DOI: 10.7860/JCDR/2024/69547.19699

Original Article

Biochemistry Section

Unveiling the Promise of Bioactive Alkaloid Compound from *Catharanthus Roseus*: An In-vitro Computational Exploration of their Molecular Docking against a Target Protein for Type-2 Diabetes

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ABSTRACT

Introduction: Molecular docking facilitates the exploration of interactions between bioactive alkaloid compounds and target proteins, offering insights into potential binding modes and affinity, crucial for drug discovery and understanding molecular mechanisms. *Catharanthus roseus* (*C. roseus*), renowned for its bioactive alkaloid compounds, emerges as a promising candidate for novel agents in diabetes management.

Aim: To study the computational methods, including in-silico molecular docking, to elucidate the interactions between bioactive alkaloids from *C. roseus* and a Type-2 diabetic target protein.

Materials and Methods: In this in-vitro study conducted in 2022 at the Department of Research, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Tamil Nadu, India. The research took place from July to October 2022. Computational techniques, particularly in-silico molecular docking, were utilised to analyse the binding affinities and potential mechanisms of action of bioactive alkaloids from *C. roseus* against a target protein associated with Type-2 diabetes. The study employed established computational protocols and software tools to predict and evaluate the binding interactions between the alkaloids and the target protein.

Results: The analysis suggests Vinblastine, Ajmalicine, and Vindolinine from *C. roseus* as potential diabetes therapeutics. Vinblastine binds strongly to Human Pancreatic Alpha-Amylase (HPA), hinting at glucose metabolism regulation. Ajmalicine and Vindolinine also interact significantly with HPA, implying antidiabetic potential. Additionally, the present study findings suggest a potential role of Reserpine in modulating HPA activity and supporting its antihypertensive effects.

Conclusion: In conclusion, the analysis highlights the potential of *C. roseus* compounds like Vinblastine, Ajmalicine, and Vindolinine in managing diabetes by interacting with HPA, indicating their potential as antidiabetic agents. Additionally, Reserpine's effect on HPA activity suggests a role in hypertension management. These findings emphasise the therapeutic potential of natural compounds from *C. roseus* for diabetes and related conditions, warranting further clinical investigation.

Keywords: Antidiabetic agent, Human pancreatic alpha-amylase, Hypertension management

INTRODUCTION

Diabetes is a prevalent chronic metabolic disorder characterised by high blood glucose levels. According to the American Diabetes Association, the global prevalence of diabetes was estimated to be 10.5% in 2020, affecting approximately 463 million people worldwide [1]. Type-2 diabetes, the most common form, accounts for around 90% of all diabetes cases [2]. It is characterised by insulin resistance and impaired insulin secretion. The management of diabetes involves various treatment approaches, including lifestyle modifications, oral medications, injectable medications (such as insulin), and, in some cases, bariatric surgery. The primary goal of treatment is to achieve glycaemic control and prevent or manage complications associated with diabetes, such as cardiovascular disease, neuropathy, and kidney disease [3,4].

In this context, natural products have gained attention for their potential as sources of novel antidiabetic agents. *C. roseus*, commonly known as Madagascar periwinkle or Vinca, is a plant species renowned for its production of bioactive compounds with pharmaceutical significance. It has been extensively studied in various research areas, including cancer treatment, cardiovascular health, and diabetes management [5,6]. Notably, *C. roseus* has shown promising potential in the field of diabetes research due to its antidiabetic activity [7]. Several bioactive alkaloid compounds derived from *C. roseus*, namely Reserpine, Vinblastine, Ajmalicine, and Vindolinine, have been investigated for their potential antidiabetic effects [8].

These compounds exhibit pharmacological activities that may modulate glucose metabolism and insulin regulation, making them attractive candidates for diabetes therapy. In recent years, in-silico molecular docking has emerged as a valuable tool for drug discovery and development. By simulating the binding interactions between small molecules and target proteins, molecular docking provides insights into their potential therapeutic effects. It allows for the assessment of binding affinity and binding modes, enabling researchers to explore the potential of bioactive compounds [6]. The present study presents to conducting in-silico molecular docking assessments of bioactive alkaloid compounds derived from *C. roseus* against a target protein (HPA) associated with type-2 diabetes. Through the analysis of binding interactions and binding energies, we aim to elucidate the therapeutic potential of these compounds in managing type-2 diabetes. The unique aspect of the present research lies in its exploration of the molecular mechanisms underlying the potential antidiabetic effects of *C. roseus* bioactive compounds using computational methods. The findings generated from the present study have the potential to shed light on new pathways for diabetes management and provide a basis for further experimental validation and optimisation of these compounds as potential therapeutic agents.

Hence, present study was conducted to study the computational methods, including in-silico molecular docking, to elucidate the interactions between bioactive alkaloids from *C. roseus* and a type-2 diabetic target protein.

MATERIALS AND METHODS

The present in-vitro study was conducted in 2022 at Department of Research, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Tamil Nadu, India. Given its bioinformatics basis, ethical approval was not required. The research took place from July 2022 to October 2022.

Protein Preparation

The Protein Data Bank (PDB) published the 3D structure of the HPA protein in the PDB format, assigned with the PDB ID: 4X9Y. To ensure the integrity of the protein macromolecules, they underwent cleaning procedures using Autodock techniques, which involved removing solvents, odd ligands, and residues. Subsequently, hydrogen atoms were added to enhance the macromolecules, and the resulting structures were saved in the Protein Data Bank, Partial Charge and Atom Type (PDBQT) format [9]. These bioactive compounds, including Reserpine, Vinblastine, Ajmalicine, and Vindolinine, were identified and documented in [Table/Fig-1] through a comprehensive literature search [10,11]. They are derived from *C. roseus*. The corresponding ligands for these components were retrieved from PubChem, utilising the Simulation Description Format (SDF) format. In order to enhance their energetic properties, PyRx (version 0.8) was utilised. This software facilitated the transformation of the ligands into their most stable configurations using the Merck Molecular Force Field94 (MMFF94) force field [12].

S. no.	Compounds	PubChem id
1.	Reserpine	5770
2.	Vinblastine	13342
3.	Ajmalicine	441975
4.	Vindolinine	24148538

[Table/Fig-1]: Alkaloids from *Catharanthus roseus* (*C.roseus*).

Molecular Docking

The molecular docking analysis in the present study utilised a modified version of the flexible docking method proposed by Trott and Olson. Specifically, the AutoDock Vina component from the Python Prescription 0.8 package was employed to conduct docking studies involving identified compounds and selected proteins. PDBQT files for the proteins were generated, encompassing partial charges and atom types based on their respective PDB files. The receptor was maintained in a rigid state, allowing complete rotational freedom for the ligand's bonds. While most parameters retained their default values, the grid box was adjusted to cover the active sites of the protein molecules. Score data files were then created for subsequent manual comparisons after completing the molecular docking experiments. For each protein-ligand complex and all phytoconstituents, ten combinations were generated, with the best docking site determined by evaluating the conformation with the lowest Binding Energy (BE, kcal/mol) and Root Mean Square Deviation (RMSD) [13]. The in-silico experiment incorporated a docking exhaustiveness of 10 and 10 modes to ensure more accurate and reliable outcomes. Interactions between ligands and proteins were visualised, analysed, and presented using PyMOL and the Discovery Studio Visualiser [14].

RESULTS

The present study conducted molecular docking simulations to explore the binding interactions between Reserpine, Vinblastine, Ajmalicine, and Vindolinine with the HPA protein.

Interactions of Reserpine with Human Pancreatic Alpha-Amylase (HPA): Molecular docking simulations revealed a strong interaction between Reserpine and HPA, with a binding energy of -9.3 kcal/mol. Key amino acids within the binding site of HPA, including HIS320, TRP174, TYP166, LYS215, ILE250, ASP212, LEU180, GLN78, and VAL178, exhibited significant interactions with Reserpine [Table/Fig-2]. These interactions involved hydrogen bonds, hydrophobic contacts, and electrostatic forces, suggesting a potential role of Reserpine in modulating HPA activity and supporting its antihypertensive effects.

Interactions of Vinblastine with Human Pancreatic Alpha-Amylase (HPA): Vinblastine demonstrated a strong interaction with HPA, particularly with amino acids ASP315, LEU180, and GLU255, with a binding energy indicating potential modulatory effects on HPA activity [Table/Fig-2]. These findings suggest a potential role of Vinblastine in influencing glucose metabolism and insulin regulation, highlighting its therapeutic potential in managing diabetes.

Interactions of Ajmalicine with Human Pancreatic Alpha-Amylase (HPA): Ajmalicine exhibited a strong binding interaction with HPA, involving specific amino acids GLN78, TRP74, and TYP77 [Table/Fig-2]. These interactions suggest potential antihyperglycaemic properties of Ajmalicine and its possible role in diabetes management by modulating HPA activity.

Interactions of Vindolinine with Human Pancreatic Alpha-Amylase (HPA): Vindolinine demonstrated a strong binding interaction with HPA, with key amino acids LEU177, ASP215, ALA213, ASP212, LEU180, and VAL178 involved in stabilising Vindolinine within the binding pocket [Table/Fig-2]. These interactions indicate the potential antidiabetic effects of Vindolinine, highlighting its therapeutic implications for managing diabetes.

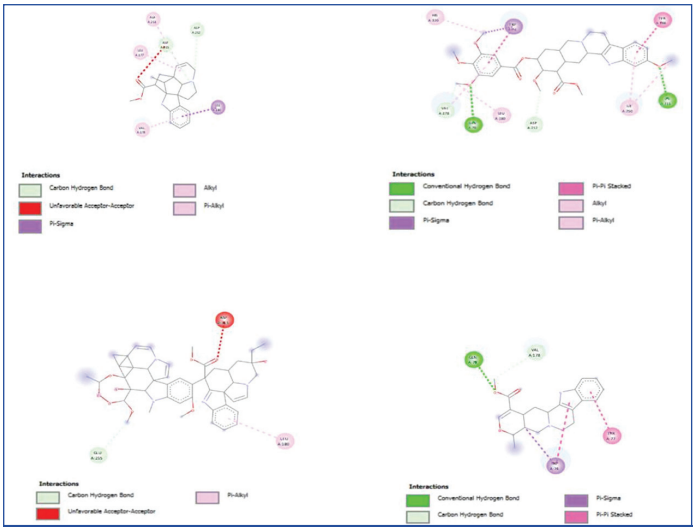
S. no.	Compound name with PubChem id	Binding energy (kcal/mol)	Interacting amino acids
1.	Reserpine (5770)	-9.3	HIS 320 TRP174 TYP166 LYS215 ILE250 ASP 212 LEU180 GLN78 VAL178
2.	Vinblastine (13342)	-9.8	ASP315 LEU180 GLU255
3.	Ajmalicine (441975)	-8.7	GLN78 TRP74 TYP77
4.	Vindolinine (24148538)	-9	LEU177 ASP215 ALA213 ASP212 LEU180 VAL178

[Table/Fig-2]: Molecular Interactions between Alkaloids from *Catharanthus roseus* (*C.roseus*) and Human Pancreatic Alpha-Amylase (HPA).

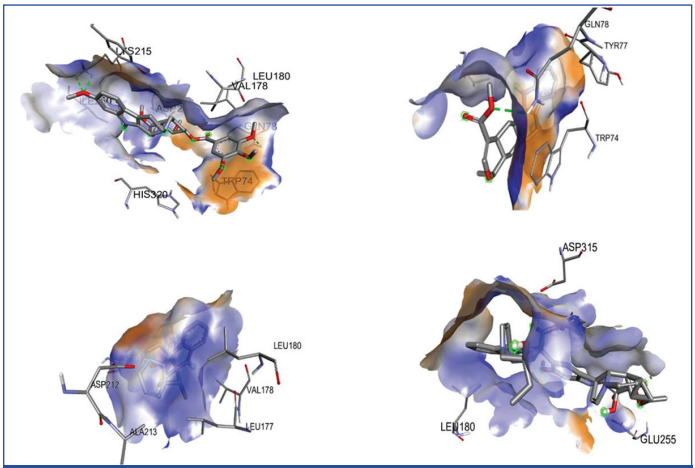
The two-dimensional interactions between HPA and the alkaloids Reserpine, Vinblastine, Ajmalicine, and Vindolinine are shown in [Table/Fig-3,4].

DISCUSSION

Catharanthus roseus, commonly known as Madagascar periwinkle or Vinca, is a plant species renowned for its production of valuable compounds with pharmaceutical significance. This plant has shown promising potential in the field of diabetes research due to its antidiabetic activity and the compounds it produces. Research has demonstrated that extracts from *C. roseus* possess antidiabetic properties, including the ability to lower blood glucose levels, improve insulin sensitivity, and protect pancreatic beta cells responsible for insulin production [6]. Among the compounds derived from *C. roseus*, Reserpine, Vinblastine, Ajmalicine, and Vindolinine have



[Table/Fig-3]: Showcases the two-dimensional interactions between Human Pancreatic Alpha-Amylase (HPA) and the alkaloids Reserpine, Vinblastine, Ajmalicine, and Vindolinine. The interactions are depicted using Biovia Discovery Studio Visualiser, highlighting the hydrogen and hydrophobic interactions.



[Table/Fig-4]: The results of the interactions were visualised using Biovia Discovery Studio Visualiser, emphasising the aromatic ligand interactions.

been investigated for their potential antidiabetic effects. While their primary focus lies in other therapeutic areas, studies have explored their impact on diabetes management [7-15]. Reserpine, known for its antihypertensive and antipsychotic properties, has shown potential in influencing glucose metabolism and insulin secretion, suggesting possible antidiabetic effects [16]. Vinblastine, primarily used as a chemotherapeutic agent, has received limited investigation regarding its antidiabetic potential. Ajmalicine, recognised for its antihypertensive and vasodilatory effects, has demonstrated antihyperglycaemic properties by enhancing glucose uptake and improving insulin sensitivity. Vindolinine, which exhibits cytotoxic activity against cancer cells, has not been extensively studied for its antidiabetic effects. Ongoing research is actively investigating the antidiabetic potential of the compounds derived from *C. roseus*. Both the plant as a whole and its extracts have demonstrated promising activity in managing diabetes [17]. However, further studies are required to fully comprehend the underlying mechanisms and evaluate the efficacy of the specific compounds present in *C. roseus* for diabetes management. Thus, the present study aimed to analyse the antidiabetic potential of the alkaloids found in *C. roseus* through in-silico methods. By conducting this analysis, the authors aimed to gain insights into the potential of these alkaloids as therapeutic agents for diabetes.

In the present study, molecular docking simulations were conducted to investigate the binding of Reserpine (PubChem ID: 5770) to the HPA protein. The results revealed a strong interaction between Reserpine and HPA, with a binding energy of -9.3 kcal/mol. Through analysis of the docking pose, significant interactions were observed between Reserpine and specific amino acids within the binding site of HPA, including HIS320, TRP174, TYR166, LYS215, ILE250, ASP212, LEU180, GLN78, and VAL178. These interactions likely played a vital role in stabilising Reserpine within the binding pocket, involving hydrogen bonds, hydrophobic contacts, and electrostatic forces. The demonstrated high binding affinity of Reserpine to HPA in the present study supports its potential antihypertensive activity, as Reserpine is known to block the release of norepinephrine from sympathetic nerve terminals, leading to vasodilation and reduced blood pressure. These findings are consistent with the established pharmacological effects of Reserpine and further support its potential therapeutic use [18]. Vinblastine (PubChem ID: 13342) is primarily known for its role as a chemotherapeutic agent in cancer treatment by disrupting cell division [19]. However, recent studies have explored its potential antidiabetic effects [20,21]. The strong interaction observed between Vinblastine and HPA, specifically with amino acids ASP315, LEU180, and GLU255, suggests a potential modulatory role in HPA activity. As HPA is involved in carbohydrate digestion and its dysregulation is linked to diabetes progression, investigating Vinblastine's impact on HPA is of interest. Further research is needed to explore the precise mechanisms underlying Vinblastine's interaction with HPA and its potential implications for glucose metabolism and insulin regulation.

The present research may shed light on Vinblastine's therapeutic potential in managing diabetes, expanding its applications beyond cancer therapy. Ajmalicine (PubChem ID: 441975) exhibited a strong binding interaction with the HPA protein, as indicated by a binding energy of -8.7 kcal/mol. The docking results revealed specific amino acids involved in this interaction, including GLN78, TRP74, and TYR77. Ajmalicine has been recognised for its potential antihypertensive and vasodilatory effects, but recent studies have also investigated its antidiabetic potential [22]. These findings suggest that Ajmalicine may possess antihyperglycaemic properties and could play a role in managing diabetes. The interaction of Ajmalicine with GLN78, TRP74, and TYR77 within the binding site of HPA may have implications for glucose metabolism and insulin regulation. GLN78 is involved in hydrogen bonding, while TRP74 and TYR77 may contribute to hydrophobic and electrostatic interactions, respectively. Vindolinine (PubChem ID: 24148538) exhibited a strong binding interaction with HPA, with a binding energy of -9 kcal/mol, suggesting its potential antidiabetic effects. The docking results revealed specific amino acids involved in this interaction, including LEU177, ASP215, ALA213, ASP212, LEU180, and VAL178. These interactions likely played a role in stabilising Vindolinine within the binding pocket, involving various types of interactions such as hydrogen bonding, hydrophobic contacts, and electrostatic forces. While Vindolinine is primarily recognised for its cytotoxic activity against cancer cells, the observed binding interaction with HPA raises the possibility of its antidiabetic potential. HPA is involved in carbohydrate digestion, and its dysregulation is associated with diabetes progression. Therefore, compounds that can modulate HPA activity may have implications for glucose metabolism and insulin regulation. The interactions observed between Reserpine, Vinblastine, Ajmalicine, and Vindolinine with HPA provide valuable insights into their potential roles in diabetes management. While these compounds are primarily known for their effects in other therapeutic areas, their strong binding affinities to HPA suggest a potential modulation of glucose metabolism and insulin regulation. Further research is warranted to elucidate the precise mechanisms underlying their interactions with HPA and their implications for diabetes treatment. These findings contribute to the growing body of evidence supporting the therapeutic potential of natural compounds, such as those derived from *C.roseus*, in managing diabetes. Additionally, the computational approach employed in the present study offers a valuable tool for screening and identifying potential antidiabetic agents, paving the way for future experimental validation and clinical translation.

Limitation(s)

Limitation is the exclusive reliance on computational methods, particularly molecular docking, which may oversimplify molecular interactions. The lack of experimental validation could limit the reliability and translation of findings. Experimental studies are crucial to confirm predicted interactions and elucidate the mechanisms underlying the therapeutic potential of bioactive alkaloids from *C. roseus*.

CONCLUSION(S)

In conclusion, the computational analysis highlights the potential of Vinblastine, Ajmalicine, and Vindolinine from *C. roseus* as therapeutic agents for diabetes management. Vinblastine exhibited a strong binding affinity to HPA, suggesting its role in modulating glucose metabolism. Ajmalicine displayed significant interactions with HPA, indicating its potential antihyperglycaemic properties. Similarly, Vindolinine demonstrated robust binding interactions with HPA, suggesting its potential as an antidiabetic compound. These findings underscore the promise of natural compounds in offering novel therapeutic avenues for diabetes. Further experimental validation and mechanistic studies are warranted to fully explore their therapeutic efficacy and safety profiles.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Jan 12, 2024
- Manual Googling: Mar 18, 2024
- iThenticate Software: May 08, 2024 (14%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? No
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jan 12, 2024**
Date of Peer Review: **Mar 15, 2024**
Date of Acceptance: **May 09, 2024**
Date of Publishing: **Aug 01, 2024**