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In-silico Dyeing strategy: Unveiling the Binding of Cotton Protein Annexin to Phyto-Pigments through Bioinformatics

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ABSTRACT

Natural dyes derived from plant sources such as fruits and flowers offer enhanced biodegradability and environmental compatibility compared to synthetic alternatives. This bioinformatics study explores the in-silico interactions of phyto-pigments with cotton-specific proteins, emphasizing their potential applications in the cotton textile industry. Utilizing an integrated bioinformatics approach including molecular docking, virtual screening, and visualization tools such as DS Visualizer, we investigated the binding interactions between phyto-pigments and Annexin, a key cotton protein. A total of seven phyto-pigments were examined: Malvidin, Peonidin, Cyanidin, Petunidin, Pelargonidin, Betanin, and Betacyanin. Among these, Betanin and Betacyanin found in beetroot and dragon fruit, respectively exhibited the strongest binding affinities with Annexin. These preliminary findings from in-silico studies suggest a promising role for these bio-pigments in the development of natural dyes with functional biological activity. The elucidation of specific binding modes and structural compatibilities reinforces the value of in-silico studies in advancing the application of bio-based dyes for sustainable textile dyeing processes. The study is conducted using bioinformatics tools and is focused solely on molecular docking simulations. No experimental dyeing or laboratory-based validation was undertaken at this stage. This bioinformatics study underscores the potential of phyto-pigments not only as eco-friendly colorants but also as agents capable of forming meaningful biochemical interactions within textile fibres. Prog Color Colorants Coat. 19 (2026), 281-296© Institute for Color Science and Technology.

1. Introduction

Cotton (Gossypium spp.) is the most widely produced natural textile fibre, characterized as a staple fibre that develops around the cotton seed (Figure 1), as seed hairs. These fibres typically measure between 22 and 32 mm in length, with each cotton seed capable of producing between 5,000 and 20,000 fibres [1, 2]. Cotton fibres, composed of 88-97 % cellulose, also contain waxes, proteins, and pectin. After harvest, lint fibres are separated from seeds for yarn production. These fibres are single-cell trichomes originating from the seed coat's outer layer [3, 4] and elongate rapidly for 20-25 days, reaching lengths of 2-3 cm [5]. Cytoskeletal development during this phase supports cell expansion, with annexin proteins-calcium-dependent phospholipid-binding proteins-playing a key role. In cotton, two annexins are fibre-specific and likely contribute to fibre elongation by stabilizing protein scaffolds [3, 5]. Annexins also support stress responses, particularly to salinity and drought, and can

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Figure 1: Cotton Seed with fibr.

represent ~0.1 % of total plant protein, varying by development and environment [6, 7]. Thus, annexins are integral to cotton fibre development and stress adaptation. Cotton fibres are living cells during the early stages of development Annexins, being abundantly present in these fibres are involved in membreane organization and structural stability. Research identifies and characterizes the annexin gene family in cotton, providing a foundation for in-silico studies of protein-pigment binding and stress response mechanisms.

Cotton is a crucial raw material in the dyeing industry due to its natural fibre properties, it allows excellent dye absorption and color retention. The versatility of cotton fibres enables a wide range of dyeing techniques and applications, making it a preferred choice for producing vibrant and durable textiles. Dyes are an essential component of the textile industry, providing color and aesthetic appeal to fabrics. However, the use of the synthetic dyes is associated with significant environmental pollution and health concerns. The dyeing process in the textile industry often requires large volumes of water, which can become contaminated with dyes, chemicals, and other pollutants, leading to severe environmental degradation when discharged untreated into rivers and streams [8]. Many synthetic dyes contain toxic compounds, including heavy metals like lead, cadmium, and chromium, as well as carcinogenic substances, posing significant risks to aquatic life and human health [9, 10]. Furthermore, residual dyes in textiles can cause allergic reactions or skin irritations in sensitive individuals [11]. In response to these challenges, many countries have established regulations to control the discharge of dye wastewater and limit the use of hazardous substances in textile production. The industry is increasingly adopting sustainable practices, such as using eco-friendly dyes, implementing closed-loop water systems, and treating wastewater prior to discharge, to mitigate these environmental impacts. Analyses of textile dye impacts on health and ecosystems reinforce the urgency for sustainable alternatives. In recent years, there has been a significant shift toward natural dyes in the textile industry. Natural dyes, sourced from plants, insects, and minerals, are celebrated for their eco-friendliness, biodegradability, and renewable properties, as well as their antibacterial benefits and safety for human health [12, 13]. Recent work highlights the shift towards biotechnological methods for sustainable dyeing, focusing on enzyme-based processes and microbial pigment production, which reduce environmental impact and improve dye-fiber interactions [14]. However, challenges such as a limited color range compared to synthetic alternatives, poor color fastness, and inconsistent quality hinder their widespread adoption [15]. Natural dyes from plants and agriculture byproducts show promising results in textile dyeing, increasing sustainability and environmental friendliness while offering performance properties for fast fashion products [16]. Additionally, natural dyes often require larger quantities and the use of mordants to improve dye adherence, which can introduce toxicity concerns [17]. Despite these limitations, there is a growing demand for natural dyes as they offer a safer and sustainable alternative.

Anthocyanins, a class of water-soluble pigments found predominantly in plants, have gathered interest in the textile dyeing industry due to their vibrant colors and natural origin [18, 19]. Key anthocyanins such as malvidin, peonidin, cyanidin, petunidin, and pelargonidin are responsible for the red, purple, and blue hues observed in various fruits and flowers, making them suitable candidates for natural dyes [20]. Anthocyanins are known for their antioxidant properties, which can enhance the durability and lightfastness of dyed fabrics [21]. As the demand for sustainable and eco-friendly dyeing processes increases, anthocyanins present a viable alternative to synthetic dyes, offering not only aesthetic appeal but also potential health benefits [22]. Studies demonstrate improved extraction and application methods for anthocyanins from sources like Hibiscus and pomegranate, optimizing color yield and fastness on cotton fabrics [23]. This paper explores the potential of seven natural dyes sourced from various botanical origins (Table 1).

Table1: Phyto-pigments present in different plant.

Sl No.	Plant	Common Name	Fruit/Flower	Pigment
1	*Syzygium cumini*	Indian Jamun Fruit	Fruit	Malvidin
2	*Delonix regiya*	Gulmohar	Flower	Peonidin
3	*Hibiscus sabdariffa*	Hibiscus	Flower	Cyanidin
4	*Vinis vitifera*	Bluish Purple Grapes	Fruit	Petunidin
5	*Punica granatum*	Pomegranate	Fruit	Pelargonidin
6	*Beta vulgaris*	Beetroot	Fruit	Betanin
7	*Selenicereus costaricensis*	Dragon Fruit	Fruit	Betacyanin

The seven pigments selected for this study-Malvidin, Peonidin, Cyanidin, Petunidin, Pelargonidin, Betanin, and Betacyanin-were chosen based on the following criteria: Natural Occurrence and Abundance: These pigments are commonly found in a wide variety of plant sources such as berries, grapes, beets, and other fruits, making them easily accessible for potential dye extraction [24]. Several of these pigments, particularly anthocyanins (Malvidin, Cyanidin, etc.) and betalains (Betanin, Betacyanin), have been traditionally used in natural dyeing and are known for their color vibrancy and bio-compatibility [25]. The selected pigments represent structurally diverse classes (anthocyanins and betalains), allowing us to explore a broad spectrum of pigment–protein interactions relevant to dye binding. Some, such as Betanin, are also noted for their relatively high stability under certain pH and temperature conditions, which is desirable in dyeing applications.

Jamun (*Syzygium cuminii*), an evergreen tree (Figure 2a) native to Southeast Asia, is characterized by its anthocyanin pigments, which serve as the primary coloring agent in its fruit [26]. *Delonix regia*, commonly known as Gul mohr or royal poinciana, is a fast-growing tree (Figure 2b) recognized for its clusters of crimson blossoms; pigments extracted from its reddish-orange flowers are valuable for textile dyeing [27]. The diverse genus Hibiscus, particularly *Hibiscus sabdariffa* (Figure 2c), yields water-soluble anthocyanin pigments that vary in color based on pH, making it a viable alternative to synthetic dyes [28]. Bluish Purple Grapes (*Vitis vinifera*) (Figure 2d), primarily cultivated for consumption, also offer dye potential through their anthocyanin content [29]. The peel of Pomegranate (*Punica granatum*) (Figure 2e) contains six anthocyanin compounds, including pelargonidin. Beetroot (Beta vulgaris), with its main coloring agent (Figure 2f), betanin, is known for its characteristic red color [30]. Dragon fruit (*Selenicereus costaricensis*), or pitaya (Figure 2g), is valued for its betacyanin pigment, which imparts a vibrant red hue, making it suitable for dye extraction [31]. In the present study the extraction of pigments (Figure 2 a, b, c, d, e, f, g) from these plant sources was conducted solely to assess their feasibility and suitability of extraction. These natural sources not only provide sustainable alternatives for textile dyeing but also highlight the rich diversity of plant-based colorants available for exploration. Furthermore, the evaluation of dye fixation to fabrics is crucial for quality control; however, current testing methods face limitations such as variability in conditions, subjective visual assessments, and discrepancies across laboratories, which complicate the accurate interpretation of results and the optimization of dyeing processes [32]. Interdisciplinary research is needed to enhance their integration into textiles and improve their sustainability [33].

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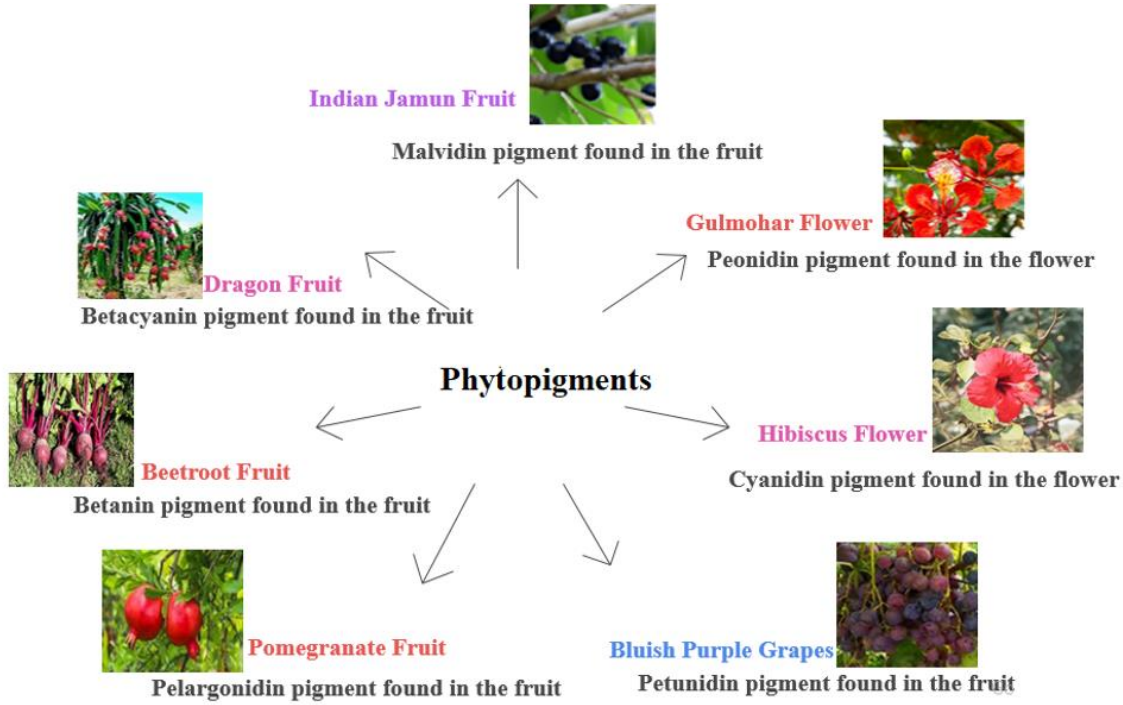


Figure 2: Phyto-pigments and their source.

In this preliminary findings, we introduce a novel approach for selecting the most suitable natural dye through comprehensive in-silico analysis, marking the first research of its kind to evaluate and predict dye binding interactions specifically with protein present in the cotton fabrics. By focusing on the molecular structures of dyes and the specific protein (Annexin) compositions within cotton fibres, we leverage advanced bioinformatics tools to deeply investigate dye-fabric binding dynamics. Recent research has focused on sustainable dyeing methods for cotton fabrics using microbial and natural pigments. These studies emphasize green processing, improved dye uptake, and multifunctional textile properties, but do not specifically address the molecular or in-silico binding of cotton annexin proteins to phyto-pigments.

While direct studies on annexin-phyto-pigment binding are limited, bioinformatics tools are increasingly used to model protein-ligand interactions, supporting the rationale for our research approach. Current research highlights significant progress in eco-friendly dyeing of cotton with natural pigments and improved textile properties. However, there is a clear gap in the literature regarding in-silico strategies to study the binding of cotton annexin proteins to phyto-pigments. Future there is a need to focus on bioinformatics and molecular modeling approaches to explore these protein-pigment interactions for innovative dyeing strategies. Understanding Annexin–pigment interactions is significant because Annexins may act as potential binding sites or facilitators for pigment adherence at the molecular level, thereby influencing pigment retention, fixation, and uniformity during bio-based dyeing processes. Furthermore, this interaction could help design eco-friendly, protein-assisted dyeing strategies that enhance the binding efficiency of natural dyes, thereby reducing environmental impact. The in-silico approach used in our study provides preliminary insights into this binding mechanism, laying the groundwork for future experimental validation and potential application in sustainable textile dyeing. Our research here work emphasizes a molecular-level assessment that not only enhances our understanding of dye interactions with cotton but also provides a foundation for optimizing dye selection, contributing to sustainable and efficient textile processing. The study is conducted using bioinformatics tools and is focused solely on molecular docking simulations. No experimental dyeing or laboratory-based validation was undertaken at this stage.

2. Experimental

2.1. Extraction of pigments from plant species

Natural pigments were extracted from various plant species using standardized methods to obtain specific anthocyanins, with the primary aim of evaluating the feasibility and suitability of the extraction process. Below are the detailed procedures for extraction of pigments from each plant species and figures are attached as Figure 2 a, b, c, d, e, f and g, respectively.

Jamun Fruit (*Syzygium cumini*)

- **Sample Preparation**: 500 g of jamun fruit was weighed and placed in a round-bottom flask.
- **Extraction Process**: 1000 mL of a solvent mixture (ethanol:water, 40:60) was added. The flask was heated in a water bath at 60 °C for 60 minutes.
- **Filtration**: The solution was filtered to obtain the dye, with malvidin identified as the primary pigment extracted.

Gul Mohar (*Delonix regia*)

- **Sample Preparation**: Fresh petals and leaves (5 g each) were collected, washed, and crushed using a mortar and pestle.
- **Extraction Process**: The crushed material was dissolved in 5 mL of a solvent mixture (distilled water:methanol) and mixed uniformly using a vortex mixer.
- **Outcome**: A small quantity of dye was extracted, with peonidin being the identified pigment.

Hibiscus sabdariffa

- **Sample Preparation**: 10 g of hibiscus calyx was weighed and transferred to a clean conical flask.
- **Extraction Process**: 100 mL of distilled water was added, and the mixture was heated for 10 minutes.
- **Outcome**: The pigment extracted was identified as cyanidin chloride.

Bluish Purple Grapes

- **Sample Preparation**: A weighed amount of grapes was extracted with distilled water in a beaker, maintaining a mass-to-volume ratio of 5 g of grapes to 20 mL of water.
- **Extraction Process**: The extraction was performed at varying temperatures (40, 60, 80, and 100 °C) for different durations (40, 60, 80, and 100 minutes) to maximize dye yield.
- **Outcome**: The primary pigment extracted was petunidin.

Pomegranates

- **Sample Preparation**: 100 g of pomegranate sample was weighed and placed in a round-bottom flask.
- **Extraction Process**: 500 mL of a solvent mixture (ethanol:water, 40:60) was added, and the flask was heated in a water bath at 60 °C for 60 minutes.
- **Filtration**: The solution was filtered to obtain the dye.

Beetroot

- **Sample Preparation**: 150 g of chopped beetroot was weighed.
- **Extraction Process**: The beetroot was treated with a solvent mixture (methanol:water, 80:20, v/v) and 50 mM ascorbic acid, adjusting the pH to 5.5. The mixture was then centrifuged at 15,000 g at 40 °C for 30 minutes.
- **Outcome**: The pigment extracted was identified as betanin.

Dragon Fruit

- **Sample Preparation**: The peel of dragon fruit was stripped and diced into small pieces, then shade-dried.
- **Extraction Process**: The dried peel was ground into powder and extracted in distilled water at a concentration of 10 g/L for 14 hours at room temperature (20 °C), followed by 1 hour each at 40, 60, and 80 °C with continuous stirring. The extracted solution was filtered using Whatman filter paper.

2.2. Bioinformatic studies

2.2.1. 2D structures of pigments

PubChem is a freely accessible database maintained by the National Center for Biotechnology Information (NCBI), which is part of the United States National Library of Medicine (NLM). PubChem supports many aspects of small molecules often ligands. In this studies, we use PubChem to download the small molecules (pigments) that bind to proteins of interest, Annexin. We have downloaded the seven different pigments in 2D SDF file format for docking with a

macromolecule protein, Annexin.

FTIR Analysis of Functional Groups in Natural Pigments has been done earlier (Harborne, J. B (1998), Naczk, M., & Shahidi, F. (2004), Stintzing, F. C., & Carle, R. (2004). Table 2 shows that Hydroxyl groups (O–H) are present in all pigments, indicated by broad a bsorption around 3300-3400 cm^{-1}**.** Aromatic C=C stretching vibrations appear between 1600-1620 cm^{-1}**.** Carbonyl (C=O) and carboxylic acids (–COOH) in betanin/betacyanin show bands near 1650 cm^{-1} and 1400 cm^{-1}. Methoxy ($–OCH_3$) groups in peonidin and petunidin are reflected in 1020-1050 cm^{-1}. C–O stretching (ether/alcohol) appears in the 1200-1300 cm^{-1} range. N–H stretching in betanin/betacyanin appears around 3400 cm^{-1}, overlapping with O–H.

The seven different pigments used in the study are downloaded in 2D SDF file format for docking with a macromolecule protein, Annexin. The pigments are sourced from the PubChem database (Figure 3 a, b, c, d, e, f and g) and are obtained in SDF (Structure Data File) format. Subsequently, these pigments are imported into PyRx, a molecular docking software. Within PyRx, users have the capability to select individual or multiple dyes for energy minimization. This process involves the optimization of dyes structures to attain a lower energy state, which enhances their potential to bind effectively with Annexin. Following energy minimization, the dyes are automatically saved in PDBQT (Protein Data Bank, Partial Charge, Atom Type) format. This formatted representation of the dyes is particularly advantageous for subsequent molecular docking simulations, enabling accurate predictions of dye-protein interactions and binding affinities.

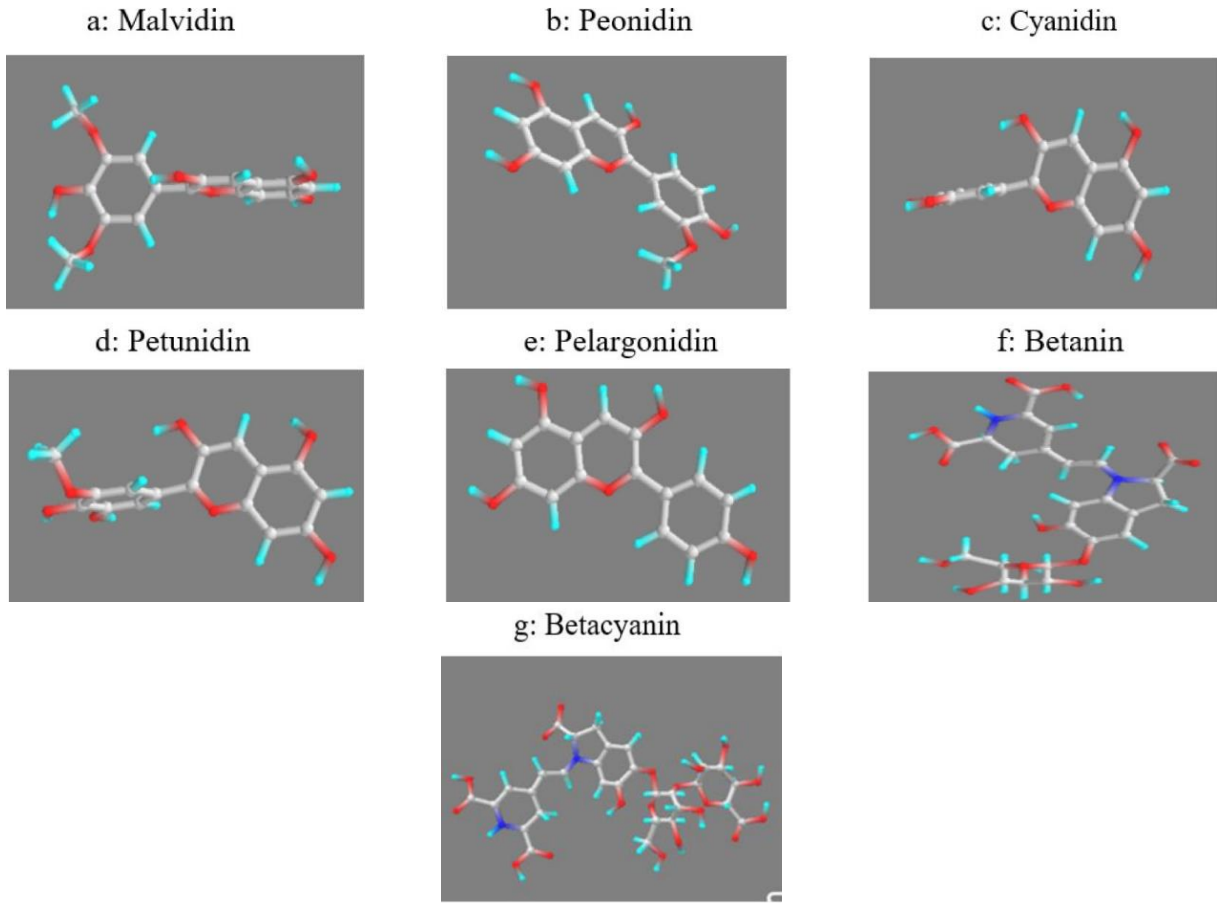


Figure 3: 2D structures of pigments (prepared ligand).

2.2.2. Docking studies

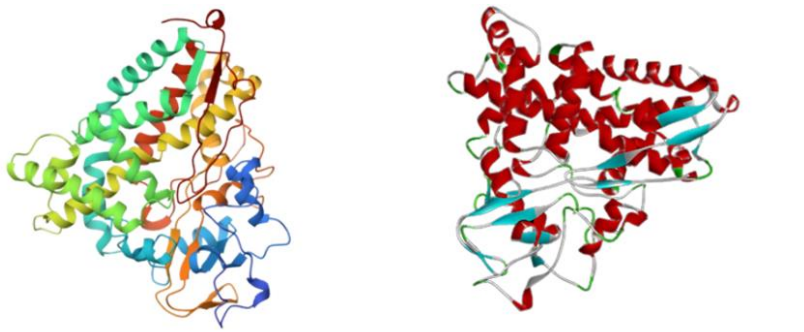
Docking is a bioinformatics technique used to forecast the most favourable alignment of one molecule with another, forming a stable complex when bound together. Molecular docking stands as a fundamental tool in structural molecular biology and computer-aided design. This approach is based on the same principle used in drug discovery, where molecular docking is employed to study interactions between drugs and proteins. Here we aim to anticipate the primary binding conuration of a pigments with a cotton protein Annexin of known three-dimensional structure. Molecular docking holds significant relevance in the field of natural dyeing, as it helps determine whether a specific dye molecule (ligand) can bind to a target protein, such as Annexin, present in textile fibres.

2.2.3. 3D Structure of annexin

The Protein Data Bank (PDB) is a database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. These data, typically are obtained by X-ray crystallography, NMR spectroscopy, or, increasingly, cryoelectronic microscopy, and submitted by biologists and biochemists from around the world the structures are freely accessible on the Internet via the websites of its member organizations. The macromolecular content of structure of annexin with ID name 1NOO (https://doi.org/10.2210/pdb1NOO/pdb) is downloaded. Total Structure Weight of Annexin is 47.37 kilo Daltons.

The 3D structure of annexin protein (Figure 4a) is retrieved from the Protein Data Bank (PDB) in the PDBQT format. This format allows for the inclusion of atomic charges, which are essential for molecular docking studies. The annexin protein is then imported into PyRx software for the purpose of adjusting its molecular properties to facilitate molecular docking simulations. Within PyRx, the imported annexin protein is designated as the macromolecule. This step is crucial for specifying the role of the protein within the docking process. Subsequently, the protein structure is further analyzed and manipulated using DS Visualizer software. The prepared annexin protein structure (Figure 4b) is then visualized and analyzed within DS Visualizer, allowing for detailed examination of the dye-protein interactions and the outcome of the docking simulations. This process is essential for understanding the potential binding modes and affinities of the selected dyeswith the annexin protein, which can have significant implications in cotton dying.

PyRx (version 0.9) is Virtual Screening software that enables biotechnologists to run Virtual Screening for Computational Discovery (https://pyrx. Sourceforge.io/downloads). PyRx facilitates the docking of small molecule dyes into the binding sites of proteins. It utilizes various algorithms and scoring functions to predict the most energetically favourable binding poses, which helps in understanding the interaction between ligands and proteins. virtual screening experiments can be done using PyRx to filter large compound libraries and by prioritizing compounds with the highest predicted binding affinities, PyRx accelerates the further docking process. PyRx offers visualization tools that enable users to analyze and visualize the docking results in 3D. This helps in interpreting the binding interactions between ligands and proteins, as well as in identifying key molecular features responsible for binding. PyRx is built on top of AutoDock, a widely used molecular docking software.



a: Structure of Annexin Protein from PDB b: Structure of prepared Annexin protein

Figure 4: Structure of annexin.

2.3. Visualization of docking

Discovery Studio (DS) Visualizer (from BIOVIA) is a free visualization tool for viewing, sharing, and analyzing proteins. It can allow us to: visualize proteins and small molecules and it helps to analyze docking result. Here Malvidin, Peonidin, Cyanidin, Petunidin, Pelargonidin, Betacyanin, and Betanin, their interaction with an annexin protein are explored through docking studies facilitated by Discover Studio Visualizer. The docking analysis within DS Visualizer involves a meticulous process (Figure 5) of predicting the preferred orientation and conformation of pigments within the binding site of the annexin protein. This predictive modeling allows to gain insights into the potential binding modes and affinity of the pigments towards the protein target. By leveraging Discover Studio Visualizer's advanced algorithms and visualization tools, we biotechnologists can examine the docking poses generated for each pigment, discerning key interactions such as hydrogen bonding, hydrophobic interactions, and electrostatic forces between the pigments and Annexin. Additionally, the software enables the visualization of binding energies, providing quantitative measures of the stability and strength of the dye-protein complexes.

2.4. Docking process

In this procedure, molecular docking is conducted using the PyRx simulation tool, an updated version of AutoDock Vina. Initially, Annexin from DS Visualizer, is loaded into PyRx The next step involves importing ligands in SDF file format, which are directly downloaded from PubChem. In this study, seven dyes are imported sequentially. Each selected dye is minimized to achieve an energetically favourable conformation and converted into the AutoDock-compatible PDBQT format. This process is repeated for all selected dyes. Subsequently, the Vina wizard is accessed, and after clicking the start button, the ligand and macromolecule are selected, and the user presses forward. A grid box appears to select receptor sites on the macromolecule for ligand binding. Initially, blind docking was performed by setting grid box to compass the entire molecule. Initially, blind docking is performed by setting the grid box to encompass the entire macromolecule. After this, clicking forward again initiates the program, which runs the docking simulation and outputs binding affinities and RMSD values for the ligands. The results are automatically saved in the directory The process is showed schematically in Figure 5.

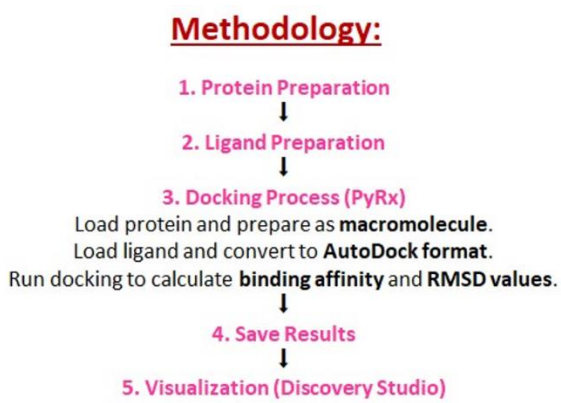


Figure 5: Docking steps in PyRx.

3. Results and Discussion

3.1. Docking studies from PyRx

The binding affinity of individual dyes with Annexin using PyRx are revealed through docking studies from PyRx. The docked result of different dyes and Annexin are shown in Figure 6a, b, c, d, e, f, g. The docking results of the individual dyes with Annexin complex obtained from PyRx are saved in PDBQT file format and subsequently analyzed using DS Visualizer.

The analysis reveals (Figure 7a, b, c, d, e, f and g) interactions between dye and Annexin. These interactions provide a detailed understanding of the binding mechanisms and stability of the ligand-receptor complex. A protein-ligand interaction, are categorized by different types of bond interactions. In this study we are trying to understand the protein Annexin's interactions with different dyes. The different types of bond interaction seen are hydrogen Bonding (H-Bonds), π-π Stacking, π-Anion Interactions, π-Alkyl Interactions, π-Sigma Interactions,Van der Waals Interactions. Hydrogen bonding interactions is a fundamental stabilizing force that provides specificity, orientation, and enhanced binding strength. The ionic and π-anion interactions contribute the highest binding affinity, while hydrogen bonds and π-π stacking add moderate to high affinity and specificity. π-π stacking increases binding affinity by adding a stabilizing interaction that enhances the receptor-ligand complex's overall strength and specificity. π-π stacking interactions are crucial for boosting binding affinity by providing a specific, stabilizing force that supports

proper dye alignment, enhances receptor selectivity, and strengthens the overall receptor-ligand interaction.π-alkyl, π-sigma, and Van der Waals interactions contribute by supporting alignment, hydrophobic stabilization, and fit, collectively enhancing the binding affinity. The π-anion, π-alkyl, and π-sigma interactions, each impact binding affinity by adding distinct types of non-covalent stabilizing forces between the ligand and receptor, influencing how strongly and selectively the ligand binds. Together, these interactions—π-anion, π-alkyl, and π-sigma—affect binding affinity by adding layers of stability, specificity, and alignment that complement stronger binding forces, like hydrogen bonds and ionic interactions. The observed unfavorable acceptor-acceptor interactions occur when two electron-rich atoms, both capable of accepting electrons, are in close proximity.

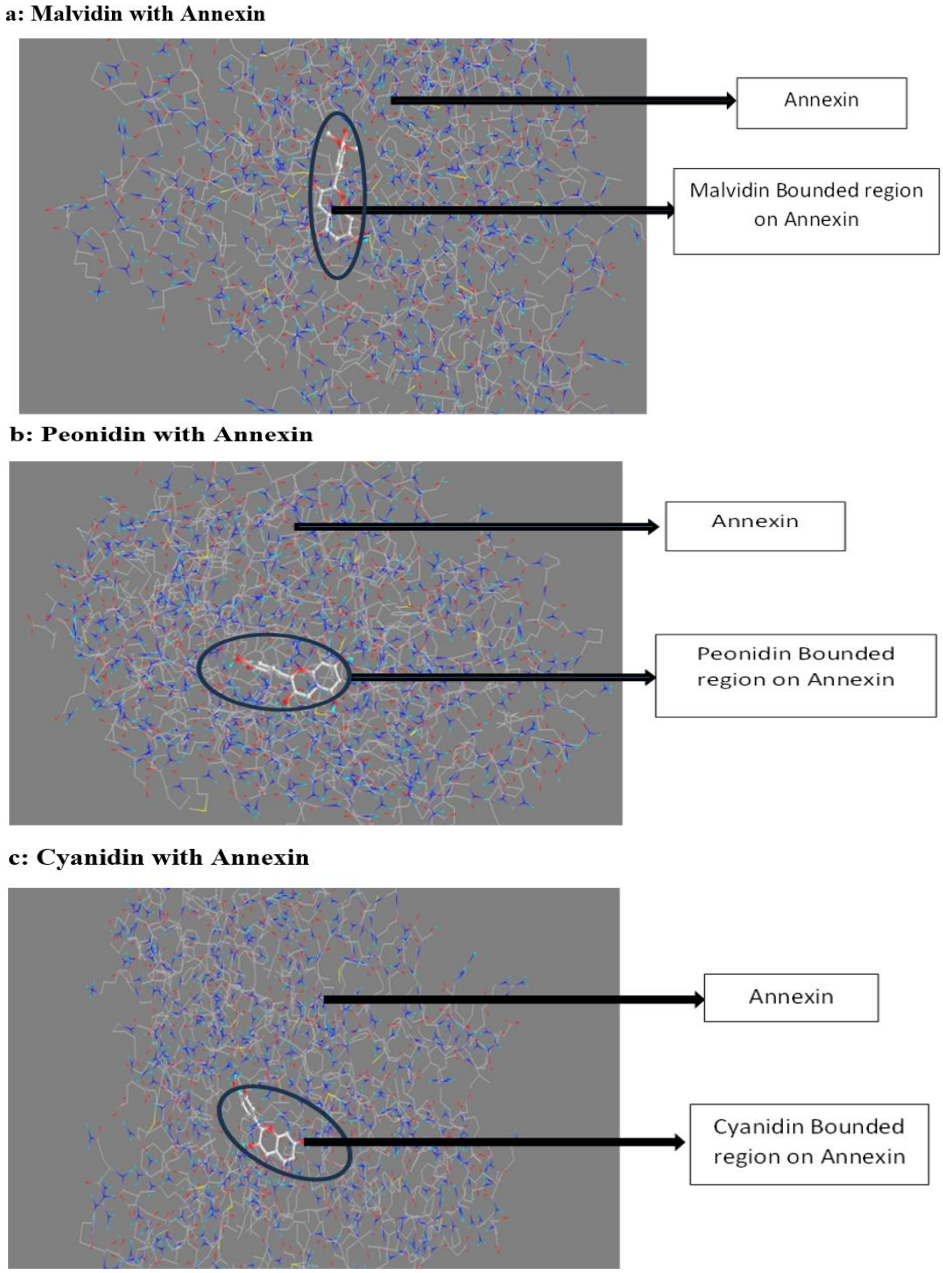
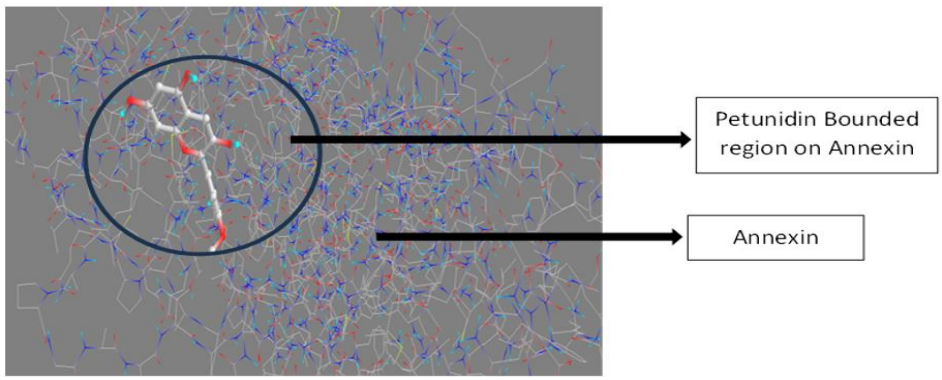
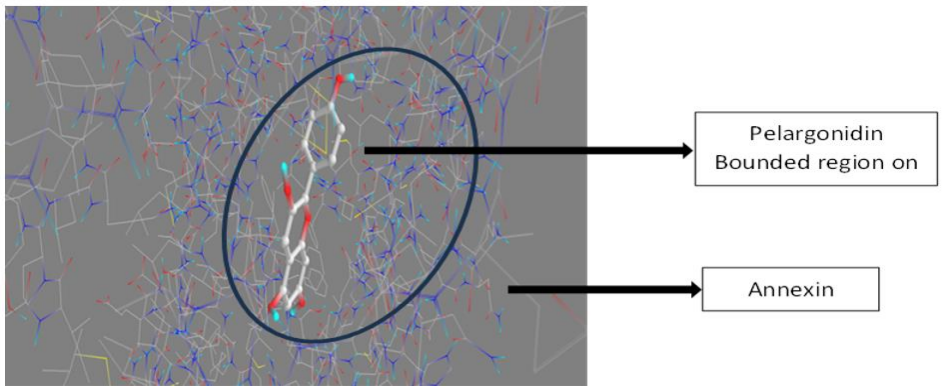


Figure 6: Docked result of dyes with protein from PyRx.

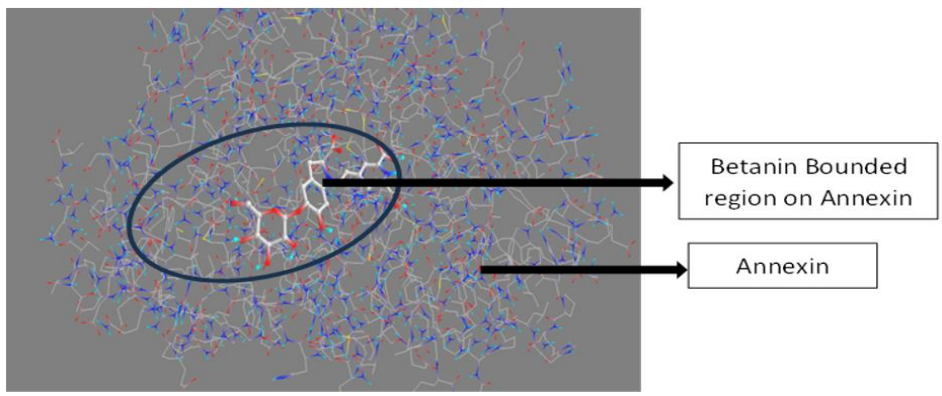
d: Petunidin with Annexin



e: Pelargonidin with Annexin



f: Betanin with Annexin



g: Betacyanin with Annexin

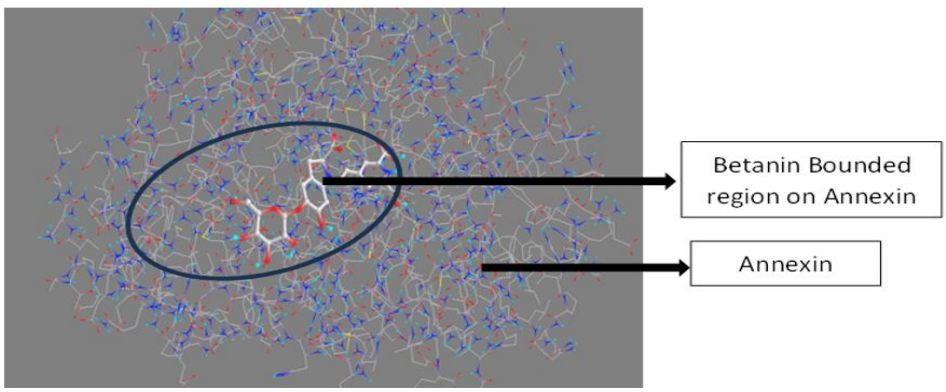
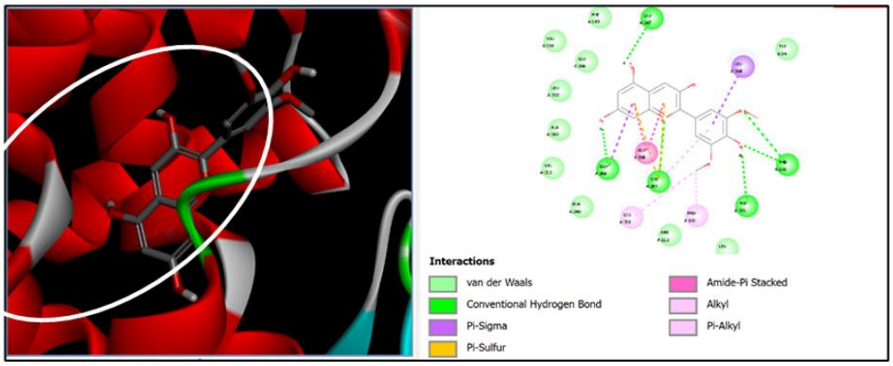
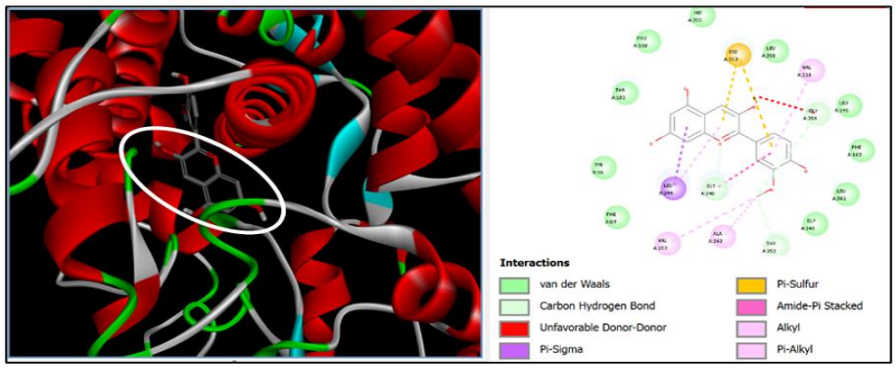


Figure 6: Continue.

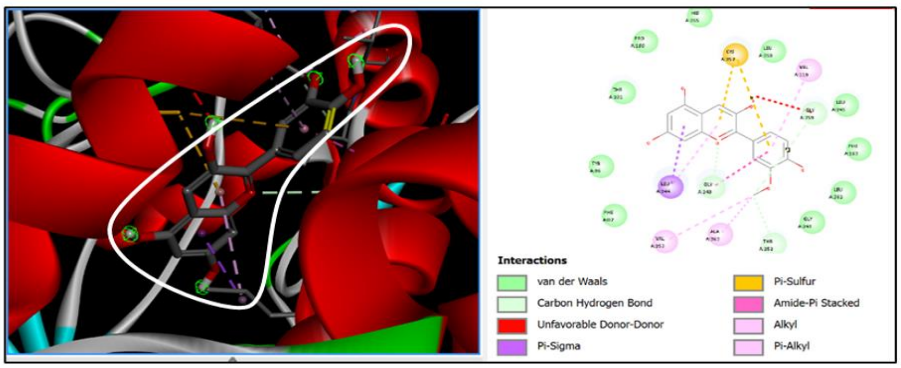
a: Malvidin with Annexin



b: Peonidin with Annexin



c: Cyanidin with Annexin



d: Petunidin with Annexin

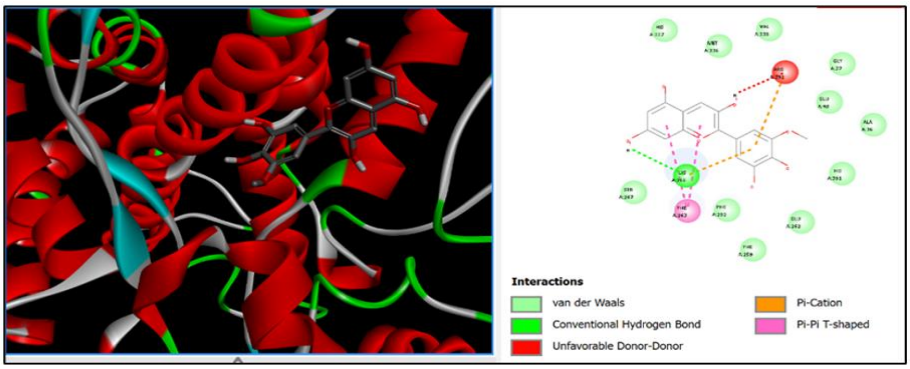
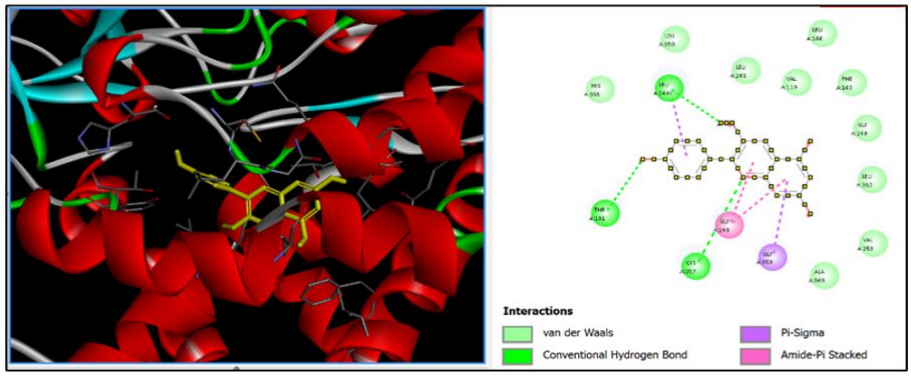


Figure 7: Visualization of docking result DS visualizer.

e: Pelargonidin with Annexin



f: Betanin with Annexin

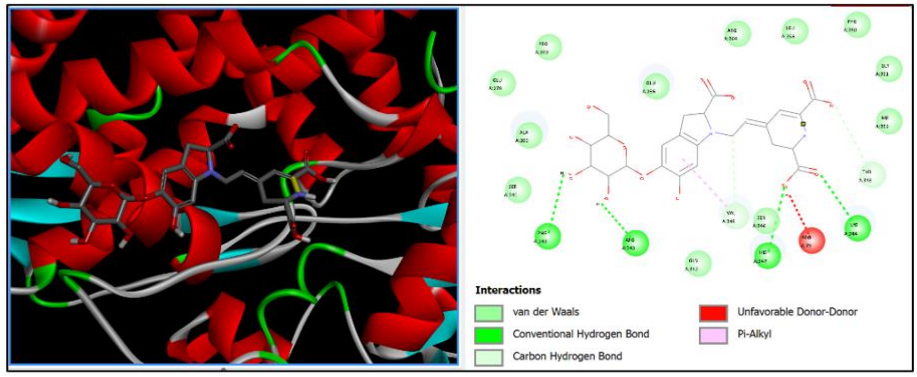


Figure 7: Continue.

These interactions are energetically unfavorable due to repulsive forces between the lone pairs on each acceptor atom. Unfavorable acceptor-acceptor interactions generally weaken binding affinity by preventing optimal interaction patterns. The interactions shown in Figure 7, collectively contribute to the binding affinity, specificity, and stability of the dye within the Annexin protein's binding site. These bond interactions along with the amino acid involved are tabulated as Table 2. This study reveals that Peonidin, Betanin, and Betacyanin exhibit a variety of bond interactions, including hydrogen bonds and π interactions, which are generally favorable for binding.Petunidin and Pelargonidin both have unfavorable Acceptor-Acceptor interactions, which may destabilize their interactions. Betacyanin has a complex interaction profile, including favorable interactions like salt bridges and attractive charges, but also includes unfavorable interactions. This complexity may affect its overall binding stability. Peonidin has a strong interaction profile with multiple favorable interactions and a good binding affinity (-8.1), making it a strong candidate. The pie chart (Figure 8) provides a visual representation of the distribution of different bond types observed in the pigment-amino acid interactions. This allows for a quick understanding of the prevalence of each type of interaction.

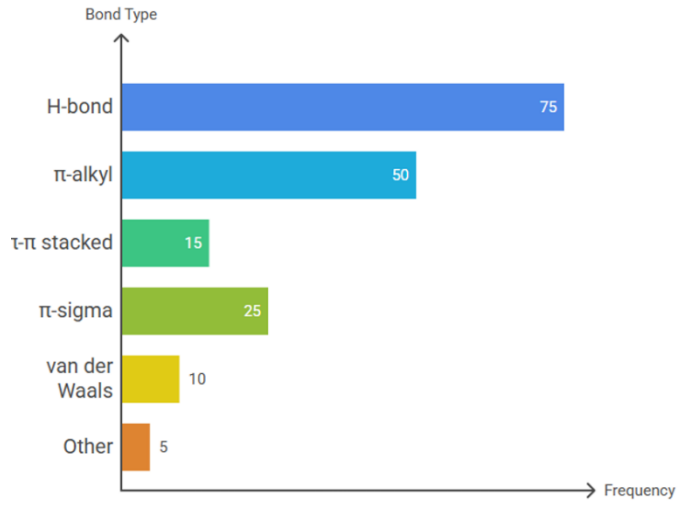


Figure 8: Distribution of bond types in pigment-amino acid interactions.

3.2. Analysis of amino acids involved in dye interactions:

The network map (Figure 9) illustrates the relationships between pigments and the amino acids they interact with. The nodes represent pigments and amino acids, while the edges represent the interactions between them. The amino acids involved in the interactions with annexin for each dye include hydrophobic amino acids (LEU, ALA, VAL, TRP, GLY, and PHE), polar amino acids (THR, GLN, and GLU), and charged amino acids (ARG, ASP, and LYS). These amino acids play a crucial role in the stability and binding affinity of the interactions between annexin and the dyes. The presence of hydrophobic amino acids helps to stabilize the interactions through hydrophobic and π-stacking interactions, while polar and charged amino acids contribute to the stability through hydrogen bonding and salt bridges. The diversity of amino acids involved in the interactions highlights the importance of various factors, such as hydrophobic effects, electrostatic interactions, and hydrogen bonding, in the binding of annexin with different dyes. The specific amino acids involved in these interactions are crucial in determining the nature and strength of the binding. Hydrophobic amino acids play a significant role in creating a stable environment for the dye, while polar and charged amino acids provide necessary interactions that enhance binding specificity and strength. For example, the combination of TRP and ARG in the interactions with Peonidin, Betanin, and Betacyanin illustrates how hydrophobic and charged interactions can work synergistically to achieve strong binding.

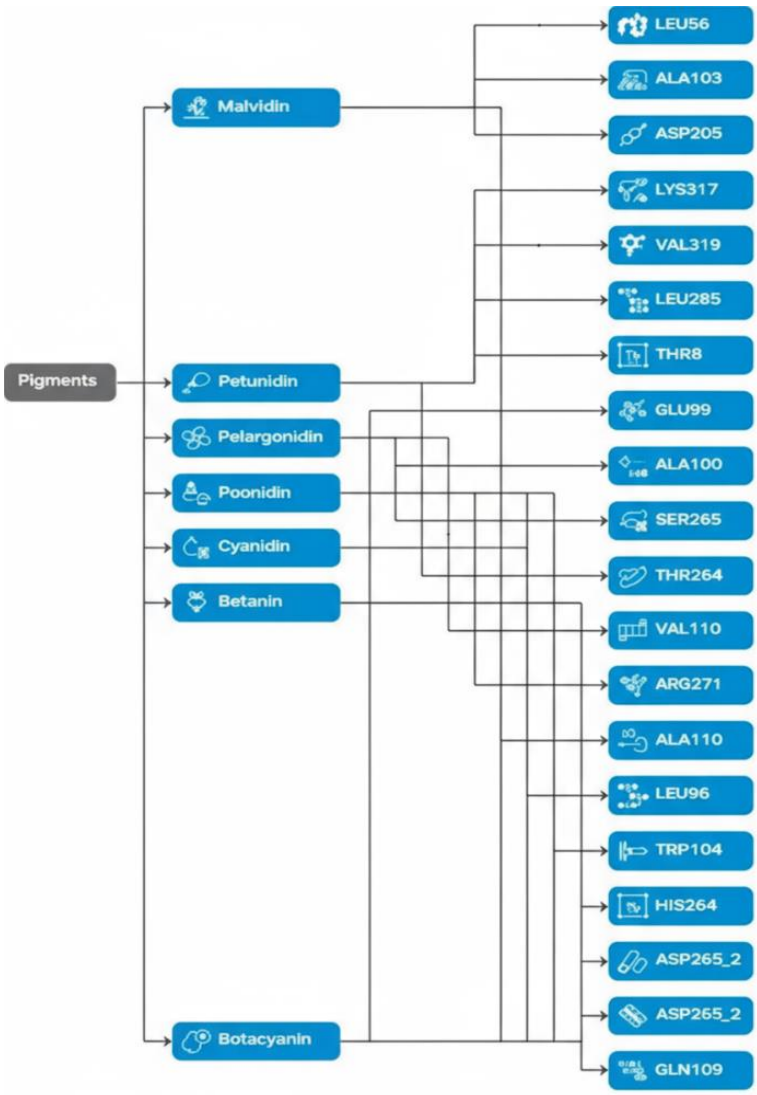


Figure 9: Pigment and amino acids interaction network.

In binding studies, Molecular Weight (MW), LogP (a measure of lipophilicity/polarity), and Topological Polar Surface Area (TPSA) are interrelated physico-chemical properties that together influence how a molecule interacts with its biological target. Lower MW and moderate polarity (Log P between -0.1 and 0.2) generally correlate with better binding affinities [29, 34]. Table 3 gives a clear relation between molecular weight (MW), polarity indicators and how these properties relate to the binding of the phyto-pigments. The more negative binding affinity values stronger is the binding; Highly polar and bulky pigments (like Betanin and Betacyanin) tend to show weaker binding, likely due to steric constraints and over-hydrophilic character, limiting efficient interaction within the annexin binding pocket. Balanced hydrophilic-hydrophobic properties (e.g., Cyanidin, Petunidin) appear to promote favourable binding through stable hydrogen bonding and better accommodation.

The more negative the binding affinity, the stronger the interaction. The lower the E-values better in molecular docking, as it suggest higher confidence in the interaction. E-values provide insight into the energetic favourability of the interactions. Lower E-values typically indicate more favourable interactions. From the Table 4 it is evident that Betanin and Betacyanin both show the strongest binding affinity of -8.6, indicating they have the potential for the most stable interactions with annexin. Betacyanin has the lowest E-value (227.43), suggesting a more favourable energetic profile compared to other dyes. The binding potential ranking of the pigments are represented as Figure 10. Betacyanin shows the strongest potential due to its highly negative binding affinity and complex interaction profile, despite the high E-value. Betanin follows closely with the same affinity but a slightly lower E-value. Petunidin ranks lower due to less favorable binding and a moderate E-value. Pelargonidin and Malvidin share the same rank due to close values.

4. Conclusion

This study provide insights into the structural basis of binding interactions between cotton protein Annexin and nature dyes. Based on the analysis, we conclude that Betanin and Betacyanin are best dyes, as both show the strongest binding affinity (-8.6), but Betacyanin has a more complex interaction profile with a lower E-value, indicating it could be energetically favorable despite some unfavourable interactions. Peonidin is also a strong contender with a good binding affinity and a favourable interaction profile. These are our final recommendation based on this study. Betanin and Betacyanin are both suitable choices, but if a simpler interaction with fewer potential destabilizing factors is preferred, Betanin may be the better option. However, if the complexity of interactions is acceptable, Betacyanin could offer a very strong binding interaction due to its favourable energetic profile. Therefore, Betanin is recommended as better choice. Further computational studies using molecular dynamics (MD) simulations help predict how dyes interact with proteins over time. These simulations can reveal the dynamics of binding and the stability of the dye-protein complex. Deeper Bio-informatic docking studies stand poised to revolutionize the textile industry by facilitating the design of sustainable dyeing processes, fostering the development of more effective dye molecules.

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