STM JOURNALS

International Journal of
Biochemistry and Biomolecules Research

ISSN: 3107-4022
Volume 3, Issue 2, 2025
July–December
DOI (Journal): 10.37591/IJBBR

https://journals.stmjournals.com/ijbbr

Research IJBBR

In-Silico Docking of *Rubia cordifolia* Phytocompounds as Potential MMP-2 Inhibitors for Varicose Vein Management

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Abstract

Varicose veins are one of the most common disorders, affecting the vein structure and function of lower limbs, there has been no absolute cure found yet. One of the underlying causes for worsening of the condition is the proteolytic activity of MMPs which are a group of enzymes that affect the extracellular matrix (ECM) surrounding the affected veins. Phytocompounds of Rubia cordifolia (Manjishta) were docked against the MMP-2 protein to check presence of protein-ligand interaction that can predict the possibility of using the best suited ligand as an inhibitor against MMP-2 protein. Out of a total of 37 phytochemicals, 17 were found to have the most drug-like properties. The ligands were retrieved after in-silico pharmacology studies and docked against the MMP-2 protein. 6 P-L were found to have lowest BI (less than or equal to –0.9) and RMSD value of 0, which corresponds to highly stable bonding between the molecules. This study gives a possibility of using the screened ligands, Munjistine, Rubiadin, 1,5-Dihydroxy-2-Methylanthraquinone, Purpuroxanthins, Alizarin and 1-Hydroxy-2-Methoxy Anthraquinone, to study their effects on reducing the activity of MMP-2, which can reduce the progression of varicose veins into chronic venous disease.

Keywords: Rubia cordifolia, MMP-2, molecular docking, varicose veins, munjistine

INTRODUCTION

Varicose veins, a common condition affecting a significant portion of the population, are characterized by abnormally enlarged, twisted, and dilated veins, often occurring in the lower extremities [1]. These vascular abnormalities can have a profound impact on an individual's quality of life, leading to various symptoms and potential complications.

The prevalence of varicose veins is estimated to range from 10% to 30% in the general population, with women (<1% to 73%) being more susceptible to the condition than men (2% to 56%) [2]. Main underlying mechanism of varicose vein development involves a multifaceted pathophysiology with the weakening of the venous wall, dysfunction of venous valves, and increased venous pressure. The complex interplay of these factors contributes to the development and progression of varicose veins, leading to the characteristic features of the disease [3]. One of the key underlying causes of varicose veins is the impairment of specific structural and functional proteins within the venous wall and valves [4]. The disruption of these proteins, such as those involved in elastin and collagen synthesis like MMP-2, also known as gelatinase A, with its ability to degrade type IV collagen, a major component of basement membranes, and denatured collagen

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Received Date: May 16, 2025
Accepted Date: June 12, 2025
Published Date: July 07, 2025

Citation: K. Anusree. In-Silico Docking of *Rubia cordifolia* Phytocompounds as Potential MMP-2 Inhibitors for Varicose Vein Management. International Journal of Biochemistry and Biomolecule Research. 2025; 3(2): 31–42p.

(gelatin), can lead to the weakening and degeneration of the venous wall, leading to the formation of varicose veins [5].

Matrix metalloproteinases, including MMP-2, are key mediators in a variety of physiological processes, such as embryonic development, wound healing, and angiogenesis, and pathological conditions, like cancer, atherosclerosis, and cardiovascular diseases [6–8].

Phytocompounds have been used as a rich source of potential therapeutic agents. These natural compounds offer several advantages over synthetic drugs, like lower toxicity, higher bioavailability, and the presence of multiple bioactive components that can together target many pathways. The use of phytocompounds for the treatment of liver diseases has been extensively studied, highlighting their potential as therapeutic agents [9]. Vascular diseases, such as cardiovascular disorders, stroke, and peripheral artery disease, are a leading cause of morbidity and mortality worldwide and, phytocompounds used as ligands have shown the potential to address vascular health, including improving endothelial function, reducing platelet aggregation, and regulating inflammation [10].

Rubia cordifolia is commonly known as Indian madder or manjistha, a perennial herb, that has been extensively used in Ayurvedic medicine for centuries for its remarkable therapeutic properties. It has a wide range of medicinal applications, as it is rich in anthraquinones, flavonoids, tannins, and iridoids, which contribute to its potent pharmacological activities [11]. These compounds have been found to possess antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and anticancer properties, making *Rubia cordifolia* a valuable addition to the Ayurvedic pharmacopoeia [12]. The roots and rhizomes of *Rubia cordifolia* are particularly prized for their ability to alleviate conditions, such as fever, stomachache, and skin infections [12]. Phytochemical analysis has revealed the presence of bioactive compounds, such as alizarin, purpurin, and lucidin, which have been shown to possess potent antioxidant and anti-inflammatory properties [13].

Molecular docking is a powerful computational technique that has become a crucial tool in the field of drug discovery [14]. This method aims to predict the binding interactions between a small molecule (ligand) and a target protein, providing valuable insights into the potential therapeutic applications of the ligand. For varicose veins, molecular docking can play a significant role in identifying potential inhibitors (ligands) that could target the underlying mechanisms of MMP-2 action that causes degradation of the extracellular matrix (ECM) [15].

The basic principle of molecular docking is its ability to predict the 3-D orientation and conformation of a molecule within the binding site of a target protein. By running a simulation of interactions between the ligand and the protein, molecular docking can provide insight into the binding affinity, the specific amino acid residues involved in the interaction, and overall stability of the ligand-protein complex.

This study aims to screen phytocompounds from the *Rubia cordifolia* to identify drug-like molecules based on standardized pharmacokinetic and drug-likeness criteria. Molecular docking studies will be performed to assess the binding affinity of these compounds with the target protein, MMP-2. This research aims to analyze the therapeutic potential of the selected phytocompounds as promising candidates for the treatment of varicose veins [16–29].

LITERATURE REVIEW

Varicose veins, characterized by their tortuous and enlarged appearance, represent a prevalent manifestation of chronic venous insufficiency, affecting a substantial segment of the adult population worldwide. The cause of varicose veins is venous valvular incompetence, which causes venous reflux, obstruction and subsequently venous hypertension [30]. One of the major factors that causes worsening of chronic venous disease is the over expression of extracellular matrix metalloproteinases. MMP-2 disrupt the venous wall by creating an imbalance between synthesis and degradation of collagen and elastin [31].

MMP-2 and its regulation in veins are of great importance, not only from the pathogenic point of view, but also due to the possibility of its clinical implications for therapy and prevention [32]. Clinical and molecular studies have linked increased venous pressure and inflammation to MMP-2 activity, with the transcription factor AP-1 acting as a key upstream regulator in smooth muscle cells of the venous wall, thereby modulating the structural integrity and functional competence of the venous vasculature [33]. Inhibition of MMP-2 activity through targeted therapeutic interventions, encompassing rationally designed small molecule inhibitors that selectively bind to the catalytic domain of MMPs, as well as RNA interference-based strategies that specifically knockdown MMP-2/9 expression, gives a convincing way to reduce venous remodeling and improve varicose vein disease's clinical aftereffects [34]. However, docking studies specifically targeting MMP-2 in the context of varicose veins remain limited, highlighting the need for computational exploration of alternative ligand libraries.

Plant-derived secondary metabolites have attracted significant attention in inflammatory disease research due to their diverse bioactivities and drug-like properties. Many phytocompounds, including flavonoids, tannins, and anthraquinones, exhibit anti-inflammatory, antioxidant, and ECM-stabilizing effects [35–37]. Natural inhibitors of MMPs have been identified in several studies, with certain polyphenols and alkaloids showing MMP-2 suppression in cancer, arthritis, and vascular models [38]. The inhibitory effect of plant-derived compounds on MMPs has been previously reported. For example, CDF, a synthetic analog of curcumin, showed effective binding to the MMP-2 catalytic domain and significantly reduced its activity in NSCLC cells through docking and functional assays [39].

Rubia cordifolia (Indian madder), a staple of Ayurvedic medicine, is rich in anthraquinones like rubiadin, alizarin, and munjistine [38]. These compounds have demonstrated a range of bioactivities including anti-inflammatory, antimicrobial, hepatoprotective, and antitumor effects [11]. While studies have reported antioxidant and vasoprotective roles of *R. cordifolia*, there is a lack of literature specifically exploring its compounds as potential inhibitors of MMPs, particularly in the context of venous disorders.

Despite the known involvement of MMP-2 in venous degeneration and the pharmacological promise of *Rubia cordifolia phytocompounds*, no prior in-silico studies have evaluated their binding interaction with MMP-2. This gap presents a unique opportunity to computationally screen these natural compounds for potential inhibitory activity, thereby laying the groundwork for future pharmacological development targeting varicose vein progression.

METHODOLOGY

Protein Retrieval

The 7JXO protein of organism Homo Sapiens by synthetic construct and expressed in *Escherichia coli* BL21(DE3), was retrieved from RCSB PDB [15] in .pdb format (https://www.rcsb.org/structure/7XJO). 7XJO is a Crystal structure of human MMP-2 catalytic domain in complex with inhibitor, that is essential in degradation of extracellular matrix proteins. Studies show that the MMP-2 may contribute to matrix remodeling in valvular disease [16].

Ligand Retrieval

37 phytochemicals were isolated from *Rubia cordifolia* (Rubiaceae) out of which 20 were manually selected by deleting duplicate records and common ions and molecules, from Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/plant-rubia-cordifolia) [17]. Canonical smiles and pubchem id of these 20 molecules were retrieved from RCSB-PDB (https://www.rcsb.org) [15] and recorded.

In Silico Pharmacology Screening of Ligands

The physico-chemical parameters of the selected ligands were retrieved in .csv format from SwissADME (http://www.swissadme.ch/) [18] using the recorded canonical smiles. Ligands were

screened based on LIPINSKI rule of 5, threshold GI absorption levels and optimum bioavailability range of 0.55 and above. The screening produced 17 ligands that showed drug-like properties.

Protein Purification

The retrieved protein must be refined into a suitable form to facilitate ease in molecular docking to the desired ligands. For this the downloaded protein structure was uploaded onto BIOVIA DS Discovery Studio (https://www.3ds.com/products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/visualization) [19] and upgraded to publication quality. The protein was stripped of water molecules, heteroatoms, ligand groups and ions to help promote the docking of the desired ligand to the protein.

As water molecules are circulated extensively throughout the body, adding polar groups in refined protein can be beneficial to better simulate the mechanism of the molecule passing through the tissues along with water. Thus, polar groups were added to the purified protein to further aid in docking and simulation.

Ramachandran plot is a method of arranging the structure of a protein by predicting the position of its amino acid residues. The positioning is based on phi (φ) and psi (ψ) (dihedral angles) torsion angles of the polypeptide backbone in a protein [20]. The purified protein was subjected to structural validation based on the Ramachandran plot [21] using an EMBL-EBI tool PDBsum (https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/) [22] and the data was recorded.

Molecular Docking

Molecular docking is a method in in silico analysis that is used to study interactions and binding affinity between protein and ligands. It is useful in analyzing behavior of the interacting molecules, also to simulate the reaction between them on whether they are receptive to the active sites of the protein and by how much intensity. The strength of bond between the protein and ligand is measured by binding affinity between each reaction, the lower the binding affinity the higher the interaction and higher the stability of bond and vice versa. Another parameter used to measure the strength of interaction was Root Mean Square Deviation (RMSD), where low RMSD indicates high stability and high RMSD would mean lower stability.

MD was performed in three stages: (i) Purification of the protein using Biovia Discovery Studio, (ii) Docking and screening through PyRx version 0.8 [23], and (iii) Visualization of top 6 different protein-ligand interactions via BIOVIA Discovery Studio [20].

The purified protein and selected 17 ligands were subjected to few modifications to facilitate easy docking, changes, such as energy minimization, conversion from .pdb file of ligand to .pdbqt where Kollman charges (q) and torsional angles (t) were added to the ligand molecules and generation of gridbox to minimize misplace of atoms during docking. Once the molecules were uploaded onto PyRx and docking was performed using Autodock Vina, 9 models of interaction between each ligand were generated out of which the only one each interaction for each ligand was considered and sent for screening. Screening of the interactions were mainly based on two scoring functions – (i) Binding affinity and (ii) RMSD Value, where 6 ligands with RMSD value of 0 and binding affinity ≤ –9 were chosen as best candidates. The interactions between these six ligands and the protein were visualized, each of them retrieved in .pdb format and the active sites involved in the interaction were recorded from BIOVIA DS Discovery Studio.

RESULTS

Protein Retrieval

MMP-2 (Figure 1) is an endopeptidase that degrades various components of the extracellular matrix (ECM), which gives support and structure to its enclosing tissues. Extreme cases of VV can cause

increase in venous pressure and vein wall tension, which has been reported to be a trigger for MMP-2 expression. This causes degradation of the ECM of affected veins and in turn damages its structural integrity leading to progressive vein dilation and spreading VV further down. Changes in the extracellular matrix are one of the main causes of VV, thus MMP-2 was chosen as a target protein to check the pharmaceutical prospects of R. cordifolia, if the plants' anti-inflammatory and venotonic [24] properties could suppress MMP-2 expression.

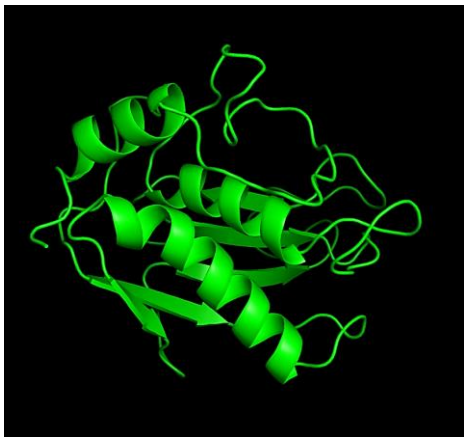

Figure 1. Purified structure of human MMP-2 catalytic domain (EntryID: 7XJO) retrieved from RCSB-PDB and purified in PyMOL Version 3.1.3.1.

In Silico Pharmacology Screening of Ligands

The screening of all 20 phytocompounds in SwissADME produced 17 ligands that showed drug-like properties. Duplicates and phytocompounds and ions common to plants were eliminated manually to retrieve ligands with high pharmacology significance. The phytochemical and physicochemical properties of selected ligands were recorded in Tables 1 and 2. Lipinski's rule of 5 is a method of screening used for valuation of drug-like properties. Table 3 depicts the ligands following the Lipinski rule thus confirming their potential as a drug.

Furthermore, pharmacokinetics of the docked ligands will show how the tissue reacts to the molecule. It was found that all the screened ligands showed high gastrointestinal absorption and bioavailability of all the molecules, that defines the concentration of drug available for therapeutic effect, was well within allowed range of 0.55 or more (Table 4).

Protein Purification and Structural Analysis

Purified MMP-2 protein consisted of only the A-chain, which is the most active portion of the protein and the other heteroatoms, water molecules, ligand groups and ions were removed. Polar groups, H-atoms, were added to make the purified protein more polar to facilitate docking.

The Ramachandran plot is used to determine the energetically acceptable regions in a protein structure when the torsions of amino acids are angled against one another. The plot was generated (Figure 2) for the purified protein using PROCHECK (https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/) [22].

Based on the phi and psi angle of the protein's tertiary structure, the protein is positioned in the Ramachandran plot. The protein had a total of 166 residues, except for glycine and proline, the rest of the amino acid residues were found to be well within the allowed region of the plot. Glycine and proline are not included in the plot as glycine is too small for it to be included and proline is considerably a larger amino acid, which would not show many changes in its position due to its size. 19 glycine and 10 proline amino acid residues were detected from the plot.

A good quality protein model should have more than 90% of its residues in the most preferred regions [A, B, and L]. However, the protein has 88.9% of its residues in the most preferred regions [A, B, and L] (Table 1), which is still a good quality model.

The structure was analyzed to have 2 sheets, 1 beta alpha beta unit, 1 beta hairpin, 1 psi loop, 1 beta bulge, 7 strands, 3 helices, 2 helix–helix interactions, 12 beta turns and 1 gamma turn (Figure 3).

Table 1. Ramachandran plot statistics of MMP-2 protein.

Regions in Plot	No. of Residues	Percentage
Most favored regions [A,B,L]	120	88.9%
Additionally allowed region [a,b,l,p]	14	10.4%
Generously allowed region [~a,~b,~l,~p]	1	0.7%
Disallowed region [XX]	0	0.0%

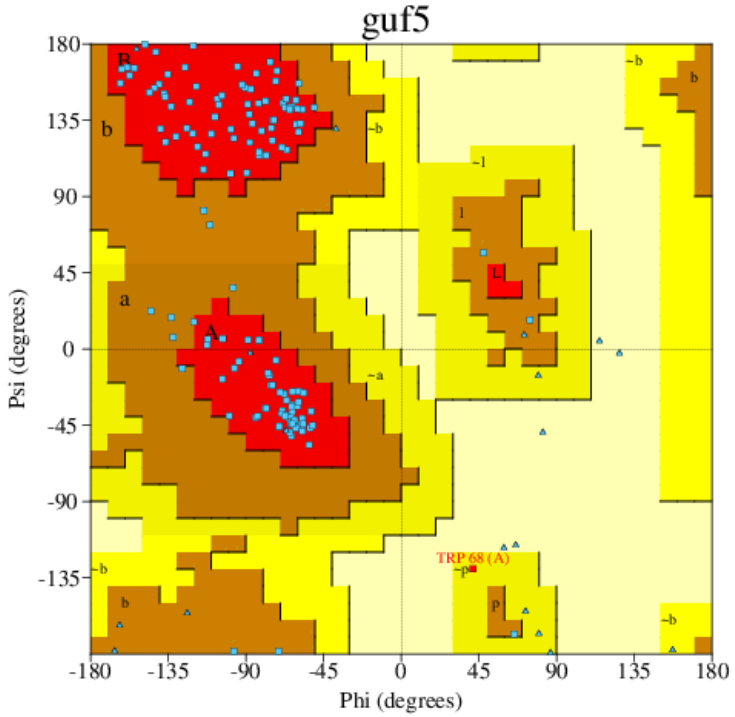

Figure 2. Ramachandran plot of MMP-2 protein using PDBsum.

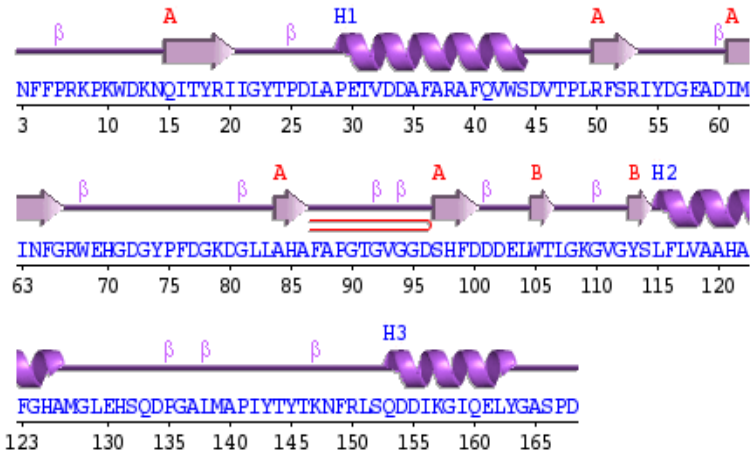

Figure 3. Secondary structure of MMP-2 protein with 166 amino acid residues.

Molecular Docking

The docking produced 9 models each for each protein-ligand interaction out of which 6 ligands most receptive to MMP-2 were screened. Based on the lowest binding affinity and 0 RMSD value, the most stable model out of the 9 models of the 6 protein-ligand interactions were selected as shown in Table 5. These ligands, Rubiadin, Munjistine, 1,5-Dihydroxy-2-Methylanthraquinone, Purpuroxanthins, Alizarin and 1-Hydroxy-2-Methoxy Anthraquinone showed most favorable drug-like properties, and their protein-ligand interactions were preceded for visualization.

Visualization

The candidate ligands and their interaction with the target protein were visualized using Biovia DS. Active sites of the protein that show affinity to the sites in the ligand were recorded and the amino acids present at the active sites that bind to the ligand were depicted as shown in Figures 3–5.

Munjistine showed binding sites at HIS121A, LEU138A, ALA140A, THR146A, THR144A, LEU117A, VAL118A, LEU83A, TYR143A (Amino acid – position of the residue – A chain) amino acid residues that are present in the protein. When checked for drug-likeness based on the Lipinski rule of 5, it is seen that munjistine is a strong candidate that may help in regulation of MMP-2 protein.

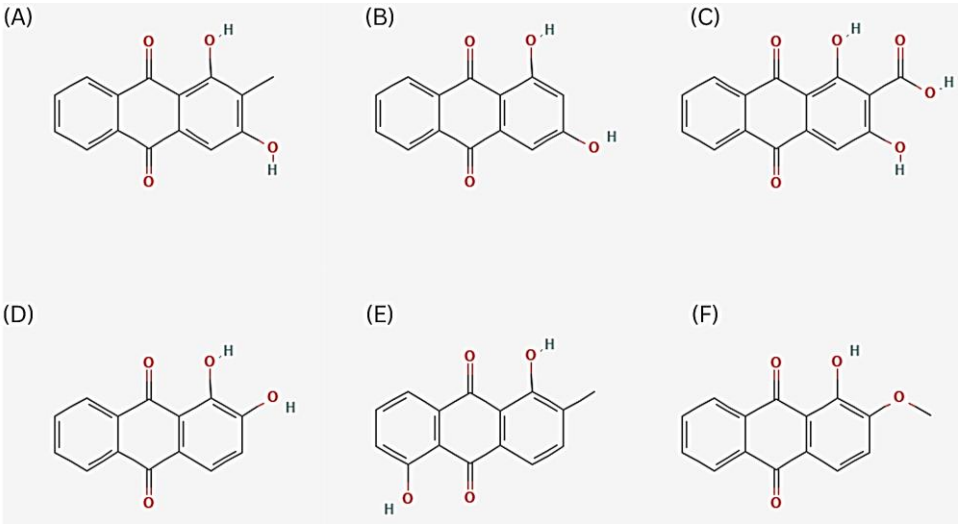

Figure 4. 2D chemical structures of selected *Rubia cordifolia* phytocompounds – Uniprot ID:124062 – Rubiadin, (B) Uniprot ID:196978 – Purpuroxanthins, (C) Uniprot ID: 160476 – Munjistine, (D) Uniprot ID: 6393 – Alizarin, (E) Uniprot ID: 182449 – 1,5-Dihydroxy-2-Methylanthraquinone, (F) Uniprot ID: 80103 – 1-Hydroxy-2-Methoxyanthraquinone.
Source: Pubchem [28].

Table 2. Pharmacokinetic and drug-likeness properties of selected *Rubia cordifolia* phytocompounds.

Ligand Name	MW	nHA	nHD	TPSA	Mlogp	ESOL	Lipinski Violations	#Rotatble Bonds	Pfizer Rule	Veber Rule
Rubiadin	254.24	4	2	74.6	0.92	Soluble	0	0	Yes	Yes
Munjistine	284.22	6	3	111.9	0.29	Soluble	0	1	Yes	Yes
1,5dihydr oxy-2-met hylanthr aquinone	245.24	4	2	74.6	0.92	Moderately soluble	0	0	Yes	Yes
Purpurox Anthins	240.21	4	2	74.6	0.67	Soluble	0	0	Yes	Yes
Alizarln	240.21	4	2	74.6	0.67	Soluble	0	0	Yes	Yes
1-hydroxy-2-methoxy anthraquinone	245.24	4	1	63.6	0.92	Soluble	0	1	Yes	Yes

Table 3. Lipinski's rule of five parameters for selected ligands.

Ligand Name	MW	nHA	nHD	Mlogp	No. of Rotatable Bonds
Rubiadin	254.24	4	2	0.92	0
Munjistine	284.22	6	3	0.29	1
1,5-dihydroxy-2-methylanthraquinone	254.24	4	2	0.92	0
Purpuroxanthins	240.21	4	2	0.67	0
Alizarin	240.21	4	2	0.67	0
1-hydroxy-2-methoxy anthraquinone	254.24	4	1	0.92	1

Table 4. ADME profiling of screened ligands based on SwissADME.

Ligand Name	GI Absorption	Pains	Brenk	Bioavailability
Rubiadin	High	1	0	0.55
Munjistine	High	1	0	0.56
1,5-dihydroxy-2-methylanthraquinone	High	1	0	0.55
Purpuroxanthins	High	1	0	0.55
Alizarin	High	2	1	0.55
1-hydroxy-2-methoxy anthraquinone	High	1	0	0.55

Table 5. Binding affinity scores of top ligands docked with MMP-2 protein.

Ligand Name	Binding Affinity
Rubiadin	–9.2
Munjistine	–9.7
1,5-dihydroxy-2-methylanthraquinone	–9
Purpuroxanthins	–9.1
Alizarin	–9.1
1-hydroxy-2-methoxy anthraquinone	–9.4

DISCUSSION

Varicose veins are a condition where the veins of lower limbs are inflamed and bulged due to various cases, such as genetic factors, proteolytic activity of matrix metalloproteinases, low blood circulation, hypoxia, and so on which leads to tissue remodeling [25]. Genetic studies, such as family studies and genome wide association studies have helped understand the disorder increasingly [26]. Here in this study, we have studied the proteolytic activity of MMP-2 that causes the degradation of affected veins, which worsens the condition. MMPs are a group of enzymes that degrade the extracellular matrix surrounding the veins. MMP-2, also called as gelatinase A, is one of the MMPs that plays a major role in triggering vein remodeling. Studies have found that increased venous filling pressure activates activator protein 1 (AP-1), which, in turn, triggers varicose remodeling by controlling MMP-2 activity [27]. Therefore, modulating the expression of MMP-2 protein may be beneficial in stopping the progression of the disease.

Roots of *Rubia cordifolia* have been known to treat many disorders and have many properties, such as blood purification, anti-inflammatory, anti-cancer, and so on. It was seen that using *Rubia cordifolia* in combination with a few other herbs showed vein strengthening properties, which was exploited for study in this molecular docking analysis.

Molecular docking of 6 ligands, Munjistine, Rubiadin, 1,5-Dihydroxy-2-Methylanthraquinone, Purpuroxanthins, Alizarin and 1-Hydroxy-2-Methoxy Anthraquinone, derived from *Rubia cordifolia* showed lowest binding affinity and RMSD value of zero. They also exhibited high drug-likeness with respect to GI absorption, Lipinski's analysis, and other previously mentioned criteria.

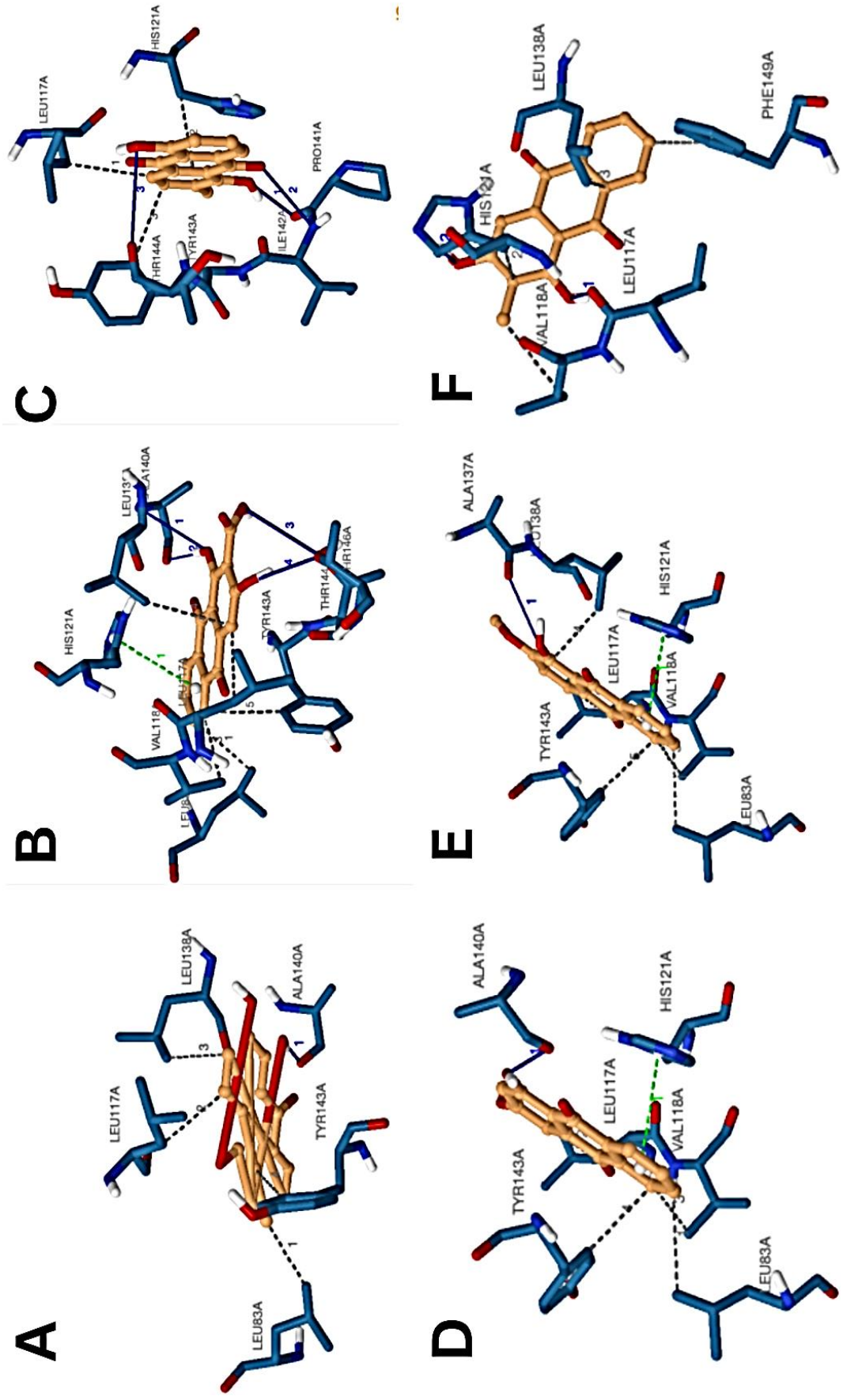

Figure 5. (A) Uniprot ID: 6393 – Alizarin, (B) Uniprot ID: 160476 – Munjistine, (C) Uniprot ID: 182449 – 1,5-Dihydroxy-2-Methylanthraquinone, (D) Uniprot ID:196978 – Purpuroxanthins, (E) Uniprot ID: 80103 – 1-Hydroxy-2-Methoxy Anthraquinone, (F) Uniprot ID:124062 – Rubiadin. The yellow colored structure depicts the ligands while the protein is shown in blue color.

Munjistin was found to have the most stable interaction with MMP-2 in this docking analysis, with the least binding affinity of –9.7. It is an anthraquinone derivative and found to have many physiological activities, including anti-inflammatory, antibacterial, antiarthritic.

Rubiadin, 1,5-Dihydroxy-2-Methylanthraquinone, Purpuroxanthins, Alizarin and 1-Hydroxy-2-Methoxy Anthraquinone all belong to anthraquinones class. Their presence and strong affinity prove the strong pharmaceutical qualities of *Rubia cordifolia.* Munjistin had the strongest bonding with MMP-2 with –9.7 binding affinity and 1,5-Dihydroxy-2-Methylanthraquinone had –9.0 binding affinity but still a stronger bond than all the other screened ligands.

Molecular docking technology has paved the way for advancements in faster drug discovery and pharmacological studies. It is a very promising field and the incorporation of AI and Machine Learning models can further enhance our understanding of complex diseases, such as cancer, autoimmune disorders, Alzheimer's and so on, to find a cure or a novel treatment.

CONCLUSIONS

In conclusion, the in-silico analysis of *Rubia cordifolia* phytocompounds against MMP-2 provides promising insights into their pharmaceutical potential for varicose vein treatment. MMP-2, a key enzyme responsible for ECM degradation and vein dilation, was successfully retrieved and purified for molecular docking studies. SwissADME screening identified 17 drug-like ligands, of which six – Rubiadin, Munjistine, 1,5-Dihydroxy-2-Methylanthraquinone, Purpuroxanthins, Alizarin, and 1-Hydroxy-2-Methoxy Anthraquinone – demonstrated strong binding affinity and pharmacokinetic suitability. Visualization of 6 most stable protein-ligand interactions based on binding affinity and RMSD value further confirmed their potential as inhibitors of MMP-2. These findings highlight the *Rubia cordifolia* as a valuable source of bioactive compounds with venotonic and anti-inflammatory properties, calling for further experimental validation.

ABBREVIATIONS

1. Activator protein 1 (AP-1).
2. Alanine (ALA).
3. Estimated Solubility in Water (ESOL).
4. Extracellular matrix (ECM).
5. Gastrointestinal (GI).
6. Histamine (HIS).
7. Hydrogen-atoms (H-atoms).
8. Leucine (LEU).
9. Matrix metalloproteinase –2 (MMP-2).
10. Molecular docking (MD).
11. Molecular weight (MW).
12. Moriguchi LogP (Mlogp).
13. Number of H Acceptor (nHA).
14. Number of H Donor (nHD).
15. Root Mean Square Deviation (RMSD).
16. *Rubia cordifolia (R. cordifolia).*
17. Threonine (THR).
18. Tyrosine (TYR).
19. Valine (VAL).
20. Varicose Veins (VV).

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