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# Characterization Of Medicinally Important Plant Protein Abrin Through In-Silico Analysis

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## Abstract

*Abris precatorius* is of great significance in medicine as it is a great source of variety of therapeutic compounds including abrin protein. Abrin exhibits anticancer and antimicrobial potential. However, its use is limited due to its toxicity. This toxicity might be reduced through genetic alterations of protein. Current study was initiated to unravel its physicochemical and structural features that might be useful for its manipulations and optimum exploitation in medicine. For this purpose, initially the amino acid sequence was obtained from NCBI (National Center for Biotechnology Information) database and subjected to characterization via SOPMA, PROTPARAM, SWISS-MODEL and TMHMM server and CB-Dock-2 docking platform. The values of alpha helix, extended strand and random coil in abrin protein were analyzed as 12.57, 29.84 and 57.59%, respectively. Protein was identified with 382 amino acids, 5.58 pI, 32.76 instability index, 79.61 aliphatic index and -0.376 GRAVY. Protein exhibited transmembrane helix and highly complex 3D configuration. Docking analysis revealed its C4 pocket as the primary binding site. The characteristics explored in current study might help in optimizing the medicinal applications of abrin protein via providing information for structural alterations.

## Introduction

Medicinal plants serve as an excellent source of bioactive molecules and therapeutics because of their rapid growth (Rathor, 2021) and the ease with which sequence and structural databases enable characterization of these plant derived biomolecules at the molecular scale (Baltoumas et al. 2021; Castro, 2023; Hosen et al. 2022; Sharma et al. 2014). Several curated nucleotide and protein sequences of a broad variety of plant species are found in public repositories like NCBI, BioPhytMol and GreenMolBD which is minable to find putative therapeutic proteins and provide annotations of their fundamental properties (Saleem et al. 2024; Sieniawska, 2024). All of these resources can be used to perform *in-silico* characterization of plant proteins comprehensively prior to costly validation in wet laboratories (Bhat et al. 2022; Nene et al. 2022).

Besides small-molecule phytochemicals, plants are also the source of numerous bioactive proteins and peptides, such as ribosome-inactivating proteins (RIPs), lectins and enzyme inhibitors, which are antimicrobial, immunomodulatory and anticancer (Fatima and Bashir 2025; Konozy et al. 2024; Padiyappa et al. 2025; Saleem et al. 2024; Wong et al. 2020).

Abrin is a type II ribosome-inactivating protein derived from *Abrus precatorius* that has been of interest due to its potential cytotoxicity, anticancer and immunotoxin properties (Lin et al. 2025). Similar to other RIPs, abrin can act as a toxin and may be used as a targeted therapy (Chopra et al. 2020). Abrus precatorius is one of its source plant. Literature documents abrin potential of cancer cells associated protein production and accelerates apoptosis (Okhale and EM, 2016; Parabakaran, 2024).
The use of modern bioinformatics tools enables the prediction of secondary structure (2D), physicochemical properties, transmembrane topology and protein-protein interaction networks using only the amino-acid sequence (Bouvier, 2021; Lu et al. 2024). In the given work, the amino-acid sequence of abrin protein was downloaded from database and was subjected to integrated in-silico workflow that included SOPMA-based 2D structure prediction, ProtParam analysis of physicochemical properties, TMHMM-based transmembrane topology prediction, SWISS-MODEL homology modelling, STRING-based interaction analysis and docking with CB-Dock2. This computational characterization offers a structural/functional framework of understanding the therapeutic potential of abrin, and future experimental efforts.

**Methodology**

Retrieve the sequence

To retrieve the sequence of protein, the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/, accessed on 31st July 2024) was accessed. Protein sequence is available under the Accession number AAL77434.1.

**SOPMA tool**

SOPMA protein secondary structure prediction tool was consulted using the Network Protein Sequence Analysis (NPS@) server at https://npsa.lyon.inserm.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html (accessed on 31st July 2024) to predict the 2D configuration of protein.

**PROTPARAM tool**

Physicochemical properties of the protein including amino acid count, molecular weight, molar extinction coefficient, isoelectric point (pI), aliphatic and instability index, and grand average of hydropathicity (GRAVY) were determined using the ProtParam tool available on the ExPASy server (https://web.expasy.org/protparam/, accessed on 31st July 2024).

**SWISS-MODEL homology modelling server**

The tertiary (3D) structure of the protein was predicted using homology modeling via the SWISS-MODEL server accessible through the ExPASy platform at https://swissmodel.expasy.org/interactive (accessed on 1st August 2024). The predicted 3D structure of protein was analyzed using QMEAN (Qualitative Model Energy Analysis) and structural validation tools to assess model reliability and functional relevance. To map interactions between plant therapeutic proteins and other biomolecules, we employed STRING v12.0 accessible at; https://string- db.org (accessed on 31st July 2024).

**TMHMM server**

The TMHMM (Transmembrane Hidden Markov Model) v1.0.39 server was used to identify the membrane topology and α-helical trans-membrane domains.

**CB-Dock**

For protein-ligand docking, the cavity-based docking platform CB-Dock-2 (http://clab.labshare.cn/cb-dock2/) was modeled to investigate interactions between plant-derived therapeutic proteins and bioactive phytochemicals.

## Results

Assessment of 2D structure

Abrin exhibited high conformational flexibility, dominated by random coils (57.59% random coil). This structural disorder likely facilitates their roles in ribosomal RNA binding and catalytic inactivation (Table 1).

**Assessment of physicochemical properties**

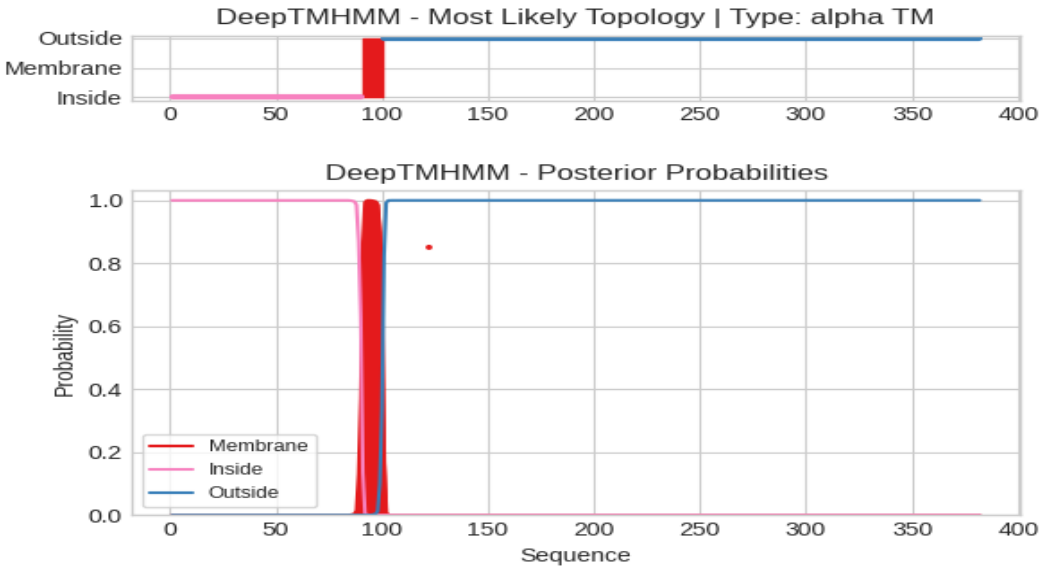
Abrin (42 acidic, 38 basic residues) exhibited approximately balanced charge ratios, suggesting theoretical pI close to neutrality, which may enhance intracellular stability and minimize the aggregation in physiological environment (Table 1).

**Table 1: Assessment of secondary (2D) configuration and physicochemical attributes of abrin protein**

| Attributes predicted | Values |
|---|---|
| SOPMA tool based analysis | |
| Alpha helix (%) | 12.57 |
| Extended strand (%) | 29.84 |
| Random coil (%) | 57.59 |
| PROTPARAM tool based analysis | |
| No. of amino acids | 382 |
| Molecular weight | 42743.50 |
| pI | 5.58 |
| Instability index | 32.76 |
| Aliphatic index | 79.61 |
| GRAVY | -0.376 |

## Prediction of trans-membrane topology

The DeepTMHMM analysis predicted the transmembrane topology of abrin as an alpha-helical transmembrane (TM) protein. The most likely topology indicated that abrin contains transmembrane segments, with regions alternating between the inside and outside of the membrane. Posterior probabilities plot showed high-confidence predictions (probabilities $\geq 0.8$) for these segments, suggesting well-defined membrane-spanning domains. The




graph displayed peaks corresponding to transmembrane helices, with the protein likely traversing the membrane multiple times between residues 0–400 (Figure 1).

Figure 1: Prediction of transmembrane topology of abrin via Deep TMHMM tool

Assessment of 3D configuration

Abrin obtained a GMQE score of 0.82, indicative of a highly reliable template alignment. This was complemented by a QMEANDisCo score of 0.77 ± 0.05, which confirmed the stereochemical quality and internal consistency of the predicted structure. Strong concordance between these two independent metrics underscores the high reliability (Figure 2a and b).


(a) (b)

Figure 2: Assessment of three-dimensional (3D) configuration of abrin protein based on SWISS-MODEL server

**GMQE and QMEANDisCo scores (b) 3D structure of abrin**

Assessment of abrin protein interaction with other proteins

The functional partner prediction for abrin presented a notably specific profile. The analysis identified a single high-confidence interaction with the protein product of locus CKAN_01586300. The exclusivity of this interaction, supported by a confidence score of 0.519, suggested a highly specialized biological function.

**Docking analysis**

Based on the molecular docking results, the analysis identified pocket C4 as the primary binding site for the ligand (Table 2). This conclusion is supported by its superior Vina docking score of -5.9 kcal/mol, which signifies the strongest predicted binding affinity among all detected cavities. The specific coordinates of the C4 pocket center (47, 15, 40) and its defined docking grid (17, 17, 17) provided a precise spatial location for the predicted binding pose. This high-confidence prediction for C4 establishes it as the most promising target for subsequent investigations, such as structure-based drug design or mechanistic studies of ligand recognition (Figure 3).

**Table 2: Top five energetically favorable binding pockets for abrin protein**

| CurPocket ID | Vina Score (kcal/mol) | Cavity Volume (Å³) | Center Coordinates (x, y, z) | Docking Grid Size (x, y, z) |
|---|---|---|---|---|
| ○C4 | -5.9 | 326 | 47, 15, 40 | 17, 17, 17 |
| ○C3 | -5.3 | 630 | 56, 24, 39 | 17, 23, 28 |
| ○C1 | -4.9 | 944 | 51, 14, 27 | 23, 23, 17 |
| ○C2 | -4.8 | 760 | 63, 12, 55 | 17, 17, 17 |
| ○C5 | -4.7 | 246 | 67, -10, 49 | 17, 17, 17 |

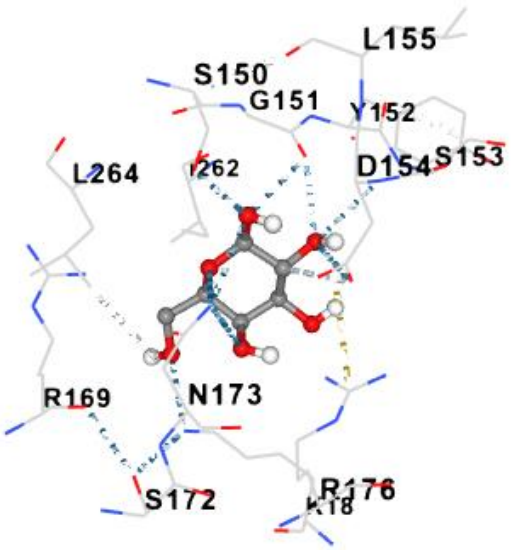



Figure 3: Identification of pocket C4 in abrin protein as the primary binding site for the ligand based on docking analysis

**Discussion**

The in-silico studies revealed that abrin has predominant random-coil secondary structure (57.59%), with minor proportions of alpha-helix and extended strand, which is in line with the existence of flexible loop regions in and around the catalytic and binding sites (Gadadhar and Karande, 2013; Geourjon and Deleage, 1995). This conformational plasticity is also common to ribosome-inactivating proteins and is believed to help them to recognize their substrate and efficiently depurinate rRNA (Gadadhar & Karande, 2013). ProtParam analysis found that the theoretical pI of abrin was neutral with a pI of 5.58, and aliphatic index was moderate implying that the protein is relatively stable in physiological conditions as well as soluble enough (Gasteiger et al., 2005).

The scores predicted via SWISSMODEL along with favorable local quality estimations indicated that core fold and active-site regions are recapitulated well and can be used in docking studies (Gasteiger et al., 2005; Kiefer et al., 2009). Only one high-confidence

interaction partner (CKAN_01586300) was identified in the STRING analysis, indicating a functionally specialized role and not a wide involvement in large protein-protein networks. CB-Dock2 docking revealed that cavity C4 was the most energetically favorable binding site whereas, residues like S150, D154, N173 and R169 were essential in the primary interaction (Szklarczyk et al., 2023). Collectively, these data showed that abrin is a conformationally adaptable but structurally robust therapeutic protein with a clear, druggable pocket, which can be used to develop it further as an anticancer immunotaxin, as well as to design safer analogs (Gadadhar and Karande, 2013).

For management and applications of abrin, precautionary measures are necessary during investigation because of the toxicity associated with this protein. Current study might help in capitalizing the applications of abrin in therapeutics through its genetic engineering.

**Statements and Declarations**

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