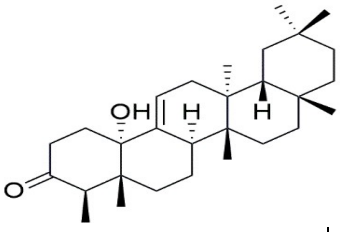
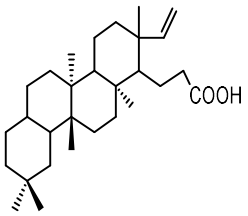
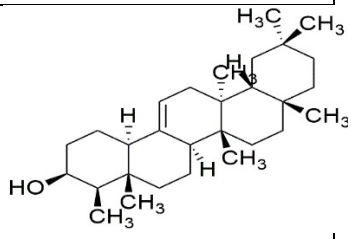
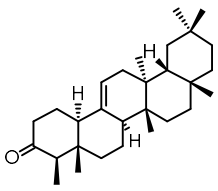
Table 1- Compound and their Structure

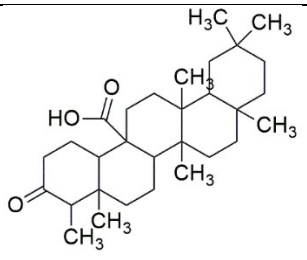
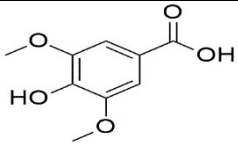
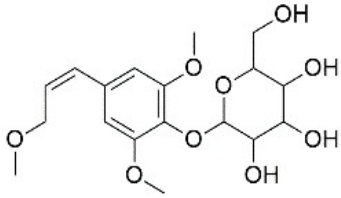
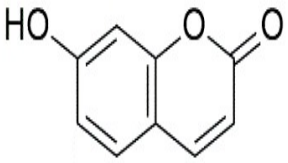
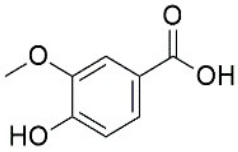
S. No.	Compounds	Structure

	3β-Acetoxyadiane-5-ene	
	3β-Acetoxy-Cycloart-24-en-23-one	
	3-O-methyl ellagic acid -4-O-α-rhamnopyranoside	
	Adian-5-ene-3β,29-diol	
	Amentoflavone	
	β-Amyrin	
	Caffeic Acid	

	Catechin	
	Chlorogenic Acid	
	Epicatechin	
	Erythrodiol	
	Ferulic Acid	
	Friedlein	
	Gallocatechin	

	Genistic Acid	
	Laminitol	
	4”’-O-methylamentoflavone	
	Methyl Putranjivate	
	Oleanolic Acid	

	Putraflavone	
	Putrajivadion	
	Putralone	
	Putranjivic Acid	
	Putrol	
	Putrone	

	Roxburghonic Acid	
	Syringic Acid	
	Syringin Methyl Ether	
	Umbelliferone	
	Vanillic Acid	

RESULTS AND DISCUSSION

Molecular docking analysis was performed to evaluate the binding affinity and interaction profile of the phytoconstituents of *Putranjiva roxburghii* with α-amylase (PDB ID: 2QV4), a key enzyme involved in carbohydrate digestion and an established therapeutic target for diabetes management. The docking scores ranged from –5.8 to –12.3 kcal/mol, indicating varying degrees of binding affinity among the investigated compounds.

Among all the screened phytoconstituents, Amentoflavone and Putraflavone exhibited the highest binding affinity with docking scores of –12.3 kcal/mol**,** followed by 4'''-O-methylamentoflavone (–9.9 kcal/mol), 3-O-methyl ellagic acid-4-O-α-rhamnopyranoside (–9.5 kcal/mol), Putrol (–9.4 kcal/mol), Putron (–9.4 kcal/mol), Catechin (–9.1 kcal/mol), and Gallocatechin (–9.0 kcal/mol).

The co-crystallized ligand of α-amylase interacts with several crucial amino acid residues, including TRP59, TYR62, GLN63, HIS101, ASN105, ALA106, VAL107, THR163, GLY164, ARG195, GLU233, HIS299, ASP300, and associated water molecules. Notably, several phytoconstituents demonstrated interactions with these key active-site residues. Amentoflavone formed hydrogen bonds with GLN63, ASP197, ASP300, ALA198, and HIS201, along with hydrophobic interactions involving TRP59 and LEU162. Putraflavone established hydrogen-bond interactions with GLN63, ASP197, ALA198, and HIS305, while also exhibiting electrostatic interactions with ASP300 and GLU233.

Similarly, 3-O-methyl ellagic acid-4-O-α-rhamnopyranoside showed multiple hydrogen bonds with GLN63, ASP197, ASP300, and GLU233, accompanied by π–π stacking interactions with TRP59. Gallocatechin formed hydrogen bonds with GLN63, ASP300, HIS299, ARG195, and GLU233, indicating strong stabilization within the catalytic pocket. Catechin, chlorogenic acid, epicatechin, and syringin methyl ether also displayed favorable interactions with several active-site residues, particularly TRP59, GLN63, GLU233, HIS299, and ASP300.

Overall, the docking results suggest that flavonoids and polyphenolic compounds from *P. roxburghii* possess significant binding affinity toward α-amylase and may act as potential inhibitors of carbohydrate hydrolysis.

Table 2: Binding energy (kcal/mol) of bio-molecules in *Putranjiva roxburghii* to Alpha amylase (2qv4)-X=-12.384, Y=48.136, Z=26.209

S. No.	Compound	Energy	Interaction	Bond
	3β-vadiane-5-ene	**-7.0**	TRP59	Pi-Sigma Bond, Hydrophobic Bond
	3β-Acetoxy-Cycloart-24-en-23-one	**-8.9**	TRP59	Pi-Sigma Bond, Hydroph

				obic Bond
			ALA198	Alkyl, Hydrophobic Bond
3	3-O-methyl ellagic acid -4-O-α-rhamnopyranoside	**-9.5**	GLN63	Hydrogen Bond
			HOH805	Hydrogen Bond
			ASP197	Hydrogen Bond
			HOH759	Hydrogen Bond
			ASP300	Hydrogen Bond
			HOH761	Hydrogen Bond
			GLU233	Hydrogen Bond
			TRP59	Pi-Pi Stacked Hydrophobic Bond
			HIS305	Carbon – Hydrogen Bond
			TYR62	Pi-Sigma Bond, Hydrophobic Bond
4	Adian-5-ene-3β,29-diol	**-7.1**	TRP59	Pi-Sigma Bond, Hydrophobic Bond
5	Amentoflavone	**-12.3**	TRP59	Pi-Sigma Bond, Hydrophobic Bond
			LEU162	Pi-Sigma Bond, Hydrophobic Bond
			TRP59	Pi-Pi Stacked Hydrophobic Bond
			GLN63	Hydrogen Bond
			HOH805	Hydrogen Bond
			HOH550	Hydrogen Bond
			HOH777	Hydrogen Bond
			HOH759	Hydrogen Bond
			ASP300	Hydrogen Bond
			ASP197	Hydrogen Bond
			ALA198	Hydrogen Bond
			HIS201	Hydrogen Bond
6	β-Amyrin	**-7.2**	HIS305	Hydrogen Bond
			TRP58	Pi-Alkyl, Hydrophobic Bond
			TYR62	Pi-Alkyl, Hydrophobic Bond
			HIS101	Pi-Alkyl, Hydrophobic Bond
			LEU165	Pi-Alkyl,

				Hydrophobic Bond
			LEU162	Pi-Alkyl, Hydrophobic Bond
			ALA198	Pi-Alkyl, Hydrophobic Bond
7	Caffeic Acid	**-6.9**	HOH805	Hydrogen Bond
			GLN63	Hydrogen Bond
			HIS299	Hydrogen Bond
			ASP197	Hydrogen Bond
			TYR62	Pi-Pi Stacked Hydrophobic Bond
			ASP300	Unfavourable Acceptor-Acceptor Bond
8	Catechin	**-9.1**	TRP59	Pi-Pi Stacked Hydrophobic Bond
			GLN63	Hydrogen Bond
			GLU233	Hydrogen Bond
			HOH759	Hydrogen Bond
			ASP300	Pi-Anion Electrostatic Bond
9	Chlorogenic Acid	**-8.7**	HOH550	Hydrogen Bond
			HOH777	Hydrogen Bond
			HOH759	Hydrogen Bond
			HOH805	Hydrogen Bond
			HIS299	Hydrogen Bond
			GLN63	Hydrogen Bond
			GLU233	Pi-Anion Electrostatic Bond
			ALA198	Pi-Alkyl, Hydrophobic Bond
			LEU162	Pi-Alkyl, Hydrophobic Bond
10	Epicatechin	**-8.8**	ASP197	Hydrogen Bond
			HOH805	Hydrogen Bond
			TYR62	Hydrogen Bond
			TRP59	Pi-Pi Stacked Hydrophobic Bond
11	Erythrodiol	**-7.0**	HOH805	Hydrogen Bond
			TRP59	Pi-Sigma Bond, Hydrophobic Bond
12	Ferulic Acid	**-6.8**	TYR62	Hydrogen Bond

			ASP300	Hydrogen Bond
			HOH805	Hydrogen Bond
			ASP197	Pi-Anion Electrostatic Bond
			LEU162	Alkyl, Hydrophobic Bond
1	Friedlein	**-8.6**	TRP59	Pi-Sigma Bond, Hydrophobic Bond
			GLN63	Hydrogen Bond
			TRP357	Pi-Alkyl, Hydrophobic Bond
1	Gallocatechin	**-9.0**	TRP59	Pi-Donor Hydrogen Bond
			TYR62	Pi-Donor Hydrogen Bond
			GLN63	Hydrogen Bond
			ASP300	Hydrogen Bond
			HIS299	Hydrogen Bond
			ARG195	Hydrogen Bond
			GLU233	Hydrogen Bond
			HOH805	Hydrogen Bond
			HOH759	Hydrogen Bond
1	Genistic Acid	**-6.0**	HOH759	Hydrogen Bond
			HOH761	Hydrogen Bond
			GLU233	Hydrogen Bond
			HIS299	Hydrogen Bond
			ASP197	Pi-Anion Electrostatic Bond
			TYR62	Pi-Pi T-Shaped Hydrophobic Bond
1	Laminitol	**-6.2**	ALA198	Hydrogen Bond
			ASP197	Hydrogen Bond
			GLU233	Hydrogen Bond
			ASP300	Unfavourable Acceptor-Acceptor Bond
1	4”’-O-methylamentoflavone	**-9.9**	HOH840	Hydrogen Bond
			HOH805	Hydrogen Bond
			HOH759	Hydrogen Bond
			GLN63	Hydrogen Bond
			TYR62	Hydrogen Bond
			TRP59	Pi-Pi Stacked Hydrophobic Bond
1	Methyl Putranjivate	**-7.4**	HIS299	Hydrogen Bond

			LEU165	Alkyl, Hydrophobic Bond
			TRP59	Pi-Sigma Bond, Hydrophobic Bond
1	Oleanolic Acid	**-7.5**	HOH759	Hydrogen Bond
			HIS305	Hydrogen Bond
			TRP59	Pi-Sigma Bond, Hydrophobic Bond
2	Putraflavone	**-12.3**	HOH805	Hydrogen Bond
			HOH759	Hydrogen Bond
			HOH777	Hydrogen Bond
			HOH550	Hydrogen Bond
			GLN63	Hydrogen Bond
			ASP197	Hydrogen Bond
			ALA198	Hydrogen Bond
			HIS305	Hydrogen Bond
			ASP300	Pi-Anion Electrostatic Bond
			GLU233	Pi-Anion Electrostatic Bond
			ARG195	Unfavourable Donor-Donor Bond
			HIS201	Unfavourable Donor-Donor Bond
			LEU162	Pi-Sigma Bond, Hydrophobic Bond
			TRP59	Pi-Sigma Bond, Hydrophobic Bond
2	Putrajivadion	**-8.4**	HIS305	Carbon – Hydrogen Bond
			TRP59	Pi-Sigma Bond, Hydrophobic Bond
			GLN63	Hydrogen Bond
2	Putralone	**-8.6**	TRP59	Hydrogen Bond
2	Putranjivic Acid	**-7.0**	LEU165	Alkyl, Hydrophobic Bond
			TRP59	Pi-Sigma Bond, Hydrophobic Bond
2	Putrol	**-9.4**	HIS305	Pi-Sigma Bond, Hydroph

				obic Bond
			TRP59	Pi-Sigma Bond, Hydrophobic Bond
			HOH805	Hydrogen Bond
2	Putron	**-9.4**	HIS305	Pi-Sigma Bond, Hydrophobic Bond
			TRP59	Pi-Sigma Bond, Hydrophobic Bond
2	Roxburghonic Acid	**-7.2**	SER108	Hydrogen Bond
			SER112	Hydrogen Bond
2	Syringic Acid	**-5.8**	GLN63	Hydrogen Bond
			ASP300	Hydrogen Bond
			TRP58	Alkyl, Hydrophobic Bond
			TRP59	Alkyl, Hydrophobic Bond
			HIS101	Alkyl, Hydrophobic Bond
			LEU162	Alkyl, Hydrophobic Bond
			ALA198	Alkyl, Hydroph
				obic Bond
			ASP197	Carbon – Hydrogen Bond
			HIS305	Pi-Sigma Bond, Hydrophobic Bond
2	Syringin Methyl Ether	**-7.0**	HOH840	Hydrogen Bond
			HOH805	Hydrogen Bond
			HOH759	Hydrogen Bond
			GLN63	Hydrogen Bond
			ASP300	Hydrogen Bond
			GLU233	Hydrogen Bond
			TRP59	Pi-Pi Stacked Hydrophobic Bond
			TYR62	Carbon – Hydrogen Bond
2	Umbelliferone	**-6.6**	HOH805	Hydrogen Bond
			TYR62	Pi-Pi Stacked Hydrophobic Bond
3	Vanillic Acid	**-5.9**	HOH805	Hydrogen Bond
			ALA198	Alkyl, Hydrophobic Bond
			LEU162	Alkyl, Hydroph

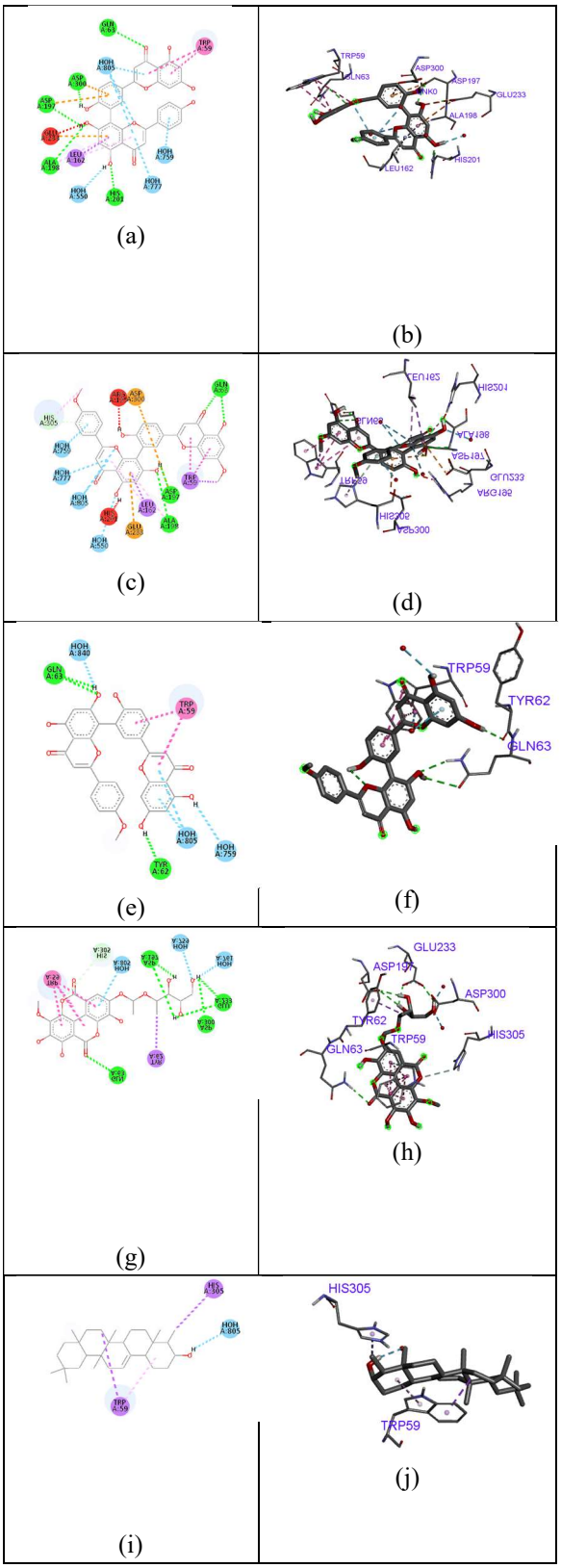
				obic Bond
			ARG195	Unfavourable Donor-Donor Bond
			ASP300	Pi-Anion Electrostatic Bond

Inhibition of α-amylase is an important therapeutic strategy for controlling postprandial hyperglycemia by reducing the breakdown and absorption of dietary carbohydrates. Therefore, compounds capable of interacting with the catalytic and substrate-binding residues of α-amylase are considered promising antidiabetic agents.

The docking study revealed that several phytoconstituents of *Putranjiva roxburghii* interact with amino acid residues that are also involved in binding the co-crystallized ligand. Among these, TRP59, TYR62, GLN63, ARG195, GLU233, HIS299, and ASP300 were the most frequently involved residues. These residues are located within or near the enzyme's active site and contribute significantly to ligand recognition and stabilization.

Amentoflavone and Putraflavone demonstrated the strongest binding affinities and formed multiple hydrogen-bond, hydrophobic, and electrostatic interactions with important catalytic residues. The extensive interaction network observed for these compounds suggests a stable ligand–protein complex and a high potential for α-amylase inhibition. Likewise, 3-O-methyl ellagic acid-4-O-α-rhamnopyranoside, catechin, gallocatechin, and chlorogenic acid exhibited favorable interaction profiles involving GLN63, GLU233, HIS299, and ASP300, residues known to contribute to substrate binding and catalytic activity.

The predominance of hydrogen bonding and π–π stacking interactions with TRP59 and TYR62 indicates that aromatic flavonoids and polyphenols can effectively occupy the substrate-binding pocket of α-amylase. Furthermore, the interaction of several compounds with catalytic residues ASP197 and ASP300 may interfere with enzymatic catalysis and contribute to inhibitory activity. The 3D and 2D docked poses of the most important phytoconstituents are given in Figure 1.



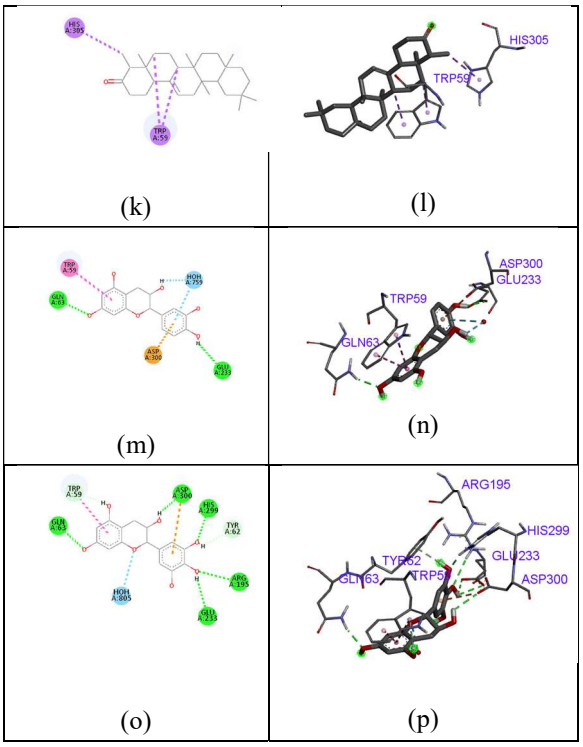


Figure 1. The 2D and 3D docked poses of the important phytoconstituents. (a) 2D pose of Amentoflavone (b) 3D pose of Amentoflavone (c) 2D pose of Putraflavone (d) 3D pose of Putraflavone (e) 2D pose of 4"'-O-methylamentoflavone (f) 3D pose of 4"'-O-methylamentoflavone (g) 2D pose of 3-O-methyl ellagic acid -4-O-α-rhamnopyranoside (h) 3D pose of 3-O-methyl ellagic acid -4-O-α-rhamnopyranoside (i) 2D pose of Putrol (j) 3D pose Putrol (j) 2D pose of Putron (d) 3D pose of Putron (k) 2D pose of Catechin (l) 3D pose of Catechin (m) 2D pose of Gallocatechin (n) 3D pose of Gallocatechin.

The superior docking scores of bioflavonoids such as amentoflavone and putraflavone may be attributed to their larger aromatic framework and multiple hydroxyl groups, which facilitate stronger hydrophobic and hydrogen-bond interactions within the active site. These findings are consistent with previous reports demonstrating potent α-amylase inhibitory activity of flavonoids and phenolic compounds from medicinal plants.

Conclusion

The molecular docking study demonstrated that several phytoconstituents of *Putranjiva roxburghii* possess strong binding affinity toward α-amylase (2QV4), suggesting their potential role as antidiabetic agents. Among the investigated compounds, Amentoflavone and Putraflavone exhibited the highest binding affinity (–12.3 kcal/mol), followed by 4"'-O-methylamentoflavone, 3-O-methyl ellagic acid-4-O-α-rhamnopyranoside, Putrol, Putron, Catechin, and Gallocatechin.

Importantly, these compounds interacted with several key active-site residues involved in co-crystallized ligand binding, including TRP59, TYR62, GLN63, ARG195, GLU233, HIS299, and ASP300. The presence of multiple hydrogen-bond, hydrophobic, and electrostatic interactions indicates the formation of stable protein–ligand complexes capable of inhibiting α-amylase activity.

Overall, the findings suggest that *Putranjiva roxburghii* is a promising source of bioactive phytoconstituents with potential antidiabetic activity. Among the screened compounds, amentoflavone and putraflavone emerged as the most promising lead molecules for further in vitro, in vivo, and pharmacokinetic investigations aimed at developing novel α-amylase inhibitors for diabetes management.

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