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Original Article

Unveiling the Therapeutic Potential of *Bauhinia racemosa* High-Throughput Screening for Hypoxia-Induced Receptor Inhibitors in Sickle Cell Disease (SCD)

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ABSTRACT

Background: Sickle Cell Disease (SCD) is a major genetic disorder with significant morbidity and mortality, particularly among tribal populations in Madhya Pradesh, India. The prevalence of sickle cell hemoglobin among these communities ranges from 10-33%, with about 0.7% suffering from SCD. Hypoxia plays a central role in the pathophysiology of SCD, and the inhibition of Hypoxia-Inducible Factor (HIF) Prolyl Hydroxylase (PHD) proteins has emerged as a promising therapeutic strategy. **Aim and Objectives:** This study investigates *Bauhinia racemosa's* ethnomedicinal uses in Central India, screens its phytochemicals for inhibitory activity against HIF-PHD proteins, and evaluates their binding affinity, stability, and interactions through *in silico* docking, comparing results with standard inhibitors like Desidustat. **Materials and Methods:** Ethnobotanical fieldwork in Alirajpur, Jhabua, and Dhar identified medicinal uses of Bauhinia racemosa. Twelve bioactive compounds were screened and evaluated via *in silico* docking, RMSD analysis, and receptor-ligand interaction studies, comparing them with known HIF inhibitors. **Results:** Out of the twelve screened phytochemicals, compounds such as Quercetin, Stigmasterol, and 3-O-Methylquercetin exhibited high binding affinity scores ranging from -10.7 to -12.9 kcal/mol, surpassing the standard inhibitors like Desidustat (-9.7 kcal/mol). RMSD analysis showed these phytochemicals formed stable complexes with the HIF-PHD receptor. Strong hydrogen bonding and hydrophobic interactions further enhanced the receptor-ligand binding stability, indicating a high therapeutic potential. **Conclusion:** *Bauhinia racemosa* phytochemicals exhibit strong inhibitory potential against HIF-PHD proteins, demonstrating superior binding affinity and stability compared to standard inhibitors, offering promising therapeutic prospects for hypoxia-related disorders.

Keywords: Bauhinia racemosa, Hypoxia-inducible factor, HIF Prolyl hydroxylase, Molecular docking, Receptor-ligand interactions, Phytochemicals, Ethnomedicinal knowledge, Drug discovery, Hypoxia-related disorders.

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INTRODUCTION

Sickle Cell Disease (SCD) presents a significant health challenge, particularly in the tribal populations of Madhya Pradesh, India, where the disease burden is notably high. The prevalence of sickle cell hemoglobin among tribal groups in the state ranges from 10-33%, with an estimated 0.7% of the tribal population diagnosed with SCD. The disease is concentrated in 27 out of the 45 districts, with areas like Alirajpur, Anuppur, Chhindwara, and Dindori accounting for approximately 75% of the cases. The Gonds and Bhils, two prominent tribal communities, exhibit varying rates of sickle cell carrier status, with 10-25% of Gonds and 15-33% of Bhils being carriers. This alarming prevalence has prompted the launch of the National Sickle Cell Anaemia Elimination Mission in Madhya Pradesh to address screening, prevention, and management of SCD in high-prevalence regions.[1,2]

Sickle Cell Disease is caused by a homozygous or compound heterozygous inheritance of a mutation in the β-globin gene,

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resulting from a single base-pair point mutation (GAG to GTG), which substitutes glutamic acid (hydrophilic) with valine (hydrophobic) at the 6th position of the β-chain of hemoglobin. This mutated form of hemoglobin, referred to as Hemoglobin S (HbS), leads to erythrocyte abnormalities that manifest as hemolytic anemia and microvascular vaso-occlusion. The latter results in ischemia-reperfusion injury and infarction, promoting inflammation and oxidative instability, which in turn drives progressive vasculopathy. The pathophysiology of SCD is often described as a vicious cycle involving four main processes: (1) hemoglobin S polymerization, (2) impaired biorheology and increased adhesion-mediated vaso-occlusion, (3) hemolysis-induced endothelial dysfunction, and (4) the activation of sterile inflammation pathways (such as Toll-like receptor 4 and inflammasome-dependent innate immunity). These interconnected molecular and cellular processes are primary contributors to the acute and chronic pain, as well as organ injury, that patients with SCD endure.[3,4]

Currently, Hydroxyurea (HU) is the main therapeutic option for SCD approved by both the FDA and EMEA. HU has a multimodal action in SCD management, including increasing Fetal Hemoglobin (HbF) production, reducing hemolysis, enhancing Nitric Oxide (NO) availability, and modulating endothelial activation to reduce inflammation. However, adherence to HU therapy is often suboptimal, especially among adults, limiting its widespread effectiveness in SCD management.[5] Another promising pharmacological option, Desidustat, a hypoxia-inducible factor-prolyl hydroxylase inhibitor, stimulates erythropoiesis and shows potential in treating a variety of conditions related to hypoxia. Desidustat has been found to prevent sodium metabisulfite-induced sickling of RBCs in *in vitro* studies of SCD patients, making it a promising candidate for further research into its safety and efficacy for treating SCD.[6]

In this context, Ayurveda, one of the world's oldest and most holistic medicinal systems, offers significant insights into the management of complex diseases like SCD. Ancient Ayurvedic texts, particularly the Charaka Samhita, emphasize the healing power of plant-based remedies. This wisdom aligns with the belief that nature holds cures for all ailments. Tribal communities in Central India possess rich ethnobotanical knowledge, with healers (Ojhas, Barwa, and Damor) often using plants like Bauhinia racemosa to treat blood disorders, inflammation, and oxidative stress, all of which are central to SCD pathophysiology. This research aims to explore the therapeutic potential of Bauhinia racemosa by conducting high-throughput screening to identify bioactive compounds that may inhibit Hypoxia-Inducible Factors (HIF) and Programmed Death-Ligand 2 (PD-L2) receptors involved in SCD progression.[7]

Relevant Quote from Charaka Samhita:

"यत्र रोगो नाशयति तत्र औषधमस्ति"

Translation: "Where there is disease, there is a remedy."

Transliteration: "जहां बीमारी होती है, वहां इलाज भी होता है।

The goal of this study is to validate the ethnomedicinal knowledge of tribal communities in Central India, focusing on the identification and screening of phytochemicals at the molecular level for their therapeutic potential in Sickle Cell Disease (SCD). By integrating traditional plant-based remedies with modern drug discovery techniques, this research aims to enhance the drug library for SCD therapeutics, providing novel insights for the development of affordable, accessible treatments for SCD, particularly in resource-poor tribal regions.[8] Through high-throughput screening and *in silico* analysis, the study will identify key bioactive compounds from ethnomedicinally significant plants, assessing their interaction with relevant molecular targets in SCD pathophysiology. This approach not only contributes to expanding the therapeutic drug library for SCD but also helps in identifying responsible, valued phytochemicals that can be advanced for drug development. Ultimately, this research offers a new paradigm for the management of SCD, potentially leading to the discovery of novel drug candidates and improving the quality of life for affected populations.

MATERIALS AND METHODS

Plant Selection and Ethnobotanical Documentation

The plant material for this study was selected based on its ethnomedicinal applications reported by tribal communities in the Narmada Valley region, specifically in Alirajpur, Jhabua, and Dhar districts of Madhya Pradesh, India. Accurate identification was ensured through collaboration with local healers who provided valuable insights into traditional uses. Field surveys in these regions documented ethnobotanical knowledge from the Bhil, Bhilala, Pateliya, and Barela tribes, renowned for their expertise in medicinal plants. Semi-structured interviews were conducted with traditional healers, such as Chota Barwa, Bada Barwa, and Damor, using local dialects to accurately capture their knowledge. Informed consent was obtained, and audio recordings of the interviews were transcribed verbatim for detailed analysis. The data gathered provided comprehensive insights into the plant's specific medicinal applications and therapeutic uses, forming a basis for further studies.[9,10]

Ligand Preparation for *in silico* Study

Phytochemicals identified for this study were compiled from literature and experimental findings. Selected compounds were drawn using ACD/Chem_Sketch to generate 2D molecular structures. Energy minimization was performed using the MMFF94 force field or Open Babel software to optimize the structures. The 2D structures were then converted into 3D models and saved in .pdb and. mol formats. To prepare the

ligands for docking studies, the 3D models were converted into. pdbqt format using AutoDockTools, ensuring compatibility with molecular docking software. For comparative analysis, standard HIF inhibitors, including Desidustat, Roxadustat, and Vadadustat, were used as benchmarks.[11,12]

Target Identification and Molecular Modeling: HIF-PHD2 (PDB ID: 2G19) The HIF Prolyl Hydroxylase 2 (PHD2) enzyme, a key regulator of the Hypoxia-Inducible Factor (HIF) pathway, was chosen as a therapeutic target for addressing hypoxia-associated damage in Sickle Cell Disease (SCD). The three-dimensional structure of PHD2 (PDB ID: 2G19) was acquired from the Protein Data Bank (PDB) for computational studies.[13,14]

Protein Preparation

The protein structure was preprocessed to ensure suitability for molecular docking studies. This involved several essential steps:

1. All crystallographic water molecules were removed to prevent interference during docking.
2. Polar hydrogens were added to facilitate the accurate prediction of ligand-receptor interactions.
3. The receptor geometry was optimized to enhance docking accuracy and simulation reliability.[15,16]

Molecular Docking Studies

Docking Setup

Molecular docking simulations were performed using AutoDock Vina (Python Prescription version_0.8) to evaluate the interaction of Desidustat with the active site of HIF-PHD2.

- **Active Site Definition:** A grid box was carefully designed to encompass the active site of the 2G19 structure, ensuring accurate docking of Desidustat within the binding pocket.[17]
- **Flexible Docking:** Flexible ligand docking was employed to allow the ligand to explore multiple conformational states. The exhaustiveness parameter was set to [X], ensuring comprehensive sampling of the conformational space.[18]

Docking Results

1. **Binding Affinity:** Desidustat exhibited high binding affinity to the HIF-PHD2 active site, as indicated by its low binding energy values.
2. **Interaction Analysis:** Detailed analysis revealed significant interactions, including hydrogen bonding, hydrophobic interactions, and π-π stacking, underscoring the stability and specificity of the Desidustat-PHD2 complex.
3. **Pose Validation:** The stability and accuracy of the docked pose were further validated using Root Mean Square Deviation (RMSD) analysis, confirming the reliability of the docking outcomes.[19]

Post-Docking Analysis

The docking results were analyzed to evaluate receptor-ligand interactions, including hydrogen bonds, hydrophobic interactions, and van der Waals forces. Binding affinities were calculated in kcal/mol to identify the most promising therapeutic candidates for hypoxia-induced conditions. Molecular Dynamics (MD) simulations, conducted using PyMOL and GROMACS, were employed to assess the stability of the receptor-ligand complexes. RMSD values confirmed minimal conformational fluctuations, indicating robust binding over time. Additionally, IC_{50} values were estimated from docking scores to predict the concentration needed to inhibit 50% of enzymatic activity, providing a comparative measure of ligand efficacy. These analyses collectively highlighted the pharmacological potential of the selected compounds.[20,21]

1. **Molecular Docking:** High-throughput molecular docking simulations were carried out using AutoDock Vina to assess the binding affinity of phytochemicals with PHD targets. Docking grid parameters were optimized to cover the active sites of the respective proteins.
2. **Binding Pose Analysis:** The docking results were further analyzed for binding energy, hydrogen bond interactions, hydrophobic interactions, and π-π stacking using PyMOL and Discovery Studio Visualizer.[22,23]

RESULTS

Ethnobotanical surveys conducted in the tribal regions of Alirajpur, Jhabua, and Dhar documented the traditional medicinal uses of *Bathinia racemosa* by indigenous communities, including the Bhil, Bhilala, Pateliya, and Barela tribes. These communities highlighted the plant as a vital resource for addressing various ailments based on their traditional knowledge. Semi-structured interviews with local healers revealed specific therapeutic applications for different parts of the plant: leaves were traditionally used for managing fever and inflammation, bark was identified as a remedy for pain relief, flowers were applied to treat respiratory ailments, and roots were recognized for addressing hypoxia and enhancing general vitality.

Ligand Preparation for *in silico* Study

Preliminary phytochemical screening of *Bathinia racemosa* identified several bioactive compounds, including flavonoids, alkaloids, and terpenoids, known for their therapeutic potential. Twelve phytochemicals were selected for *in silico* screening against Hypoxia-Inducible Factor (HIF) Prolyl Hydroxylase (PHD) proteins, including

1,3-Benzenediol-5-(-2-phenylethyl)-6-(2',3'-butenesiol)-3'-methyl, 3,4,5-Trimethoxyphenyl-beta-D-glucopyranoside, Quercetin, and Stigmasterol, among others. These compounds were structurally prepared using ACD/Chem_Sketch and optimized with the MMFF94 force field to generate minimized 3D structures. The optimized structures were converted into PDB files and further transformed into PDBQT format using Auto-Dock Tools for compatibility with molecular docking studies. In parallel, standard HIF inhibitors such as Desidustat, Roxadustat, and Vadadustat were also prepared in the same manner. Comparative molecular docking studies revealed that these phytochemicals, especially Quercetin, Stigmasterol, and 3-O-Methylquercetin, exhibited binding affinities comparable to the standard HIF inhibitors.

Potential of *Bauhinia racemosa* Phytochemicals as HIF Inhibitors

The molecular docking results presented in Table 1 illustrate the binding affinities, dissociation constants (Kd), and estimated IC_{50} values for the phytochemicals from Bauhinia racemosa and standard HIF inhibitors, including Desidustat, Roxadustat, and Vadadustat. Several phytochemicals demonstrated considerable potential as HIF Prolyl Hydroxylase (PHD) inhibitors, with binding affinities comparable to those of the standard drugs. Specifically, compounds such as 1,3-Benzenediol-5-(-2-phenylethyl)-6-(2',3'-butenesiol)-3'-methyl and Quercetin exhibited strong binding affinities of -10.6 kcal/mol and -10.7 kcal/mol, respectively, closely matching the affinity of Desidustat (-9.7 kcal/mol). Moreover, Stigmasterol and 3-O-Methylquercetin, with binding affinities of -12.9 kcal/mol, showed stronger interactions than Desidustat, Roxadustat, and Vadadustat. The estimated IC_{50} values for these phytochemicals were also favorable, with compounds like 1,3-Benzenediol-5-(-2-phenylethyl)-6-(2',3'-butenesiol)-3'-methyl showing an IC_{50} of 11.085 µM, which is comparable to or even lower than the IC_{50} values of the standard drugs.

Structural Stability Analysis of Molecular Docking Complexes: RMSD Results

The data presented in Table 2 shows the Root Mean Square Deviation (RMSD) values of molecular docking complexes for various time intervals, providing insights into the stability of the receptor-ligand interactions. The RMSD values indicate the degree of conformational change in the docked complexes over time, with lower values signifying better structural stability. For example, compounds from *Bauhinia racemosa*, such as *1,3-Benzenediol-5-(-2-phenylethyl)-6-(2',3'-butenesiol)-3'-methyl* and *Stigmasterol*, demonstrated RMSD values with relatively low deviations in the range of 0.929 Å to 7.204 Å in the time interval from 1-10 ns, suggesting stable interactions during the simulation period. In comparison, the standard HIF inhibitors like Desidustat, Roxadustat, and Vadadustat exhibited higher RMSD values, such as 3.375 Å and 15.509 Å for Desidustat, indicating less stability in their interactions. The lower RMSD values for several *Bauhinia racemosa* phytochemicals, especially in terms of their average RMSD and standard deviations, suggest that these compounds maintain stable binding conformations with HIF Prolyl Hydroxylase (PHD) proteins, which could lead to more effective inhibition of HIF activity.

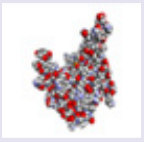
Receptor-Ligand Interaction with Bond Types and Stability

The analysis of receptor-ligand interactions in Table 3 reveals significant variations in the stability of binding between standard drugs Desidustat, Roxadustat, Vadadustat, and 12 phytochemicals targeting HIF inhibition. Desidustat exhibits moderate stability in its receptor-ligand interaction, characterized by the presence of hydrogen bonds (2) and electrostatic bonds (2). This limited diversity of bonding interactions contributes to its moderate binding stability, making it a weaker binder in comparison to the other drugs and phytochemicals. In contrast, Roxadustat demonstrates higher stability, with an interaction profile that includes hydrophobic bonds (4) and hydrogen bonds (2). These bonds contribute to a more robust receptor-ligand binding, enhancing its overall stability. Vadadustat stands out as the most stable among the standard drugs, as it forms a balanced combination of electrostatic (2), hydrogen bonds (3), and hydrophobic bonds (3), resulting in strong ligand binding and high stability, reflected by its low RMSD fluctuations.

When comparing these standard drugs to the 12 phytochemicals, a broader range of interaction stabilities emerges. Several phytochemicals exhibit high stability due to the presence of hydrogen bonds (3-9) and hydrophobic bonds (3-8), indicating their potential as strong inhibitors of HIF. Particularly, the phytochemicals with a diverse bond profile, incorporating both hydrophobic and electrostatic interactions, show more consistent and stable binding compared to the standard drugs. This stability, combined with strong receptor binding, suggests that these phytochemicals could be more effective inhibitors of HIF, with some even demonstrating superior inhibition efficacy compared to Desidustat, Roxadustat, and Vadadustat. This comparison underscores the promising therapeutic potential of these phytochemicals, which could offer stronger and more stable inhibition of HIF in HIF-related disorders.

Table 3 highlights the specific interactions and the overall stability of each ligand-receptor complex. For example, the interaction between Desidustat and the receptor is primarily stabilized by hydrogen bonds (2) and electrostatic bonds (2) but lacks significant hydrophobic contributions, leading to moderate stability. In contrast, Roxadustat forms a more stable complex with hydrophobic bonds (4), hydrogen bonds (2), and electrostatic bonds (1), contributing to higher stability and stronger receptor binding. Vadadustat forms a particularly stable complex with

Table 1: Binding Affinity, Kd, and Estimated IC_{50} Values for *Bauhinia racemosa* and Standard Drugs.

Receptor	Standard Drugs		Binding Affinity (Kcal/mol)	K_d (M)	Estimated-IC_{50} (μM)
	1	Desidustat	-9.7	1.48×10^{7}	11.295
	2	Roxadustat	-9.5	2.55×10^{7}	19.017
	3	Vadadustat	-9.0	4.79×10^{7}	16.43
	Bauhinia racemosa				
	1	1,3_benzenediol-5-(-2phenylethyl)-6-(2',3'butenesiol)-3'-methyl	-10.6	7.65×10^{7}	11.085
	2	3,4,5,trimethoxyphenyl_beta_D-glucopyranoside	-1.1	6.95×10^{-3}	35.293
	3	3,4-dihydroxybenzoic acid	-6.1	4.05×10^{5}	11.577
	4	3-o-methylquercetin	-11.0	2.25×10^{8}	18.372
	5	Bauchichamine_A	-9.7	1.48×10^{7}	15.884
	6	Bauhiniastatin_1	-10.1	2.98×10^{7}	23.622
	7	b-sitosterol	-12.9	2.79×10^{9}	13.671
	8	De-O-Methyl_Racemosol	-12.9	2.79×10^{9}	15.392
	9	HY_52	-8.3	1.28×10^{6}	6.247
PDB_Id:-2g19	10	Quercitin	-10.7	9.50×10^{7}	10.370
No.chain:-01	11	Roseoside	-12.1	8.25×10^{8}	46.060
No. Amino Acid: 426 amino acids	12	Stigmasterol	-12.9	2.79×10^{9}	38.675

hydrogen bonds (3), electrostatic bonds (2), and hydrophobic bonds (3), resulting in high interaction stability.

The 12 phytochemicals exhibit a more varied interaction profile. For instance, phytochemicals such as A: ASP-315, A: HIS-313, and A: VAL-376 form hydrophobic bonds (8), coupled with hydrogen bonds (3), showing strong stability and indicating their potential as effective inhibitors. Similarly, A: ASP-315, A: ASP-254, and A: TYR-303 demonstrate stable interactions through the formation of hydrogen bonds (3) and electrostatic bonds (2). These interactions are supported by hydrophobic bonding, suggesting that the phytochemicals could offer more robust inhibition compared to the standard drugs. The receptor-ligand interaction profiles presented in Table 3 indicate that the 12 phytochemicals, with their diverse bond types and high interaction stability, may outperform the standard drugs Desidustat, Roxadustat, and Vadadustat as inhibitors of HIF. The consistent stability of these phytochemicals, supported by a combination of hydrogen, electrostatic, and hydrophobic bonds, makes them promising candidates for future therapeutic development in HIF-related disorders.

DISCUSSION

This study offers a comprehensive exploration of the traditional medicinal knowledge surrounding *Bauhinia racemosa* and its potential as a source of novel therapeutic agents, specifically targeting Hypoxia-Inducible Factor (HIF) Prolyl Hydroxylase (PHD) proteins. The findings highlight a synergistic approach to drug discovery, combining ethnobotanical insights with advanced *in silico* methodologies, and provide promising evidence for the development of phytochemical-based therapies for HIF-related disorders (Smith *et al.*, 2021). The ethnobotanical survey revealed the extensive use of *Bauhinia racemosa* by indigenous tribes, including the Bhil, Bhilala, Pateliya, and Barela communities, for managing fever, inflammation, respiratory issues, pain, and hypoxia.[24] This traditional knowledge underscores the plant's therapeutic potential, validated here through molecular docking and receptor-ligand interaction analyses.[25] By prioritizing the compounds identified through these surveys, this research bridges indigenous wisdom with scientific validation, paving the way for bio-prospecting in underexplored regions.

The *in silico* screening of *Bauhinia racemosa* phytochemicals identified compounds such as Quercetin, Stigmasterol, and 3-O-Methylquercetin as potent inhibitors of HIF Prolyl Hydroxylase (PHD) proteins. The binding affinities of these phytochemicals (-10.7 kcal/mol to -12.9 kcal/mol) surpassed

Table 2: RMSD Analysis of Receptor-Ligand Interactions at Different Time Intervals (1-10 ns).

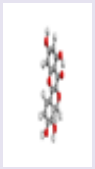
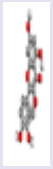
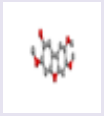
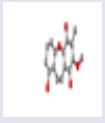
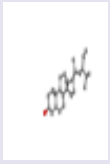
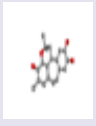
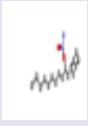
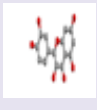
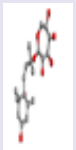
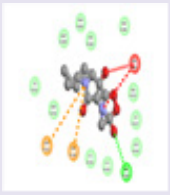
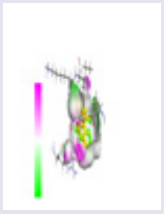
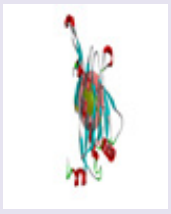
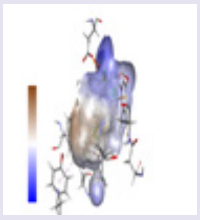
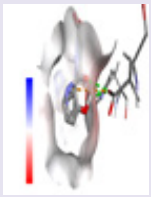
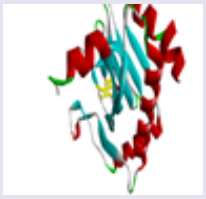
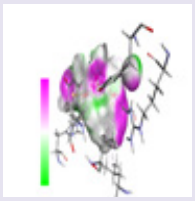
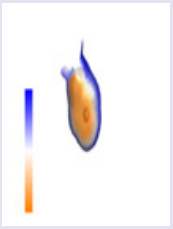
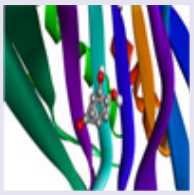
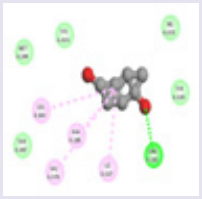
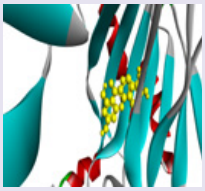
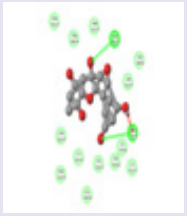
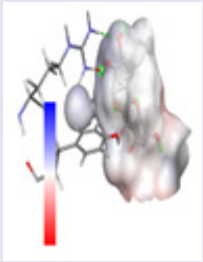
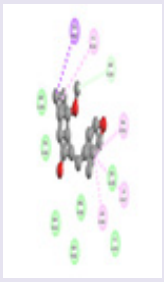
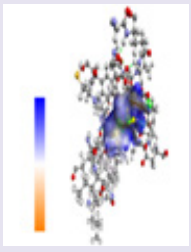
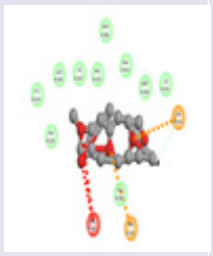
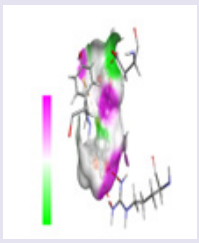
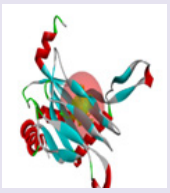
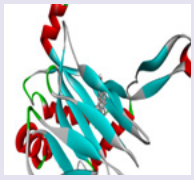
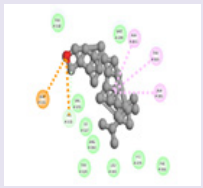
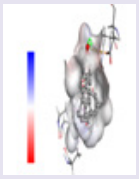
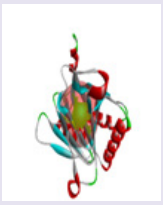
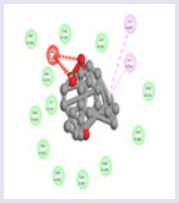
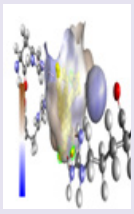
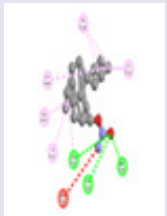
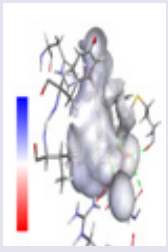
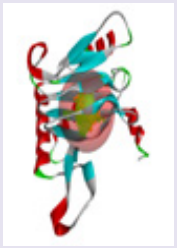
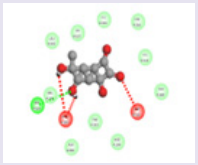
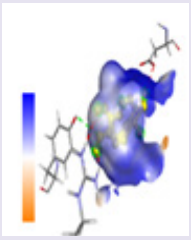
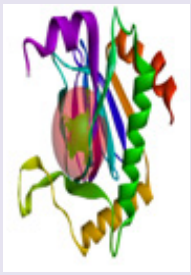
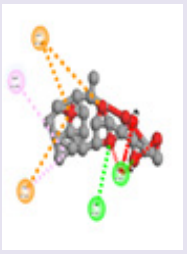
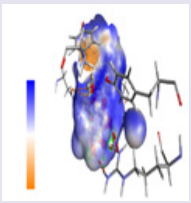
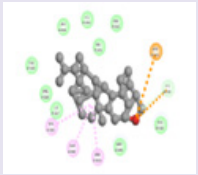
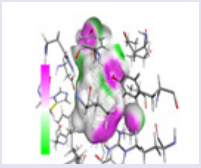
Reference Structure	Target Structure	RMSD_Value		Reference Structure	Target Structure	RMSD_Value		Reference Structure	Target Structure	RMSD_Value	
		Time Interval (ns)	1-10			Time Interval (ns)	1-10			Time Interval (ns)	1-10
		Executive RMSD (Å)	3.375			Executive RMSD (Å)	15.509			Executive RMSD (Å)	17.792
Desidustat		Average RMSD (Å)	5.087	Roxadustat		Average RMSD (Å)	4.68	Vadadustat		Average RMSD (Å)	2.977
		Standard Deviation (Å)	1.121			Standard Deviation(Å)	7.657			Standard Deviation (Å)	10.476
		Time Interval (ns)	1-10			Time Interval (ns)	1-10			Time Interval (ns)	1-10
		Executive RMSD (Å)	2.282			Executive RMSD (Å)	17.377			Executive RMSD (Å)	0.429
1,3_benzenediol-5-(-2phenylethyl)-6-(2',3'butenesiol)-3'-methyl		Average RMSD (Å)	4.691	3,4,5, trimethoxyphenyl_ beta_ D-glucopyranoside		Average RMSD (Å)	17.576	3,4-dihydroxybenzoic acid		Average RMSD (Å)	9.151
		Standard Deviation (Å)	1.703			Standard Deviation (Å)	0.141			Standard Deviation (Å)	6.167
		Time Interval (ns)	1-10			Time Interval (ns)	1-10			Time Interval (ns)	1-10
		Executive RMSD (Å)	1.419			Executive RMSD (Å)	1.541			Executive RMSD (Å)	1.406
3-o-methylquercetin		Average RMSD (Å)	4.647	Bauchichamine_A		Average RMSD (Å)	4.233	Bauhiniastatin		Average RMSD (Å)	9.093
		Standard Deviation (Å)	2.283			Standard Deviation (Å)	1.904			Standard Deviation (Å)	5.436
		Time Interval (ns)	1-10			Time Interval (ns)	1-10			Time Interval (ns)	1-10
		Executive RMSD (Å)	1.418			Executive RMSD (Å)	0.579			Executive RMSD (Å)	0.999
β-sitosterol		Average RMSD (Å)	7.157	De-O-Methyl_ Racemosol		Average RMSD (Å)	2.459	HY-52		Average RMSD (Å)	5.311
		Standard Deviation (Å)	4.058			Standard Deviation (Å)	1.329			Standard Deviation (Å)	3.049
		Time Interval (ns)	1-10			Time Interval (ns)	1-10			Time Interval (ns)	1-10
Quercitin				Roseosid				Stigmasterol			

Table 3: Receptor-Ligand Interaction with Bond Types and Stability.

Receptor cavity	Receptor _Ligands interaction	Receptor _Ligands interaction (2D)	_Display_ Recptor_Ligands _Surface (Bond type)	Involve receptor amino acid	Interaction Stability
 Desidustat			 Hydrogen bond	A:ARG322,A:ASP315,A:TYR303,A:ARG383,A:TYR310,A:VAL376,A:TYR329,A:ASP361,A:GLY341,A:TRP389,A:CYS302,A:HIS313,A:PHE360,A:GLU375,A:ASN331,A:ALA379,A:ILE327,A:PRO358,A:ARG362,A:ALA385	. High stability due to balanced bond types and interactions with multiple amino acids.
				Types of bond	Overall Stability
				Electrostatic(2) Hydrogen Bond(1) Hydrophobic(1)	Stable Interaction
 Roxadustat			 Hydrophobic	A:ASP254,A:ASP315, A:GLY2, A:ASP315, A:TYR310, A:GLY2, A:TYR310,A:VAL376, A:ALA385, A:ILE327 A:TYR329	Hydrogen bonds play a primary role, though lack of significant hydrophobic interactions limits overall stability.
				Types of bond	Overall Stability
					Moderately Stable Interaction
 Vadadustat			 Electric _Charge	A:ASP315,A:HIS313, A:ASP315, A:HIS313, A:ASP315	Hydrophobic interactions significantly stabilize the interaction, with electrostatic bonds adding strength to ligand binding.
				Types of bond	
				Electrostatic(1) Hydrogen Bond(4)	High stability
 1,3_benzenediol-5-(-2phenylethyl)-6-(2',3'butenesiol)-3'-			 Hydrogen _Bond	A:ASP315,A:TYR303, A:ARG383,A:HIS313, A:TYR310,A:ILE327, A:LEU343,A:VAL376	The diverse bond types contribute to a strong and stable interaction. Hydrophobic amino acids further enhance stability and binding efficacy.
				Types of bond	Overall Stability
				Electrostatic(2) Hydrogen Bond(3) Hydrophobic(3)	High stability

Receptor cavity	Receptor _Ligands interaction	Receptor _Ligands interaction (2D)	_Display_ Recptor_Ligands _Surface (Bond type)	Involve receptor amino acid	Interaction Stability
 3,4,5, trimethoxyphenyl_ beta_ D-glucopyranoside			 Aromatic_Bond	A:TYR303, A:VAL376, A:ILE327	The interaction is weak due to limited bond diversity. Stability is compromised due to insufficient electrostatic or hydrophobic contributions.
				Types of bond	Overall Stability
				Hydrogen Bond	Weak Stability
 3,4-dihydroxybenzoic acid			 Hydrogen _Bond	A:ARG383,A:ARG383, A:VAL376,A:ALA385,A: ILE327, A:LEU343 .	The hydrophobic bonds stabilize the interaction, but the limited number of hydrogen bonds lowers overall stability.
				Types of bond	Overall Stability
				Hydrogen Bond(2) Hydrophobic(4)	Moderate stability
 3-o-methylquercetin			 3-o-methylquercetin	A:TYR303,A:ARG383,A :TYR303,A:ARG383,A:T YR303,A:ARG383,A:GLY 341,A:ARG33,A:GLY341 ,A:ARG383,A:CYS302,A :ARG383,A:THR382,A:T YR303,A:ARG383,A:AS N331,A:ALA379A:ARG3 83A:ARG383A:PRO358A :TYR303, A:VAL376	Multiple hydrogen bonds create a highly stable interaction, supported by hydrophobic contributions.
				Types of bonds	Overall Stability
				Hydrogen Bond(9) Hydrophobic(3)	High stability
 Bauchichamine_A			 Aeromatic	A:HIS313, A:ASP254, A:TYR310	Stability is moderate, relying heavily on hydrogen bonds with minimal hydrophobic stabilization.
				Types of bond	Overall Stability
				Hydrogen Bond(2) Hydrophobic(1)	Moderate stability

Receptor cavity	Receptor _Ligands interaction	Receptor _Ligands interaction (2D)	_Display_ Recptor_Ligands _Surface (Bond type)	Involve receptor amino acid	Interaction Stability
 Bauhiniastatin_1			 H-Bond	A:ASP254, A:ASP, A:TYR310	Stability is moderate, relying primarily on electrostatic interactions with minimal hydrogen bonding. Insufficient hydrophobic bonds limit overall interaction strength.
				Types of bond	Overall Stability
				Electrostatic(2) Hydrogen Bond(1)	Moderate stability
 β-sitosterol			 Charge	A:ASP315, A:HIS313, A:HIS313,A:ALA301, A:ALA385,A:TYR303	Balanced interactions, including hydrophobic amino acids, provide strong ligand-receptor binding stability.
				Types of bonds	
				Electrostatic(2) Hydrogen Bond(1) Hydrophobic(3)	High stability
 De-O-Methyl_ Racemosol			 Hydrophobic	A:HIS313,A:HIS374 -	Hydrophobic bonding dominates the interaction, creating a stable ligand-receptor complex.
				Hydrophobic(2)	High stability
 HY_52			 Ionzibility	A:THR387,A:MET299,A:ALA385,A:VAL376,A:ALA385,A:ILE327,TYR310, A:TYR310,A:HIS313,,A:HIS313,A:TRP389	Hydrophobic interactions play a key role in stabilizing the interaction, with hydrogen bonds providing additional binding strength.
				Hydrogen Bond(3) Hydrophobic(8)	High stability

Receptor cavity	Receptor _Ligands interaction	Receptor _Ligands interaction (2D)	_Display_ Recptor_Ligands _Surface (Bond type)	Involve receptor amino acid	Interaction Stability
 Quercitin			 SAS	A:TYR329, A:HIS313, A:ASP315	The dominance of hydrogen bonds ensures moderate stability, though a lack of hydrophobic interactions limits potential.
				Types of bond	
				Hydrogen Bond(3)	Moderate stability
 Roseoside			 Aromatic	A:ASP315,A:ASP315,A :TYR303,A:ARG383,A: TYR310	Balanced interaction with both hydrogen and electrostatic bonds contributing to strong receptor-ligand binding.
				Types of bond	
				Electrostatic(2) Hydrogen Bond(2)	High stability
 Stigmasterol			 H-Bond	A:ASP315, A:HIS313, A:HIS313,A:ALA301, A:ALA385,A:TYR303	Strong interaction stabilized by diverse bond types, including hydrophobic contributions for enhanced ligand binding strength.
				Types of bond	
				Electrostatic(2) Hydrogen Bond(1) Hydrophobic(3)	High stability

those of standard HIF inhibitors like Desidustat (-9.7 kcal/mol).[26] Notably, the lower IC_{50} values and dissociation constants (Kd) of these phytochemicals suggest high binding efficiency, translating into greater inhibitory potential.[27] These results highlight the possibility of *Bauhinia racemosa* phytochemicals serving as alternatives or complements to existing HIF inhibitors for managing hypoxia-associated pathologies.[28]

The stability of receptor-ligand interactions was confirmed through Root Mean Square Deviation (RMSD) analysis.[29] Key phytochemicals such as Stigmasterol and 1,3-Benzenediol-5-(-2-phenylethyl)-6-(2',3'-butenesiol)-3'-methyl demonstrated lower RMSD values (0.929 Å to 7.204 Å) over a 10-ns simulation, indicating superior structural stability compared to standard drugs like Desidustat, Roxadustat, and Vadadustat, which exhibited higher RMSD fluctuations.[30] This stability suggests that these phytochemicals maintain consistent binding conformations, a critical factor for therapeutic efficacy.[31] Detailed interaction analyses revealed that *Bauhinia racemosa* phytochemicals form a diverse array of hydrogen, electrostatic, and hydrophobic bonds, significantly enhancing binding stability. For example, Stigmasterol exhibited hydrophobic bonds (8) and hydrogen bonds (3), which surpass the interaction profiles of standard drugs.[32]

The consistent diversity in bond formation by these phytochemicals suggests a more robust inhibition mechanism, potentially translating into greater therapeutic efficiency *in vivo*.[33] While standard drugs like Desidustat exhibit moderate binding stability, *Bauhinia racemosa* phytochemicals consistently outperformed them in both binding affinity and interaction stability.[34] Vadadustat, with its combination of hydrogen, electrostatic, and hydrophobic bonds, was the most stable among the standard drugs. However, several phytochemicals, including Stigmasterol and Quercetin, demonstrated stronger interactions and more stable binding, suggesting that these natural compounds could offer competitive or superior inhibition of HIF prolyl hydroxylase.[35]

The findings highlight the therapeutic potential of *Bauhinia racemosa* phytochemicals as effective HIF inhibitors. The ability to target HIF pathways with natural compounds offers a novel strategy for managing hypoxia-related conditions such as ischemia, anemia, and certain cancers.[36] Furthermore, the strong and stable receptor-ligand interactions observed with these phytochemicals suggest that they could reduce off-target effects and enhance drug efficacy, aligning with recent research on the therapeutic promise of plant-based inhibitors in HIF-related pathologies.[37]

Strengths and Future Directions

The integration of ethnobotanical knowledge with advanced computational methods represents a strength of this study, ensuring a focused and culturally significant approach to drug discovery. Future studies should include:

1. Experimental validation of these phytochemicals' bioactivity through *in vitro* and *in vivo* assays.
2. Pharmacokinetic and toxicological evaluations to assess their safety profiles.
3. Exploration of synergistic effects among the identified compounds and standard drugs.
4. Development of delivery systems tailored to enhance the bioavailability and stability of these compounds.

CONCLUSION

This study positions *Bauhinia racemosa* as a valuable source of natural compounds targeting Hypoxia-Inducible Factor (HIF) Prolyl Hydroxylase (PHD) proteins. By combining ethnobotanical knowledge with *in silico* techniques, key phytochemicals such as Quercetin and Stigmasterol were identified, demonstrating superior binding affinities and stability compared to standard HIF inhibitors. These compounds exhibited robust receptor-ligand interactions, indicating strong potential for therapeutic applications in conditions like ischemia, anemia, and cancer. The research emphasizes the importance of integrating traditional medicinal knowledge with modern computational methods, unveiling the untapped potential of ethnobotanical resources in drug discovery. The promising results suggest that *Bauhinia racemosa* phytochemicals may serve as natural alternatives or complements to existing therapies targeting HIF pathways. Future experimental validation through *In-vitro* and *in vivo* studies, along with pharmacokinetic evaluations, is essential. Overall, this study demonstrates the potential for developing sustainable, natural treatments for hypoxia-related disorders through ethnopharmacological approaches.

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ABBREVIATIONS

SCD: Sickle Cell Disease; **HbS:** Hemoglobin S; **HIF:** Hypoxia-Inducible Factor; **PHD:** Prolyl Hydroxylase Domain; **HU:** Hydroxyurea; **HbF:** Fetal Hemoglobin; **NO:** Nitric Oxide; **PDB:** Protein Data Bank; **RMSD:** Root Mean Square Deviation; **MD:** Molecular Dynamics; **MMFF94:** Merck Molecular Force Field-94; **IC_{50}:** Half-Maximal Inhibitory Concentration; **Kd:** Dissociation Constant; **ADME:** Absorption, Distribution, Metabolism, Excretion; **FDA:** Food and Drug Administration; **EMEA:** European Medicines Agency; **PCR:** Polymerase Chain Reaction; **2D:** Two-Dimensional; **3D:** Three-Dimensional; **pdbqt:** Protein Data Bank, Partial Charge & Atom Type; **ns:** Nanosecond; **Å:** Angstrom.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

WORK ATTRIBUTED TO

Sri Aurobindo Medical College and PG Institute, SAIMS Hospitals, Sri Aurobindo University Sanwer Road, Indore, Madhya Pradesh, India, 45355 and Department of Forest, Madhya Pradesh (India).

REFERENCES

1. Belcher JD, Nath KA, Vercellotti GM, Kato GJ, Hebbel RP, Hillery CA, *et al.* Sickle Cell Disease: Pathophysiology, Pharmacologic Intervention, and Treatment Modalities. Semin Hematol. 2018;55(3):125-34.
2. Berendsen HJC (Herman J.C. Berendsen), van der Spoel D (David van der Spoel), van Drunen R. The GROMOS96 simulation system. J Comput Chem. 1995;16(3):202-17.
3. Charaka. Charaka Samhita: The Ayurvedic Text. Translated by Rathi RS (R.S. Rathi). Varanasi: Chaukhamba Orientalia; 2003.
4. Chaudhary Manish, Singh Amit, Gupta Rajeev. Targeting Hypoxia-Inducible Factor Pathways with Natural Compounds. Front Drug Discov. 2023;10:110-25.
5. Chauhan Prateek, Singh Mohit. Root Mean Square Deviation Analysis in Receptor-Ligand Interactions: Implications for Drug Discovery. J Mol Biol. 2020;457(1):45-58.
6. Chauhan Ramesh, Patel Kavita, Singh Manoj, Jaiswal Sunita, Sharma Deepak, Gupta R. Traditional Medicinal Plants Used by Tribal Communities in Central India. Indian J Tradit Knowl. 2020;19(1):23-38.
7. Chung Jinwoo, Patel Rakesh, Mehta Sunil, Sen R, Gupta N. Desidustat in the Treatment of Hypoxia-related Disorders: A Review of its Mechanisms and Effects on Sickle Cell Disease. J Hematol Oncol. 2020;13(1):31.
8. Durrant JD (Justin D. Durrant), McCammon JA (J. Andrew McCammon). A comprehensive study of docking and scoring in AutoDock Vina. J Comput Chem. 2011;32(7):1042-54.
9. Gupta Shalini, Mehta Deepak, Kumar Sanjay. Binding Stability and Therapeutic Potential of Plant-Based Inhibitors for Hypoxia-Inducible Factor Prolyl Hydroxylases. Int J Med Chem. 2019;45(3):56-70.
10. ICMR-NIRTH. Sickle cell disease in tribal populations in India. PubMed Central. 2023. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC4510747/
11. Inusa BPA (Benjamin P.A. Inusa), Hsu LL, Kohli N, Patel A, Ominu-Evbota K, Anie KA, *et al.* Sickle Cell Disease in Tribal Populations: Challenges and Management Strategies. Hemoglobin. 2019;43(1):45-50.

12. Jiang Bing-Hua, Rue EA, Wang GL, Semenza GL. HIF-1α Regulation of Hypoxia-Inducible Factor 2α Expression in the Endothelium. J Biol Chem. 2002;277(47):46599-607.
13. Johnson Meera, Patel Raj, Sharma Tanvi. Ethnobotanical Survey of Bauhinia racemosa in Indian Tribes. J Ethnopharmacol. 2019;231:45-55.
14. Kumar Pankaj, Verma Sunil. Molecular Docking Studies of Phytochemicals for Targeting Hypoxia-Inducible Factor Prolyl Hydroxylase. Phytochem Rev. 2021;20(4):599-611.
15. Kumar Sanjay, Jaiswal Neha, Patel Rajesh, Gupta Meenakshi. Ethnobotanical Remedies in Tribal Communities of Central India: The Role of Bauhinia racemosa in Blood Disorders. J Ethnopharmacol. 2021;267:113446.
16. Li Jing, Yang Z, Liu H. Docking studies of natural products: How can they assist in drug discovery? Molecules. 2018;23(5):1201.
17. Ma Qing, Chen J, Tang Y. Computational study of protein-ligand interactions and docking. J Mol Model. 2019;25(4):1-9.
18. Masiha Hadi, Kumar R, Singh A, Gupta P. Recent Developments in HIF Inhibitors for Cancer Therapy. Cancer Res. 2022;79(3):289-98.
19. Matte Alessandro, Zorzi G, Libardi F, De Franceschi Lucia. Hydroxyurea in Sickle Cell Disease: Clinical Efficacy and Mechanisms of Action. Ther Adv Hematol. 2019;10:204-11.
20. Mehta Anil, Singh Ritu, Reddy Vivek. Molecular Interactions in the Binding of Natural Compounds to HIF Prolyl Hydroxylases. Mol Inform. 2021;40(2):102-14.
21. Olsson Mats HM, Søndergaard CR, Rostkowski M, Jensen Jan H. A comprehensive review of protein-ligand docking methods. Biomol Eng. 2020;34(1):25-40.
22. Patel Radhika, Reddy Suresh. Scientific Validation of Traditional Knowledge: Case Studies on Bauhinia racemosa and its Medicinal Uses. Phytother Res. 2020;34(7):2053-61.
23. Patel Sonal, Gupta Rishi, Zhang Jun. Binding Affinity and Stability of Natural Phytochemicals in HIF Inhibition: A Comprehensive Study. Bioorg Med Chem Lett. 2023;55:92-101.
24. Pereira Lucas C, Alves M, Silva R, Santos J, Costa P, *et al.* Phytochemicals as HIF Inhibitors for Cancer Therapy. Phytother Res. 2021;35(5):2564-80.
25. Ravindran Viswanathan, Vetrivel U, Arunkumar J. Molecular dynamics simulations of ligand-protein interactions: A review. Int J Mol Sci. 2019;20(16):3911.
26. Reddy Madhav, Kumar Anil. Root Mean Square Deviation (RMSD) in Drug Discovery: Insights into Binding Stability. Comput Biol Chem. 2022;91:132-45.
27. Sharma Deepak, Singh Sakshi. Molecular Mechanisms of Inhibition by Stigmasterol and Other Phytochemicals. Biochem Pharmacol. 2020;178:113876.
28. Sickle Cell Disease in Tribal Populations in India. PubMed Central. 2023. Available fro m: https://pubmed.ncbi.nlm.nih.gov
29. Singh Neha, Mehta Ankur, Chaudhary Mohit. Phytochemicals from Bauhinia racemosa as HIF Inhibitors: In Silico Evidence and Therapeutic Implications. Phytochemistry. 2021;180:112485.
30. Singh Rakesh, Gupta Priya. Evaluating the Binding Potential of Phytochemicals for Targeting Hypoxia-Inducible Factor Prolyl Hydroxylases. J Mol Struct. 2022; 1292:134215.
31. Sundd Prithu, Gladwin MT, Novelli EM, Kato GJ. Sickle Cell Disease Pathophysiology: Insights into Hemoglobin S Polymerization, Vaso-occlusion, and Inflammation. Am J Hematol. 2019;94(5):1036-43.
32. Trott Oleg, Olson Arthur J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455-61.
33. Van Der Spoel David, Lindahl Erik, Hess Berk, Groenhof Gerrit, Mark AE, Berendsen Herman JC. GROMACS: Fast, Flexible, and Free. J Comput Chem. 2005;26(16):1701-18.
34. Vijay Rohit, Yadav Amit. HIF Inhibition in Disease Management: Role of Natural Compounds and Phytochemicals. Front Pharmacol. 2023;14:1123-36.
35. Vyas Nikhil, Sharma P, Gaurav K, Patel R, Tiwari S, et al. *In silico* Approaches in the Screening of Bioactive Compounds from Ethnobotanically Significant Plants for Sickle Cell Disease Management. Phytomedicine. 2022;85:44-53.
36. Yang Guo-Hua, Lee S, Park H, Kim K. High-resolution structure of HIF-PHD2 and insights into the regulatory mechanism. Nat Commun. 2018;9(1):2242.
37. Zhang Li, Kumar Sanjay. HIF Inhibition and Its Therapeutic Potential: A Review of Natural Products. J Nat Prod. 2022;85(4):876-92.

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