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Natural Phytochemicals as Modulators of Hypoxia Inducible Factors: A Bioinformatics Analysis

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Abstract

Introduction: Hypoxia-inducible factor 1 alpha (HIF-1α) is critically involved in regulating cellular responses to low oxygen conditions, and its dysregulation has been implicated in multiple malignancies. In these malignancies, HIF-1α activates a cascade of molecular events that promote tumorigenesis, making it an attractive therapeutic target. This study aimed to identify natural compounds with the potential to inhibit HIF-1α activity.

Methods: We sourced chemical structures of potential inhibitors from the PubChem database and performed molecular docking simulations using PyRx. The binding affinities and interactions between the compounds and HIF-1α were analyzed through PyMOL visualization.

Results: Among the natural compounds tested, Berberine exhibited the most promising inhibitory activity, showing the strongest binding affinity with a docking score of -8.6 kcal/mol. These results suggest that Berberine binds effectively to key amino acid residues within the catalytic site of HIF-1α, potentially hindering its activity.

Conclusion: This study highlights Berberine as a candidate for further investigation as a therapeutic agent, with potential for *in vivo* and *in vitro* testing.

Keywords: Molecular docking, Simulation, Tumor hypoxia, Phytochemicals, Berberine

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Introduction

For decades, hypoxia has been a major focus of research in the tumor microenvironment, where it exerts diverse impacts on cancer cells (1,2). While normal tissues receive sufficient oxygen supply to meet cellular demands, diffusion of oxygen from blood capillaries into tumor masses of 1-2 mm^3 is restricted owing to leaky neovascularization, causing hypoxic areas within the tumor mass (3).

Breast cancer is the most common cancer affecting females globally and is the foremost cause of cancer-related mortality (4,5). Hypoxic sub-regions are present in roughly one-quarter to two-fifths of invasive breast tumours, highlighting oxygen deprivation as a major pathological trait of the breast tumours (6).

Polarographic measurements indicate that healthy breast tissue has a median oxygen tension of about 65 mmHg, whereas malignant breast lesions often show markedly reduced median pO2 values around 10 mmHg, with a substantial proportion of tumours exhibiting extremely low oxygen levels below 2.5 mmHg (7,8). Clinically, intratumoral hypoxia implicates poorer prognosis in breast cancer and exerts an adverse prognostic effect regardless of tumour stage, nodal status, and histological grade (9, 10).

At the time of breast cancer diagnosis, elevated HIF-1 expression correlates with high risk for tumour recurrence and metastasis, as well as with poorer overall outcomes

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(11,12). Triple-negative breast cancers, in particular, tend to exhibit increased expression of HIF-1–regulated genes, underscoring the importance of this pathway in aggressive disease subtypes (13).

HIF-1 activity underpins multiple hallmarks of breast cancer biology, including pathological angiogenesis, the Warburg effect, sustenance of cancer stem-like cells, epithelial–mesenchymal transition, invasion, metastatic dissemination, and chemoresistance. Through hypoxia-driven transcriptional control of protein-coding genes and diverse non-coding RNAs, HIF-1 significantly affects breast cancer cell migration, invasion, and metastatic spread. Consequently, HIF-1 is widely regarded as an attractive molecular target in breast cancer.

For many years, plant-derived bioactive compounds have been employed both to maintain health and treat a wide range of ailments (14,15). Berberine, curcumin, genistein, quercetin, and resveratrol have significant anticancer activities through unique molecular mechanisms. Berberine inhibits cancer development through induction of DNA damage, RAD51 expression regulation, and induction of apoptosis in ovarian, breast, and osteosarcoma cells (16). Curcumin disrupts tumorigenesis by affecting cancer stem cell pathways and PI3K/AKT and Wnt/β-catenin signaling, frequently synergizing with other natural or chemotherapeutic agents (17). Genistein inhibits epigenetically through control of DNA methylation and histone modification, inhibiting cell growth in hormone-dependent malignancies such as breast and prostate cancers (18). Quercetin inhibits growth of cancer cells, angiogenesis, and apoptosis through oxidative stress modulation and downregulation of BCL2, with synergistic effects when used with curcumin or resveratrol (19). Resveratrol acts as an anticancer and chemopreventive agent against breast, prostate, and oral cancers by triggering p53, suppressing EMT, and regulating oncogenic noncoding RNAs and histone deacetylases (20).

These compounds were specifically chosen on the basis of sound experimental evidence of their capacity to modulate hypoxia-regulated molecular pathways and specifically suppress HIF-1α expression or activity. Each compound belongs to a dominant phytochemical class with established safety profiles and complementary mechanisms of anticancer action, such as epigenetic modulation, redox modulation, and antiangiogenic activity. Their choice facilitates a comparative assessment of different structural classes for possible inhibitory consistency towards HIF-1α signaling, hence their inclusion in this work. The present study investigated the anti-tumor potency of several nutraceuticals employing an in silico approach.

Methods

Preparation of Protein Structure

The crystallographic structure of HIF-1α in three dimensions (PDB ID: 1H2K) was downloaded from the RCSB Protein Data Bank. All non-essential elements, such as co-crystallized ligands and water molecules, were deleted to pre-adapt the receptor for docking. Polar hydrogens were inserted, and Gasteiger partial charges were calculated using AutoDockTools by following the "Edit → Add Polar Hydrogens → Compute Gasteiger Charges" sequence. The charge-assigned and processed receptor structure was then exported in the PDBQT format for further docking simulations.

Preparation of Ligand

The phytochemicals, including resveratrol, genistein, quercetin, berberine, and curcumin, were retrieved from the PubChem database in SDF format. These structures were subsequently converted to PDB files using an online SMILES translator before docking analysis. The ligands prepared were transferred into AutoDockTools, and automated computation of partial Gasteiger charges was performed. The corrected molecules were then optimized and transformed into PDBQT format before being transferred into PyRx for docking simulation (21,22).

Molecular Docking

Molecular docking was carried out in PyRx using the AutoDock 4.0 engine to assess the binding of the selected ligands to HIF-1α. During docking, the ligands were treated as flexible, whereas the receptor structure was kept rigid. Grid parameters were generated with the AutoGrid module implemented in PyRx, and docking poses were examined to identify amino acid residues within the HIF-1α binding pocket that interacted with each ligand. Docked conformations with a positional root-mean-square deviation (RMSD) below 1.0 Å were clustered and considered to represent favourable binding orientations. Among the resulting poses, the ligand exhibiting the lowest (most negative) binding energy score was taken to indicate the highest predicted binding affinity for HIF-1α.

Results

Molecular docking was carried out using the PyRx platform. Among the generated poses, the conformation with the lowest docking score was considered the most likely binding orientation (Figure 1). The docking scores and hydrogen-bond interactions of the top five shortlisted compounds are summarized in Table 1.

Molecular docking profile of phytochemicals with the HIF target, showing predicted binding affinity (docking score, kcal/mol) and key hydrogen-bonding residues with their bond distances (Å) in the binding pocket.

All ligands were successfully positioned within the HIF-1α active site and were found to engage in hydrogen-bonding interactions with residues in the binding pocket. The five phytochemicals exhibited favourable docking scores ranging from −8.6 to −7.6 kcal/mol, with more negative values reflecting stronger predicted binding affinity.

Each compound formed multiple hydrogen bonds with HIF-1α, consistent with stable accommodation in the active site. Among them, berberine showed the most favourable binding energy (−8.6 kcal/mol) and established two hydrogen bonds with GLN147 and GLN203, both at distances below 3 Å, indicative of strong intermolecular

Table 1. Molecular docking of selected phytochemicals with the HIF target protein.

S. No	Compound	Docking score (kcal/mol)	Key hydrogen bond partners (residues)	H-bond distances (Å)
1	Berberine	−8.6	GLN147, GLN203	2.2, 2.7
2	Curcumin	−8.2	GLU202, GLN239	1.5, 2.4
3	Genistein	−7.8	GLN147, ASP201, ARG238, GLN239	2.7, 2.4, 2.0, 2.2
4	Quercetin	−8.0	SER91, THR149, ASP201, ARG238	2.2, 2.2, 2.5, 2.3
5	Resveratrol	−7.6	ARG238, GLN239	1.4, 2.2

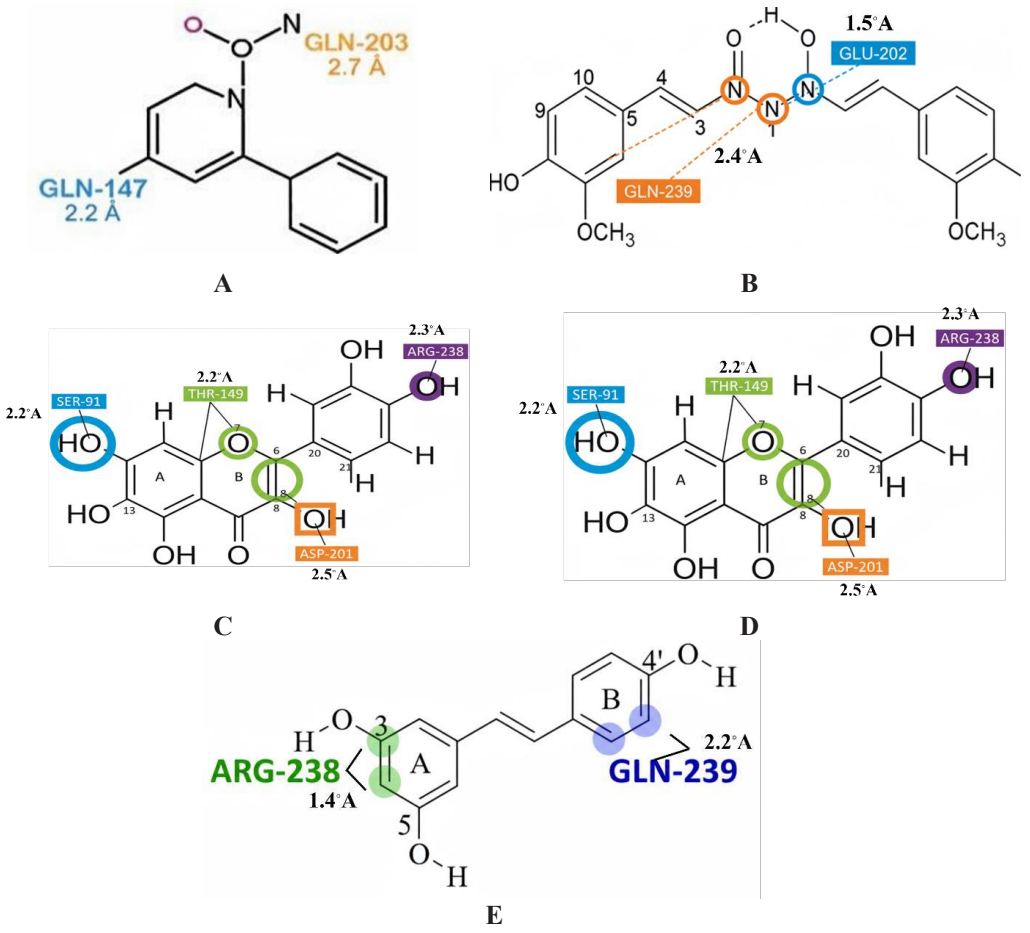


Figure 1. Representative docking poses of HIF-1α in complex with berberine (A), curcumin (B), genistein (C), quercetin (D), and resveratrol (E)

interactions. Collectively, these findings suggest that all five ligands adopt energetically favourable binding modes with HIF-1α.

Discussion

HIF-1α is a crucial protein that transduces cellular responses to low oxygen tensions and is crucial for cancer development, such as breast cancer, through the regulation of angiogenesis, metabolism, and survival signaling (23,24). The HIF-1α subunit binds to several co-factors and DNA and transactivates genes in response to hypoxia (25). Targeting HIF-1α with small molecule inhibitors has been recognized for the purpose of therapeutic intervention in pathologies of hypoxia (26).

The Representative binding modes of HIF-1α in complex with the top-ranked phytochemicals Berberine (A), Curcumin (B), Genistein (C), Quercetin (D), and Resveratrol (E), as predicted by molecular docking, are shown in Figure 1. Of the five natural compounds investigated, berberine has the greatest binding affinity to HIF-1α with a binding score of -8.6 kcal/mol, where stable hydrogen bonds were formed with GLN-147 and GLN-203. This robust binding is associated with its widely documented induction of apoptosis and modulation of DNA repair proteins such as RAD51 in cancers, and it is possible that direct suppression of hypoxia signaling is part of its anticancer action (26,27). Curcumin revealed a binding score of around -8.0 kcal/mol, consistent with its function in the modulation of cancer stem cell pathways and interloping of PI3K/AKT signaling, which suggests that its modest affinity to HIF-1α would complement its wider pro-apoptotic and anti-inflammatory effects (28).

Genistein, quercetin, and resveratrol had binding scores ranging from -7.6 to -7.9 kcal/mol, in line with their antioxidant activity and epigenetic repression of tumor suppressor genes via DNA methylation and histone modification (29,30). Their docked structures had multiple hydrogen bond interactions in the active site

of HIF-1α and attest to their effectiveness in inhibiting angiogenesis and oxidative stress, which are important in cancer development. Together, these in silico findings add to the biological significance of these phytochemicals' anticancer activity, with promising potential molecular basis of interaction with HIF-1α to inform rational design of natural product-derived inhibitors (26,31).

This study also has important limitations. This investigation centered mainly on hydrogen bonding interactions between natural compounds and the active site of HIF-1α via rigid docking protocols of AutoDock, which themselves assume a static protein conformation, and may miss vital conformational flexibility of the receptor and induced-fit effects. The lack of molecular dynamics simulations inhibits insight into the temporal stability and dynamic behavior of the complexes in physiologically relevant environments, which are essential for precise binding affinity predictions and realistic interaction mapping. Also, no ADMET profiling was performed, limiting the evaluation of pharmacokinetic and toxicity properties required for drug development potential. Recent progress has indicated that integration of machine learning, flexible docking, molecular dynamics, and binding free energy calculations may improve inhibitor discovery and characterization of HIF-1α, including side-by-side comparison with prototype compounds. Integration of these advanced in silico methods is important for detailed mapping of ligand-receptor binding modes, systematic assessment of amino acid contacts, and lead prioritization for experimental confirmation.

Conclusion

This research proves that the natural bioactive molecules berberine, curcumin, genistein, quercetin, and resveratrol have high binding affinities to the HIF-1α active site, which is confirmed by molecular docking studies. The stable hydrogen bond interactions, especially with residues GLN-147 and GLN-203 in the case of berberine, show their ability to regulate hypoxia signaling pathways involved in cancer development. These results support and build upon earlier experimental accounts of their antioxidant, apoptotic, and epigenetic modulatory activities, placing these compounds firmly in the running as developmental candidates as HIF-1α inhibitors. Although this computational method offers insightful mechanistic information, additional studies merging dynamic simulations with experimentally validated determinations are necessary to provide full disclosure of binding effectiveness and therapeutic value. This research provides foundational grounds for rational design of new natural product-inspired anticancer agents for hypoxic tumor microenvironments.

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Competing Interests

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

Data Availability Statement

Access to the study's underlying data can be provided by the corresponding author upon a justified request.

Ethical Approval

The study was approved by the Ethics Committee of Meenakshi Academy of Higher Education and Research, Chennai, Tamil Nadu, India (Ethical code: MAHER/IEC/PHD/77/NOV24).

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