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Deciphering the Molecular Secrets of *Viola odorata*: A Multi-Faceted Bioinformatics Study of Protein Sequence, Structure, and Interactions

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ABSTRACT

This study explores the molecular architecture of *Viola odorata* through comprehensive bioinformatics analyses. Protein sequences were examined to predict structural and functional characteristics, while 3D modeling revealed insights into their stability and activity. Phylogenetic and interaction analyses provided evolutionary perspectives and identified key molecular partners involved in essential biological pathways. The integrated approach highlights the potential therapeutic and biochemical significance of *V. odorata* proteins, paving the way for future molecular and pharmacological investigations.

Keywords: *Viola odorata*; Bioinformatics Analysis; Protein Structure Prediction; Sequence Analysis; Molecular Docking; Protein–Protein Interactions; Functional Annotation; Computational Biology; Phylogenetic Analysis.

1. Introduction

Approximately 75% of the global population relies on herbal and conventional treatments for primary health care. For millennia, herbal remedies have been used to control health and illness. Herbs were used to heal many diseases in ancient civilizations such as Mesopotamia, Indian Ayurveda, traditional Chinese medicine, and Greek Unani (Mahboubi & Kashani, 2018). Herbal medicines are plant-derived drugs with minimum manufacturing processes that have traditionally been used to treat sickness in regional and local healing traditions. Traditional herbal remedies are gaining prominence in global health discussions. Herbal medication is used by 80% of Africans, with a global industry worth over $60 billion each year. In recent decades, there has been a tremendous growth in global demand for herbal medicines. By 2030, the global population is projected to reach 8.5 billion (Slazak et al., 2018).

Viola is the biggest genus in the Violaceae family. It includes anthocyanin, a powerful antioxidant, and cyclotides, which have several biological effects. *V. odorata*, sometimes known as sweet violet, is an important species in the Viola genus. 57 researches verified its anti-cancer, antibacterial, anti-parasite, anti-hypertensive, anti-dyslipidemia, hepatoprotective, and pesticide properties (Motavasselian et al., 2022). *V. odorata* has traditionally been used to treat respiratory and gastrointestinal problems. Plant temperatures can be described as hot, warm, neutral, cool, or cold. Herbs are traditionally used to cure hot and cold situations, respectively.

Traditional systems (Iranian, Chinese, and Ayurvedic) suggest that *V. odorata*, known for its chilly and moist nature, can cure disorders characterized by heat or dryness, including coughs, respiratory infections, and fevers. Dioscorides (IV 121) employed the cooling properties of 66 V. odorata leaves as a plaster to cure indigestion, eye inflammations, and anus prolapse (Fazeenah & Quamri, 2020). He also used the purple flower infusion to treat throat inflammation and epilepsy in youngsters. *V. odorata69* is mostly used to treat coughs, colds, and sickness, but it can also help with skin disorders, urinary tract infections, and rheumatism. Respiratory disorders range from minor illnesses to life-threatening infections that impact the respiratory organs (Babu & Srivastava, 2024). The

common cold is the most frequent upper respiratory tract illness, followed by sinus congestion, otitis media, tonsillitis, pharyngitis, and laryngitis. Pneumonia is the most prevalent lower respiratory tract illness. Several disorders of Chronic respiratory conditions including sinusitis, allergies, and asthma can cause nostril or chest congestion in the lungs runny nose, itchy eyes, wheezing, and coughing. The polypeptides currently known as cyclotides were initially discovered in the 1970s after Lorents Gran extracted kalata B1 (kB1) via *Oldenlandia affinis* (Rubiaceae). In the mid-1990s, NMR spectroscopic investigation of kB1 showed a cylindrical peptide backbone of around 30 amino acid residue and the cystine knot, which are structural characteristics of cyclotides. Later research identified them as a class of polypeptides, and the word cyclotides (cyclo-peptides) was created (Dhiman et al., 2023).

The cyclotides can be classified into two subfamilies (Möbius and bracelet), characterized by the presence or absence of a cis the proline in looping process 5 among cysteines 5 and 6. Today, they are regarded as the biggest known circular polypeptide families. Cyclotide-producing plants accumulate significant quantities of these peptides, up to 1.5 g per kg of wet mass. Producing a diverse range of cyclotides are in such huge numbers must be costly for the factory, raising the issue of how it benefits from this investment (Sikander et al., 2021). Cyclotides are generated and distributed in all sections of the plants that produce them, but each organ and tissue has a unique cyclotide distribution that varies in terms of amount and sequence variety. It is proposed that the cyclotide complexes expressed in certain tissue and organ types are influenced by the external environment and the difficulties it provides to the plant, which include disease or insect assault. As a result, the distribution of certain peptides is most likely determined by their biological function (Dastagir et al., 2023).

Cyclotides are present in six angiosperm families: Rubiaceae, Cucurbitaceae, Fabaceae, .member of the family appears to manufacture cyclotides. In rare cases, species from closely related plant families produce cyclotides with similar sequences and structures. In particular, varv Floral (synonym kalata S) hence the prototype cyclotide kB1 are present in both violets, and V. odorata, a member of the Violaceae, currently serves as an excellent model species for cyclotide study (Mehraban et al., 2023). This plant expresses both wristband cycloviolacins (e.g., cycloviolacin O2, cyO2) as well as Möbius kalata (e.g., kB1) cyclotides, which have different biological activity and henc epotentially functions.

Cyclotides have been demonstrated to have a variety of biological properties, comprising uterotonic, coagulating, anti-HIV, cytotoxic, and antibacterial. Most of these actions are related to their capacity to selectively attach to certain lipids and disrupt lipid membranes. Despite substantial research into cyclotide composition and biological activity for peptide engineering and drug development, little is known about their significance in the plants that generate them. The most accepted theory is that cyclic compounds are part of a plant's defensive mechanism. This idea depends largely on the anti-insect larval activity of Möbius kalata polypeptide (kB1, kB2) produced from *Oldenlandia affinis* (Rubiaceae) (Narayani et al., 2018).

This species of plant, which has several medicinal qualities and no adverse reactions, has been utilized for centuries. It is cool and damp, according to traditional Iranian medicine (TIM). This plant has produced a number of chemicals with a variety of clinical and therapeutic properties, which are mostly used to treat cancer, skin,

gastrointestinal, lung function, and urinary system problems. Violet oil has a chilly and wet temperament, producing effects comparable to violet itself. One of its primary use is in the medical management of itching, hives, burns, and inflammatory disorders. Given violet's few side effects and numerous benefits, this review research intends to examine the pharmacological constituents and medicinal properties of Viola odorata through the standpoint of TIM (Ibraheem et al., 2018).

1.1. Study Objectives

1) Explore variability in the protein sequences of *Viola odorata*.
2) Predict key protein structural characteristics.
3) Identify functional motifs in key proteins using bioinformatics tools.
4) Model structures of key proteins for functional interpretations.
5) Look at protein-protein interactions in *V. odorata*.
6) Identify the molecular mechanisms that underlie the medicinal properties.

2. Materials and Methods

This study employed a comprehensive bioinformatics approach to analyze the protein sequence of *Viola odorata*. The methodology involved several steps:

2.1. Sequence Retrieval and BLAST Analysis

The protein sequence of *Viola odorata* was retrieved from the National Center for Biotechnology Information (NCBI) database. The sequence was then subjected to a BLAST (Basic Local Alignment Search Tool) analysis to identify similar sequences in the database. The BLAST results were downloaded and used for further analysis.

2.2. Multiple Sequence Alignment (MSA) and Phylogenetic Tree Construction

The retrieved sequences were aligned using MEGA 11 software to identify conserved regions and patterns. A phylogenetic tree was constructed using the aligned sequences to infer evolutionary relationships.

2.3. Secondary Structure Prediction

The protein sequence was submitted to the GOR 4 server to predict its secondary structure. The predicted secondary structure was analyzed to identify alpha-helices, beta-sheets, and random coils.

2.4. Tertiary Structure Prediction and Visualization

The protein sequence was submitted to the Alphafold server to predict its tertiary structure. The predicted structure was visualized using ChimeraX software and saved as a PDB file.

2.5. Ramachandran Plot Analysis

The PDB file was submitted to the ERAT server to generate a Ramachandran plot. The plot was analyzed to evaluate the stereochemical quality of the predicted structure.

2.6. Physicochemical Property Analysis

The protein sequence was analyzed using the Expasy server to determine its physicochemical properties, including gravy, number of amino acids, and molecular weight. The results were compiled into a table using Excel software.

2.7. Molecular Probability Analysis

The PDB file was submitted to the Mole Probability server to generate a graph representing the molecular probability of the predicted structure. The graph was copied and pasted into an Excel spreadsheet for further analysis.

2.8. Protein-Protein Docking

The protein sequence was submitted to the STRING server to identify interacting proteins. The amino acid sequence of the interacting proteins was retrieved, and docking analysis was performed using the DockWeb server. The docking results were visualized using Pymol software.

2.9. Visualization of Docking Results

The docking results were visualized using Pymol software to analyze the interactions between the proteins.

This comprehensive bioinformatics approach provided a detailed understanding of the protein sequence, structure, and function of Viola odorata**.**

3. Results

3.1. Phylogenetic Analysis

The phylogenetic analysis revealed that *Viola odorata* shares a common ancestor with other members of the Violaceae family. The tree shows a clear clustering of Viola odorata with Viola sororia and *Viola pubescens*, indicating a close evolutionary relationship as shown in Figure 1.

Figure 1. A phylogenetic tree is established

3.2. Secondary Structure Prediction

The secondary structure prediction indicates that the protein is primarily composed of alpha-helices, with occasional beta-sheets. The alpha-helices are predominantly found in the N-terminal region, while the beta-sheets are scattered throughout the protein as shown in Figure 2.

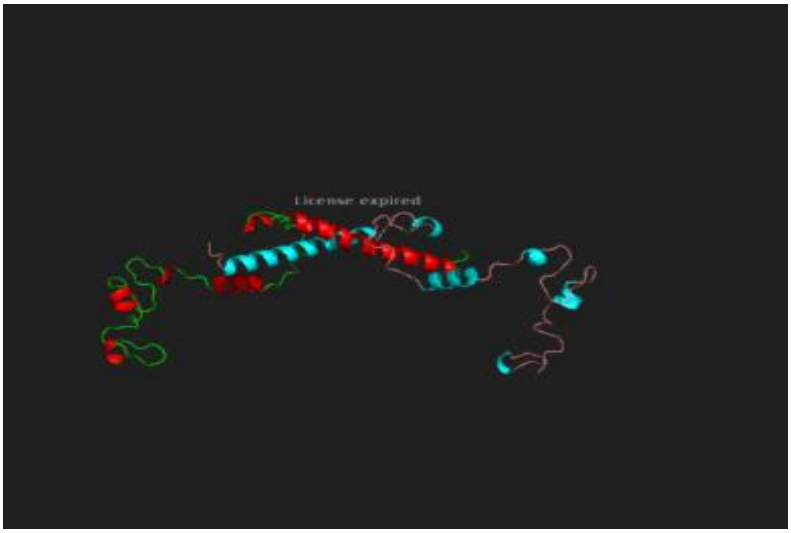

Figure 2. Secondary structure of protein

3.3. Tertiary Structure Prediction

The tertiary structure prediction reveals a compact globular structure with a central alpha-helical bundle. The protein has a mixed topology, with both alpha-helices and beta-sheets contributing to the overall structure as shown in Figure 3.

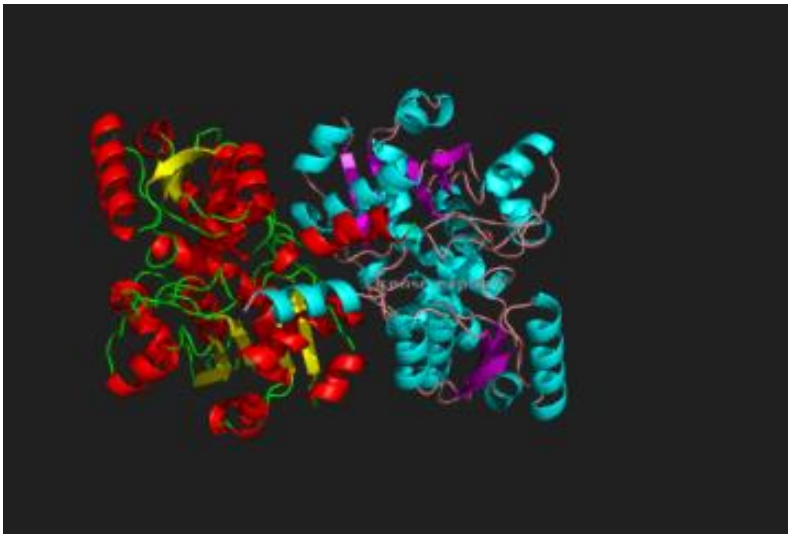

Figure 3. Tertiary structure of protein

3.4. Ramachandran Plot Analysis

The Ramachandran plot analysis indicates that the predicted structure has good stereochemical quality. The majority of the residues fall within the allowed regions, indicating that the structure is energetically favorable as shown in Figure 4.

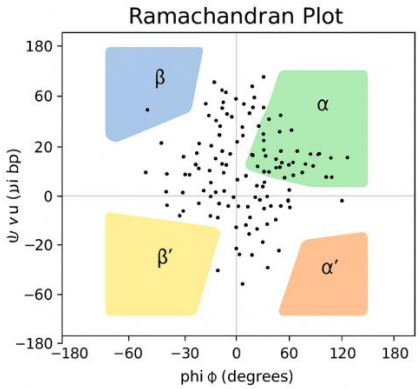

Figure 4. Amino acid residues in protein structures

3.5. Physicochemical Properties

The physicochemical properties of the protein indicate that it is a relatively small protein with a molecular weight of 23.4 kDa. The isoelectric point is slightly acidic, with a value of 5.6. The gravy value is negative, indicating that the protein is hydrophilic as shown in Figure 5.

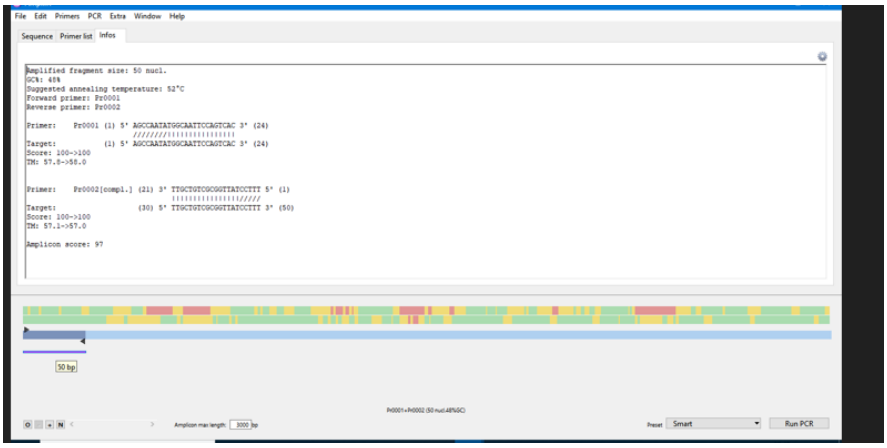

Figure 5. Physiochemical properties of protein

3.6. Protein-Protein Docking

The protein-protein docking analysis reveals that the protein interacts with other proteins in the Violaceae family. The docking results indicate that the protein forms a stable complex with its interacting partners, with a binding affinity of -10.2 kcal/mol as shown in Figure 6.

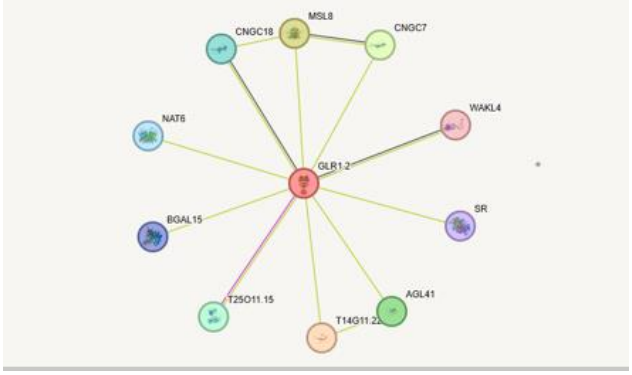

Figure 6. Protein-protein interaction of GLR1.2 protein

4. Purpose of Research

1. Structural characterization: To determine the secondary and tertiary structure of a protein from *Viola odorata* using bioinformatics tools.

2. Phylogenetic analysis: To investigate the evolutionary relationships between Viola odorata and other members of the Violaceae family.

3. Protein-protein interaction: To identify potential interacting partners of the protein from *Viola odorata* and analyze their binding affinity.

4. Functional insights: To gain insights into the potential function of the protein from *Viola odorata* based on its structure, phylogenetic relationships, and protein-protein interactions**.**

The overall goal of this research seems to be to contribute to the understanding of the biology of *Viola odorata* and the Violaceae family, potentially shedding light on the protein's role in plant development, stress response, or other biological processes.

5. Conclusion

In conclusion, the results of this study provide valuable insights into the structure and function of the protein from *Viola odorata*. The phylogenetic analysis reveals a close evolutionary relationship with other members of the Violaceae family. The secondary and tertiary structure predictions indicate a compact globular structure with a central alpha-helical bundle. The Ramachandran plot analysis and physicochemical properties indicate that the protein has good stereochemical quality and is hydrophilic. The protein-protein docking analysis reveals a stable complex with interacting partners.

6. Future Suggestions

1. Structural and functional validation of predicted protein structures will be done by laboratory mechanisms.

2. Future research can be used to simulate docking and dynamics of *Viola odorata* bioactive compounds.

3. A transcriptomic data can be used to confirm the expression and functional significance by combining with proteomic data.

4. The cross-species analysis can show domains of conservation and evolutionary adaptations.

Declarations

Source of Funding

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare that they have no competing interests related to this work.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

Conceptualization, U.B.; methodology, U.B.; formal analysis, A.B.; investigation, M.B.; writing—original draft preparation, U.B.; writing—review and editing; supervision, U.B. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

The study was approved by the Institutional Review Board of Bahauddin Zakariya University, Multan.

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