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Original Article

IN SILICO EXPLORATION OF BERBERINE AS A POTENTIAL WOUND HEALING AGENT *VIA* NETWORK PHARMACOLOGY, MOLECULAR DOCKING, AND MOLECULAR DYNAMICS SIMULATION

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ABSTRACT

Objective: Wound healing remains a complex biological process crucial for tissue repair and homeostasis. Our goal in this paper is to focus on the application of advanced computational techniques to explore the potential of naturally occurring compound berberine in addressing molecular targets related to wound healing.

Methods: Network pharmacology, molecular docking analysis, *in silico* ADMET prediction, and extensive 100 ns molecular dynamics simulations was performed to gain a holistic understanding of the therapeutic potential of berberine against molecular targets involved in wound healing. This study predicted drug-likeness scores, potential side effects, ADMET profiles, carcinogenicity, MolLogP, molecular volume analysis, and molecular polar surface area for berberine.

Results: Findings of the study revealed that berberine displayed a remarkable binding affinity for the epidermal growth factor receptor (EGFR), with a binding energy of-8.14 kcal/mol, surpassing the crystal ligand's binding energy of-7.15 kcal/mol. This indicates a strong potential for berberine in modulating EGFR-related pathways critical for wound healing. The culmination of the investigation was a 100 ns molecular dynamics simulation, which demonstrated consistent binding and stability over time, reinforcing the potential of berberine as a wound healing agent.

Conclusion: The integration of gene expression analysis, enrichment studies, network analysis, molecular docking, and molecular dynamics simulations unveiled crucial mechanisms underlying efficacy of berberine as a potent wound-healing agent.

Keywords: Molecular docking, Network pharmacology, Gene ontology, Berberine, Wound healing

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INTRODUCTION

Phytochemicals-based medicines have been widely applied for centuries for primary medical and health care of society and community. Nearby 80% of the human community in the world depends on phytomedicines or herbal medicines as their primary preventive medicine. The treatment of many diseases and disorders with herbal medicines is observed as very secure with no or minimal side effects [1]. Berberine is a natural compound found in several plants, including goldenseal, Oregon grape, barberry, and tree turmeric [2]. In recent years, berberine has been the subject of much scientific research, and its potential health benefits have been further investigated [3]. Computational studies, also known as *in silico* investigations, have become a mainstay of contemporary science across a wide range of disciplines [4–6]. In order to better understand complicated processes, current studies use computer-based simulations, modeling, and data analysis [7]. They make it possible to quickly find prospective medication candidates by analyzing huge chemical archives. For the purpose of verifying experimental designs and testing hypotheses, *in silico* investigations are an invaluable resource [8, 9].

Berberine has been reported to be one of the potential candidates having wide therapeutic usage and found beneficial in conditions like blood sugar control [10]. In several clinical trials, berberine has been shown to be as effective as some diabetes medications in lowering blood sugar levels in people with type 2 diabetes [11]. Wound healing is a highly complex and dynamic process that involves a series of intricate cellular and molecular events. Berberine is believed to exert anti-inflammatory effects. Inflammation is a fundamental component of the initial stages of wound healing. The anti-inflammatory properties of berberine may help regulate the inflammatory response at the wound site, leading to tissue repair. Berberine possesses antioxidant capabilities, which play a vital role in shielding the wounded tissues from oxidative damage [12]. Oxidative stress can hinder the healing process, and berberine's antioxidant effects may help mitigate this obstacle. Moreover, berberine is thought to enhance blood flow to the wound site. Improved blood circulation aids in the delivery of essential nutrients and oxygen to the injured tissues. Furthermore, berberine has been suggested to stimulate collagen production. Collagen is responsible for providing structural integrity to the newly formed tissue, and the ability of berberine to promote its production is of significant importance. Berberine may contribute to reducing scarring following wound closure. Scarring can affect both the functional and cosmetic aspects of the healed tissue, and potential of berberine in minimizing scarring holds promise for improving the overall quality of wound healing outcomes [13].

Wound healing is a complex and highly regulated biological process that is crucial for the restoration of tissue integrity and function [14]. This work explores the various aspects covering a computer model-based approach to examine a potential phytochemical agent's healing duration and effectiveness using lead bioactive agents' ability to interact with the relevant receptors involved in the wound-healing process. Wounds are disruptions in the structural and functional integrity of tissues, often resulting from physical trauma, surgical procedures, or underlying medical conditions [15]. They can be classified into several categories based on their etiology, including acute wounds (e. g., cuts and burns) and chronic wounds (e. g., diabetic ulcers and pressure sores) [16, 17]. Key players include Fibroblasts, Keratinocytes, Macrophages, and Growth factors like Transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), and epidermal growth factor receptor (EGFR) are crucial for cell proliferation, angiogenesis, and tissue repair [18, 19]. The literature review suggested that the use of synthetic and natural molecules as a substitute advantageous wound healing treatment because of various mechanisms like modification, blocking, and repression [20, 21]. This research aims towards current evidence on the health benefits of berberine as a potential wound-healing agent with the help of *in silico* methodologies. The most likely mechanism for its treatment was

studied using a network pharmacology, molecular docking, and molecular dynamics simulation approach to understand interactions between berberine and a modulated protein and also predict its pharmacokinetic properties [22, 23]. Network pharmacology is an interdisciplinary field that helps to integrate bioinformatics, network analysis, and systems biology to study ligand-target interactions within biological networks [24, 25]. It is used to comprehend the complex interactions between ligand, targets, and biological systems at a network level. Molecular docking is another computational technique which is widely used to predict the preferred binding orientation of ligand with molecualr target [26-28]. MD simulation is a computational method used to study the movements and interactions of atoms and molecules over time [29–31]. It helped to simulate the behavior of a complex system by solving the classical equations of motion for all atoms in the system [32–34]. The primary objective of MD simulation in the current study is to model and analyze the dynamic behavior of protein-ligand complex systems at an atomic level [35–38].

MATERIALS AND METHODS

ADMET profile and drug-likeness prediction

The pharmacokinetic and drug-likeness profile of berberine was predicted. Druglikeness assessment was done employing "Lipinski's rule of 5" principle. The prediction was done using MolSoft. Likewise, the Qikprop module of Schrödinger was used to predict the absorption, distribution, metabolis, and excreation (ADME) profile of berberine phytoconstituents. Furthermore, in the study, Cheminformatics tools were employed to assess topological polar surface area (TPSA) for the purpose of predicting the possible bioavailability, molecular volume correlation analysis, and determining MolLogP values of berberine.

Gene enrichment and expression analysis

The DIGEP-Pred tool (https://www.way2drug.com/ge/), which incorporates a training set of proteins for regulatory protein analysis was used to screen gene enrichment and expression. The activity of berberine with a Pa (probability) threshold exceeding 0.5, along with the identification of its interacting partners was conducted using the search tool for the retrieval of ınteracting genes/proteins (STRING). Subsequently, pathways related to wound healing and its outcomes were identified through the kyoto encyclopedia of genes and genomes (KEGG) pathway database (https://www.genome.jp/kegg/), curated by KEGG.

Pathways and network ınvestigation

In this study, a set of proteins associated with wound healing was subjected to screening via the STRING database. Berberine-modulated pathways were identified through gene enrichment analysis. KEGG pathway analysis was performed to discern pathways relevant to wound healing. Afterward, a network incorporating protein structures, identified pathways, and berberine was constructed utilizing Cytoscape 3.5.1. The entire network was visually interpreted based on the number of edges, using a color and node size scale with correlating edge counts. The most consistent node was denoted by a prominent node symbol.

Molecular docking

The 3D structure of berberine was retrieved from the PubChem database, which was further subjected to energy minimization using OPLS 2005. The crystal structure of the kinase domain from the epidermal growth factor receptor (EGFR) (PDB ID: 1M17) with a resolution of 2.60 Å was downloaded from the RCSB protein data bank. In this study, the native ligand Erlotinib was extracted from the downloaded protein structure and preserved for subsequent docking analysis. Water molecules and other heteroatoms were removed from the protein structure [39]. The docking environment was optimized to a pH of 7.4 using Schrödinger's Maestro Molecule Builder, and an Ionizer was employed to determine protomers and ionization states for the docked ligands. Utilizing Glide XP docking precision, the prepared ligands were docked into the generated receptor grid. The docking study between the target protein and berberine was executed using Schrödinger Suite's GLIDE tool. Subsequently, the Schrödinger Suite was utilized to scrutinize the interactions within each complex and ascertain the 3D poses indicative of molecular recognition interactions.

Molecular dynamics simulations

The MD simulation was conducted using Schrödinger, LLC's Desmond 2020.1 software. Employing the OPLS-2005 force field and the TIP3P water model, the system was enclosed within a periodic boundary solvent box to cover the entire complex structure [22, 40]. A 0.15 M Na+ion concentration was introduced through NaCl solutions to mimic physiological conditions and neutralize charges. Equilibration began with a 10 ns simulation in the NVT ensemble to stabilize complexes [41]. Subsequently, a 12 ns equilibration phase, coupled with energy minimization, was performed in the NPT ensemble. The Nose-Hoover chain approach maintained 1 bar pressure with a 1.0 ps relaxation time for temperature regulation [42]. A consistent 2 fs time step was maintained throughout the simulation. Pressure control utilized the Martyna-Tuckerman-Klein chain coupling barostat method with a 2 ps relaxation time [43, 44]. A 100 ns production run was done to capture system dynamics. MD trajectory analysis was done with parameters like root mean square deviation (RMSD), root mean square fluctuation (RMSF), and intermolecular interactions [45, 46].

RESULTS AND DISCUSSION

ADMET and carcinogenicity studies

Berberine exhibits notable lipophilicity and a heightened capacity to traverse the blood-brain barrier, suggesting a low likelihood of central nervous system harm. Table 1 presents a comprehensive overview of the ADMET prefilling results for berberine, including associated probability scores. This increased lipophilicity can be attributed to the presence of a hydrophobic moiety. Notably, the investigation of berberine's aqueous solubility indicates a Level 4.95, indicating poor solubility in aqueous environments. Furthermore, the model for plasma protein binding predicts that berberine binds to plasma proteins with high affinity, indicating 100% binding. Importantly, the assessment of berberine's influence on cytochrome P450 2D6 (CYP2D6) revealed an inhibitory effect. The proximity of the carcinogenicity score to one within table 1 is a critical factor affecting the accuracy of cancer prediction. A score closer to one increases the probability of accurately predicting cancer, whereas a score nearer to zero diminishes this predictive accuracy. Berberine indicated nonmutagenic to AMES and mouse carcinogenicity. This parameter has direct implications for berberine's pharmacokinetics and can influence its distribution and metabolism within the body.

MolLogP, MolPSA, and MolVol analysis

Berberine, with a molecular weight of 336.12 g/mol, exhibits favorable properties for drug development. It is characterized by 4 hydrogen bond acceptors and no hydrogen bond donors, which can influence its interactions with biological molecules. Furthermore, it boasts the highest drug-likeness score (0.77), indicating its potential as a promising candidate for pharmaceutical applications. Table 2 provides a comprehensive summary of berberine's molecular attributes, including its molecular weight, number of hydrogen bond acceptors and donors, MolLogP, MolPSA, MolVol, and drug-likeness information. Total polar surface area (TPSA) is a crucial parameter for evaluating drug bioavailability, and berberine's TPSA values fall within an acceptable range, suggesting that it may possess favorable pharmacokinetic properties. The molecular volume (M. V.) of a compound can significantly impact its behavior during transportation, particularly concerning intestinal absorption. Smaller molecules tend to have enhanced permeability, allowing them to enter cells more rapidly. Berberine's strong M. V., as indicated in table 2, suggests its potential to efficiently traverse biological membranes. In addition to the aforementioned attributes, the lipid-soluble nature of berberine is a noteworthy characteristic, enabling it to readily cross cell membranes. This property is critical for its effectiveness as a drug candidate, as it influences its distribution and access to intracellular targets. To further assess Berberine's overall drug-likeness, we employed cheminformatics tools available on the web. These analyses were based on the quantification of hydrogen bond acceptors, donors, M. V., and log P, all of which play pivotal roles in determining a

molecule's suitability as a drug candidate. In the scientific context, these findings underscore the potential of berberine as a valuable candidate for drug development. Its molecular attributes, such as its favorable drug-likeness score, appropriate TPSA values, and robust M. V., suggest its potential as a drug with good bioavailability and cellular permeability. Furthermore, its lipophilic nature enhances its ability to cross cell membranes efficiently, making it a promising compound for pharmaceutical research.

Table 1: Berberine predicted carcinogenicity and ADMET solubility amounts: [[4] high solubility, [3] and [2] moderate solubility, [1] less solubility, [0] low solubility]

Properties	Predicted values
BBB level	0.57
HIA	0.74
HOA	0.514
CYP 3A4	Inhibitor (0.54)
CYP 2D6	Inhibitor (0.70)
PPB %	100 (0.851)
Solubility level	-2.94
Ames mutagenesis	Non-mutagen
Mouse Carcinogen	Negative
Rat Carcinogen	Positive
Carcinogenicity	Non-Carcinogen (0.97)
HERG inhibitor	Non-Inhibitor (0.72)
TA100-NA	Positive

Table 2: Molecular weight, Molecular formula, number of hydrogen bond acceptors (NHBA) and donors (NHBD), MolLogP, MolPSA (molecular polar surface area), MolVol (molecular volume), and drug-likeness of berberine

Ligand	Molecular formula	Mol weight (>500)	NHBA (>10)	NHBD (>5)	Log P (>5)	MolPSA	MolVol	DLS
Berberine	$C_{20}H_{18}NO_4$	336.12	4	0	4.39	33.45	332.12	0.77

Analysis of network, enrichment, and gene expression

The network analysis conducted has unveiled a complex web of interactions and pathways that illuminate role of berberine in the process of wound healing. Huang *et al.* in their recent publication, reported the network pharmacology-based apprach and identified some key molecualr targets for berberine [47]. Molecular docking study also reported in their work [47]. Berberine, a naturally occurring isoquinoline alkaloid, has been gaining attention for its potential therapeutic properties, including wound healing. This network analysis provides valuable insights into the molecular mechanisms through which berberine exerts its effects. Gene set enrichment analysis revealed the influence of protein expressions on fifteen distinct pathways. Notably, wound healing is a highly orchestrated process involving various molecular pathways, and the identification of these pathways is crucial for understanding the mechanistic actions of berberine. One critical pathway that emerged as highly influenced is the Epidermal Growth Factor receptor tyrosine kinase domain interaction pathway. This pathway plays a central role in cell proliferation, migration, and differentiation, processes integral to wound repair. It's worth noting that berberine's ability to modulate these pathways underscores its potential in promoting tissue regeneration. A closer examination of protein interactions within these pathways revealed that berberine has a significant impact on several key protein molecules. For instance, it interacts with EGFR, which is known to play a pivotal role in wound healing by regulating cell growth and migration. Berberine's influence on ERBB and ERBB1 further strengthens its connection to the EGFR signaling pathway. Ligand-receptor interactions are vital for cellular communication and are integral to wound-healing processes. Berberine's modulation of these interactions, particularly with proteins like TP53 (p53), HER (Human Epidermal Growth Factor Receptor), and others, suggests that it affects multiple facets of wound healing, including cell cycle regulation and DNA repair. Furthermore, the high edge count associated with berberine's interactions indicates a broad range of effects on these pathways, reinforcing its potential in mediating wound healing processes. Berberine's influence on proteins like CDC25A, ATR (Ataxia Telangiectasia and Rad3-Related Protein), and CHEK1 (Checkpoint Kinase 1) underscores its impact on cell cycle regulation, which is crucial for proper tissue repair. In the scientific context, berberine's multifaceted modulation of these wound-healing pathways aligns with previous research on its therapeutic potential. Berberine has been shown to promote wound closure, enhance collagen deposition, and reduce inflammation, all key aspects of the wound healing process. While these computational findings provide valuable insights, it is essential to acknowledge that further experimental studies, including *in vitro* and *in vivo* investigations, are necessary to validate berberine's efficacy in wound healing. Additionally, understanding the precise mechanisms through which berberine exerts its effects on these proteins and pathways will be a pivotal next step. Table 3 presents the resulting data from the enrichment analysis of modulated proteins.

Table 3: Enrichment analysis of modulated proteins

Term descriptions	Gene count	Matching proteins
Epidermal Growth Factor receptor tyrosine kinase domain	6	EGFR, ERBB, MCM6, GMNN, ERBB1, TP53,HER
Ras signaling pathway	11	CSF1R, KIT, PIK3CB, PRKACA, MET, MAPK10, ABL1, PIK3CD, CDC42, IKBKB, NTRK1
PI3K-Akt signaling pathway	11	CDK4, CSF1R, KIT, PRKACA, MET, MAPK10, MAPKAPK2, MKNK1, CDC42, IKBKB, NTRK1,
cAMP signaling pathway	9	PIK3CB, CHRM1, PRKACA, ROCK2, ADORA2A, MAPK10, PIK3CD, ROCK1, GRIA1
Insulin resistance	6	RPS6KB1, PIK3CB, MAPK10, PTPN1, PIK3CD, IKBKB
Estrogen signaling pathway	4	PIK3CB, PRKACA, PGR, SRC
Gap junction	4	HTR2B, GRM5, PRKACA, SRC
HIF-1 signaling pathway	4	RPS6KB1, PIK3CB, MKNK1, PIK3CD
Calcium signaling pathway	5	HTR2B, GRM5, CHRM1, PRKACA, ADORA2A

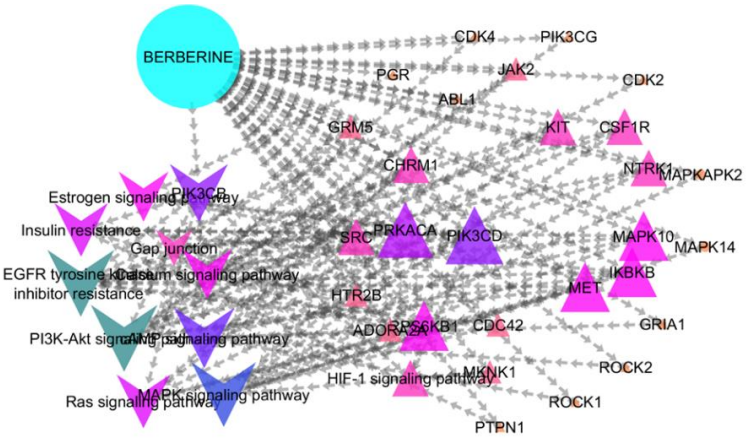

Fig. 1: Network representation between berberine, targets, and pathways interaction

Molecular docking analysis

In the pursuit of understanding the binding interactions between berberine and the chosen protein, molecular docking was executed to predict the most favorable conformational positions within the active regions of target protein. The wound healing potenatial of berberine is widely explored in previously reported literatures [48, 49]. The assessment of the resulting docked complexes was based on the consideration of lowest energy values (Kcal/mol) and the examination of bonding patterns, encompassing hydrogen, hydrophobic, and electrostatic interactions. Huang *et al.* in their recent publication, reported the docking study of berberine and found the promising binding energy of berberine against PDB Ids including 1O43, 1I7I, 1NHZ, 5JQH, 5WIU, 2BK4, 3QM4, and 2ILT [47]. Current work focus on the exploration of binsing affinity and interactions of berberine against EGFR. Berberine, the focal point of this investigation, exhibited a binding site with a notable binding affinity value of-8.14 kcal/mol. This value signifies the strength of attraction between berberine and the target protein. Further insight into the interaction is gleaned from the Glide energy calculation, which amounted to-61.28 kcal/mol, emphasizing the favorable energetics of this binding event. The intricate interplay between berberine and the target protein also revealed the formation of two hydrogen bonds with specific residues. These interactions are of great importance in molecular recognition and play a critical role in the stabilization of the complex. The residues Asp831 and Lys721 were identified as the partners in these hydrogen bond interactions. In parallel, the reference ligand, erlotinib, also underwent molecular docking, resulting in a binding affinity value of-7.15 kcal/mol. The comparative Glide energy calculation, which yielded a value of -55.09 kcal/mol, showcases the favorable nature of erlotinib's interaction with the target protein. Remarkably, similar to berberine, erlotinib established two hydrogen bond interactions with specific residues during the docking process. The residues Cys773 and Met769 were identified as the counterparts involved in these hydrogen bond interactions. Fig. 2 represents 2D and 3D binding interactions. These findings highlight the strong affinity of both berberine and erlotinib for the target protein, as substantiated by their favorable binding affinity values and Glide energy scores. The formation of hydrogen bonds further emphasizes the specificity and stability of these interactions, as they involve key residues in the active site of the protein. In a broader context, molecular docking is an essential tool in rational drug design and the exploration of potential therapeutic agents. It enables the assessment of ligand-protein interactions and aids in the prediction of binding affinities. The strong binding affinities observed in this study suggest that berberine and erlotinib may have significant potential in modulating the target protein's activity. However, it is essential to recognize that while molecular docking provides valuable insights into binding interactions, further *in vitro* and *in vivo* studies are needed to confirm the practical implications of these interactions in a biological context. Additionally, understanding the structural basis of these interactions can guide the development of novel compounds with enhanced binding affinity and specificity, potentially leading to the discovery of new therapeutic agents. This research underscores the importance of computational methods in drug discovery and the promising prospects for berberine and erlotinib in the context of the target protein.

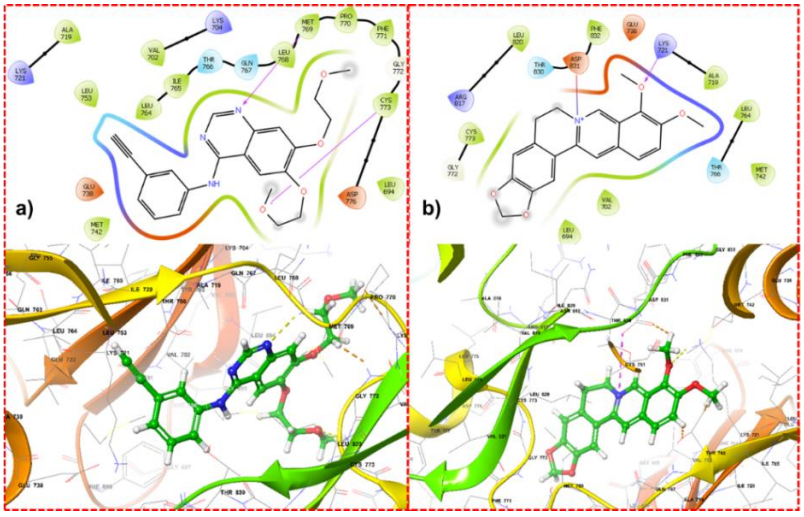

Fig. 2: Binding interaction of a) redocked cocrystal ligand and b) berberine with docked EGFR

Molecular dynamics simulation

The binding affinity and interactions between berberine and EGFR (PDB 1M17) were initially assessed through a molecular docking study. However, this approach provided a static view of the binding interactions, lacking the exploration of dynamic variations. MD simulation of the docked berberine-EGFR complex was conducted over a 100 ns timespan to address this limitation of the docking study. The resultant MD trajectory was subsequently subjected to statistical analysis and Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), evaluation of protein-ligand contacts, and examination of ligand properties in a dynamic environment was carried out. These parameters were utilized to ascertain the stability of the complex and to uncover any conformational changes occurring during the simulation. The RMSD analysis served as a valuable statistical parameter for assessing the stability of the berberine-EGFR complex over the 100 ns MD simulation. RMSD values were used to estimate the degree of deviation in the structure of berberine from the initial complex. Notably, the RMSD values for berberine exhibited fluctuations spanning from 0.3 Å to 2.4 Å. This dynamic behavior indicated a notable shift in the complex structure around the 45 ns mark as showed by a sharp scattering of RMSD values. This deviation from the initial structure may suggest a conformational change in the binding interaction between berberine and EGFR during that specific period of the simulation. In contrast, the RMSD analysis of the protein backbone displayed a more centric behavior with minimal scattering as shown in fig. 3a. This consistent RMSD pattern suggests that the overall conformation of the EGFR protein remained relatively stable and maintained its structural integrity during the entire 100 ns MD simulation. RMSF offered a dynamic perspective on the fluctuations within the complex. RMSF plot highlighted regions of high flexibility to conformational changes as shown in fig. 3b. The stability and flexibility of the berberine-EGFR complex were elucidated by considering the RMSD and RMSF results. Moreover, the analysis of protein-ligand contacts helped to reveal key interaction sites between berberine and EGFR. Examination of protein-ligand contacts emphasized specific interactions that persist or evolve during the simulation, providing a dynamic understanding of the binding. MET769 exerted strong binding with the formation of hydrogen contact with the docked ligand as indicated in fig. 3c. The MD simulation allowed for a comprehensive evaluation of the complex's behavior in a dynamic context. Ligand exerted a good profile for the radius of gyration and stable confirmation throughout the simulation as shown in fig. 3d. This dynamic information is crucial for understanding the molecular underpinnings of the complex and may guide further lead optimization efforts.

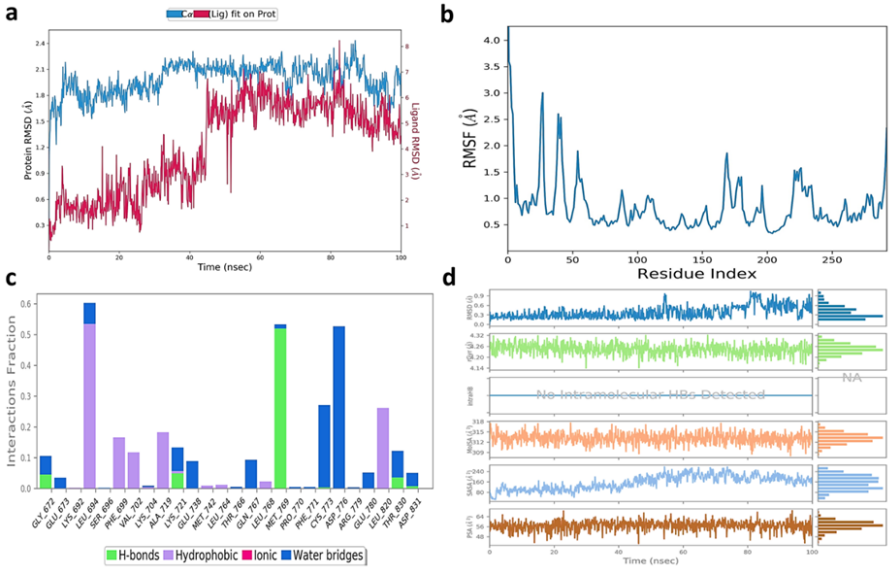

Fig. 3: a) RMSD, b) RMSF, c) protein-ligand contacts, and d) ligand properties estimated for simulated protein-ligand complex using 100 ns MD simulation

CONCLUSION

In the present work, the role of berberine as an adjuvant in the treatment of wound healing is analyzed through *in silico* gene expression, enrichment, and network analysis methods. The molecular docking results concluded that the plausible potential of berberine as a promising adjuvant in wound healing treatment and further, the plasma protein binding model predicts strong receptor binding, good absorption, a low BBB, and low toxicity. The current results must be further verified using carefully constructed wet lab techniques, which is the future focus of the project, as they are entirely based on database searches and knowledge-based computer simulations.

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Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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