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Research Article

Exploring the Potential of *Hemigraphis alternata* Leaves: Docking and Molecular Dynamics Analysis Targeting Cyclooxygenase-2 (COX-2)

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Abstract: Cyclooxygenase-2 (COX-2) mediates various physiological responses, including inflammation. In traditional medicine, the leaves of *Hemigraphis alternata* are commonly used to treat inflammation. The leaves have yielded 22 secondary metabolites that have been isolated. This investigation is designed to identify the compounds that exhibit the highest affinity for COX-2 through computational analysis. The main objective is to develop these compounds into anti-inflammatory agents. The molecular docking procedure using DOCK 6.9 and molecular dynamics simulations using GROMACS 5.1.2 were used to screen test compounds. DOCK 6.9 assessed binding affinity and ligand-receptor interactions, whereas GROMACS 5.1.2 examined the interactions, Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Molecular Mechanics-Poisson Boltzmann Surface Area (MM-PBSA), hydrogen bonds, and radius of gyration. The validation of the docking method resulted in an RMSD value of less than 2 Å, indicating that the docking protocols are suitable for screening potential ligands. *Phytol* exhibited the most attraction compared to the test and reference substances (*diclofenac sodium*), with a binding affinity of -32.85 kcal/mol to COX-2. The MM-PBSA analysis revealed that *phytol* exhibited a greater affinity to COX-2 than the reference, as evidenced by binding free energy of -36.259 kcal/mol. These data suggest that *phytol* has the potential to be investigated as a possible source for the generation of COX-2 inhibitors.

Keywords: affinity, docking, molecular dynamics, COX-2, *Hemigraphis alternata*

Introduction

The plant known as *Hemigraphis alternata* belongs to the family of plants known as Acanthaceae. Ivy metal leaf, purple waffle plant, cemetery plant, aluminium plant, red flame, and Java ivy are other names for this creeping herb [1]. It reaches 15 to 30 cm and is an adaptable and low-crawling perennial herb [2]. This plant has several active components, including terpenoids, phenols, tannins, flavonoids, coumarins, saponins, carboxylic acid, and cinnamic acid [3]. In folk medicine, the leaves of the *H. alternata* are used for a wide range of purposes, including treating wounds, ulcers, inflammation, diabetes mellitus and promoting urination [4]. There are 22 secondary metabolite compounds that have been successfully isolated from *H. alternata* leaves in water, ethyl acetate, and n-hexane extracts [5].

Cyclooxygenase type 2, also known as COX-2, is an enzyme responsible for catalysing the transformation of arachidonic acid into prostaglandins within the body. Cells involved in inflammation, such as macrophages, are responsible for the production of COX-2, which is then rapidly activated by inflammatory mediators, mitogens (tumour necrosis factor, TNF), and hormones (epidermal growth factor, EGF) [6]. Recent studies have shown that COX-2 mediates various physiological responses in the organism, such as reducing the risk of gastric ulcers [7], and providing antipyretic and analgesic reaction in conditions like osteoarthritis, rheumatoid arthritis, or acute pain [8], an effective adjuvant strategy for targeting cancer [9], epilepsy management [10], cardiovascular protection [11], and significant effects on psychiatric disorders [12].

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Computer-aided drug design (CADD) or in silico study reduces animal use in pharmacological research and helps rationally generate safe novel drugs [13]. This method can identify potential leads from many compound databases by narrowing the search for potential hits [14]. This method can produce rapid, high-throughput predictions based on compound structures. The two most common computational methodologies utilized in drug discovery are molecular docking and thermodynamic-based molecular dynamics (MD) simulation [15].

Molecular docking is a computational method in bioinformatics that involves simulating and predicting the interactions between molecules. Molecular docking is used to study the conformation and orientation of molecules in a target's binding site, also known as "pose". Searching algorithms and scoring functions are crucial components of docking applications. Search algorithms produce potential positions, which are evaluated based on scoring functions [16]. The conformational search method is utilized to locate the optimal location for the receptor and ligand to bond. How strongly the molecules are bonded is what the scoring function determines [17]. In virtual screening, molecular docking drastically enhances screening efficiency compared to the conventional screening approach. This method is promising for medicinal chemistry applications, especially structure-based rational drug design [18]. MD simulations are increasingly indispensable for drug discovery and investigating protein dynamics, functions, structures, and interactions. Integrating structural data from high-resolution approaches with MD simulations has improved our comprehension of disease processes in the body [19]. MD and docking approaches exhibit a robust and mutually beneficial interaction. These methods aid in determining the structural properties of various protein complexes, including protein-ligand, protein-peptide, protein-protein, protein-lipid, and protein-nucleic acid complexes [20].

The development of medicines that can inhibit COX2 becomes a critical concern. Nevertheless, such development necessitates a significant investment of both time and resources. In the earliest phases of drug development, CADD may be the optimal decision for the identification of potential pharmaceutical candidates. This work utilized molecular docking and MD analysis to determine the molecule with the strongest affinity for COX-2 out of the 22 compounds present in the leaves of *H. alternata*.

Materials and Methods

Materials

The following pieces of software were utilized in this investigation, DOCK 6.9, Chimera 1.13, Automated Topology Builder (ATB) (https://atb.uq.edu.au/), GROMACS 5.1.2, and Discovery Studio Visualizer 2020. The Protein Data Bank (http:///rcsb.org/pdb) obtained the three-dimensional (3D) structure of the COX-2 receptor. The PDB code for this receptor is 3LN1. In the meantime, the 3D forms of 22 different ligands and a reference chemical (*diclofenac sodium*) were obtained from PubChem (http://PubChem.ncbi.nlm.nih.gov).

Molecular Docking Study

Docking experiments were conducted utilizing DOCK 6.9 and a flexible ligand docking approach [21]. DOCK 6.9 explores the search space utilizing the anchor-and-grow methodology. The standard grid-based scoring system organizes and categorizes ligand conformations. It generated experimental-like poses in seven out of eight complexes, demonstrating robust sampling power [22] . It computes hierarchical databases with advanced functionalities such as torsion minimization [21]. The flexible ligand docking methodology used in DOCK 6.9 may enhance target accuracy [23].

The first stage in docking with the DOCK 6.9 program is protonating the ligand and receptor, then creating the receptor surface using Chimera. The DOCK 6.9 application generated spheres, sphere selectors, generated box, generated grid, and performed molecular docking [24]. The docking approach was confirmed by re-docking the native ligand to the receptor (self-docked) to the receptor. This procedure was performed to obtain a Root Mean Square Deviation (RMSD) of ≤ 2 Å [25]. Celecoxib is the native ligand bound to COX-2 (PDB code: 3LN1). Due to the significant number of scoring functions and crystallographic structures that come in various conformations, it is essential to do early docking method validation before virtual screening [26].

Following the completion of the validation step, 22 test ligands and the reference were docked against COX-2. The grid score value represents the result obtained from molecular docking conducted with the DOCK 6.9 program [27]. The ligand exhibiting the lowest grid score possesses the highest potency as it necessitates less

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energy to attach to the receptor [28]. Afterward, the molecular docking outcomes were represented using the Biovia Discovery Studio Visualizer [29].

Molecular Dynamics Simulation

This study employed MD simulations utilizing GROMACS 5.1.2. GROMACS is a widely used, free, and open-source software for biomolecular dynamics simulations, noted for its efficiency, accuracy, and comprehensive range of simulation options. The application provides two execution choices depending on the type of simulation to be performed [30]. The initial scenario concerns the simulation of an isolated biomolecule (apoprotein), whereas the subsequent case involves simulating the dynamics of a macromolecule interacting with a tiny molecule (protein-ligand) [31].

The MD simulation was executed through a series of sequential steps, generating receptor topologies using pdb2gmx, creating ligand topologies with ATB, forming boxes, solvation, adding ions, minimizing energy, equilibrating temperature, equilibrating pressure, and production. The parameters of MD results that were examined included RMSD, RMSF (Root Mean Square Fluctuation), MM-PBSA (Molecular Mechanics-Poisson Boltzmann Surface Area), hydrogen bonds and radius of gyration [32]. In this study, molecular dynamic simulations were run at 310 K, 1 atm pressure, a GROMOS96 54a7 force field, a triclinic box, and a simulation time of 20 ns.

A restriction of CADD is the intricacy of molecular dynamics. The method is computationally intensive and contingent upon the dimensions of the simulated system, with analysis durations varying from hundreds of nanoseconds to microseconds. The issue is in the restricted time frame, which is frequently insufficient for analyzing protein folding that might span from milliseconds to seconds. It may result in the "undersampling" of protein conformations. This computational approach can enhance medication development and transform clinical.

Result and Discussion

Molecular Docking Study

The process of molecular docking is a technique that uses an automated computer algorithm to make a prediction regarding the correct orientation of a ligand to a receptor when they are attached to each other to form a stable complex. Three fundamental characteristics are computed during the process. These characteristics include the conformational geometry, the score in terms of binding and free energy, and the ligand orientation in relation to the receptor. When carrying out a docking study, it is essential to select the searching algorithms and scoring functions with great care to ensure they are successfully validated for the specific ligand-protein system being investigated [33].

The searching algorithm and sampling method used in the docking process of 22 test compound and reference against COX-2 are genetic algorithm (GA) and anchor-and-grow, respectively. GA is one of the heuristics searching algorithms. The heuristic searching algorithm searches for binding areas for small-molecule compounds, which generally have a specific binding pocket so that the conformational space search range can be determined [34]. The anchor-and-grow is a Breadth First Search (BFS) algorithm utilized to sample the conformation of small molecules. It makes use of a scoring mechanism that is based on footprint similarity [17]. The capability of the anchor-and-grow search routine to recreate experimental poses is 16% higher than that of the hierarchical database (HDB) [21].

The validation results of the docking method produce an RMSD value of 0.8256 Å (Fig. 1). Molecular docking validation aims to compare the crystallography and experimental conformation of the native ligand-receptor complex. The native ligand that had been separated from the receptor was re-docking to validate the molecular docking method [35]. The conformation is stated to be getting better with a decrease in the RMSD value because the ligand position from the re-docking results is close to the ligand position from the crystallography result. The obtained RMSD value is < 2 Å, allowing the docking method to screen test ligands [36].

Molecular docking relies heavily on the receptor's flexibility. Three different types of docking approaches can be applied in docking simulations. These methodologies are rigid body docking, flexible ligand docking, and flexible docking, also commonly called soft docking [33]. The DOCK 6.9 software only supports rigid body

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docking and flexible ligand docking. This study used flexible ligand docking. Flexible ligand docking fixes the receptor's conformation yet allows the ligand to change within a range. Non-critical elements affected by this adjustment are bond angle and bond length alterations. This docking approach is frequently used to model docking between small molecules and macromolecules like proteins [18].

Hydrogen is added to the ligand to add a charge during the docking simulation session. Providing charges to the ligands is a crucial aspect. Considering that the ligand molecule is not neutral under physiological conditions, this can be accomplished by adding or withdrawing hydrogen atoms. Receptor production follows a similar pattern, intending to minimize interference with the active site by optimizing the amount of water molecules in the receptor crystal structure [33].

Docking simulations were conducted on 22 test compounds and compared with the COX-2 receptors. Molecular docking can be used to determine the binding relationship between ligands and receptors at both the molecular and atomic levels. Scientists in the field of drug development frequently examine ligand-receptor interactions to ascertain the binding affinity of a chemical molecule, the selectivity of a drug toward various targets, and the efficacy of drugs concerning a particular target. Various binding conformations can be generated by docking technologies to precisely locate the ligand at the predicted or designated binding site on the protein. To precisely measure interactions, scoring functions can transform geometries into energy values [37].

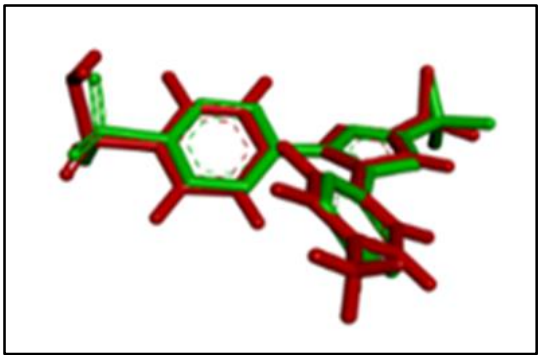

Figure 1. The conformation of *celecoxib* before (green) and after (red) re-docking to COX-2

Table 1. Results of molecular docking of test and reference compounds

Compound	Grid Score (kcal/mol)
Z-2-dodecenol	-21.13
Nerolidol	-21.53
5-hydroxy-1,2-dimethyl-4-nitro-1H-indole	-17.75
15-chloro-4-pentadecyne	-31.35
4-(2-methoxyphenyl)-piperidine	-14.24
Cyclobutanol	-9.95
2-propylmanonic acid	-16.63
1-hexadecyne	-20.42
2-hexylacrylonitrile	-16.63
Acrylonitrile β-[3-(2,2-dimethylcyclopropyl)-2,2-dimethylcyclopropyl	-16.30
10-undecyn-1-ol	-17.39
Phytol	-32.85
2,7-dioxa-tricyclo[4.4.0.0(3,8)]deca-4,9 diene	-10.41
15-chloro-4-pentadecyne	-20.63
2-methylenecholestan-3-ol	-26.67
L- Alanine	-12.84

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2,3-dihydro-2,5-dimethyl-5H-1,4-dioxepin	-14.08
Glycylsarcosine	-18.26
5-Hydroxymethylfurfural	-13.78
Levodopa	-18.79
(4abeta)-8abeta-methyldecalin-1,8-dione	-13.55
n-Hexadecanoic acid	-29.64
9,9-dimethoxybicyclo[3.3.1]nonane-2,4-dione	-16.47
Diclofenac sodium (reference)	-19.73

DOCK 6.9 utilizes grid score as its default scoring function. The grid score is computed by adding the total van der Waals and electrostatic energies. The Van der Waals grids were calculated using 6-9 Lennard-Jones exponents on an all-atom protein model. Coulomb's law and a distance-dependent dielectric, ε(r) = 4r, were used to calculate electrostatic grids [21]. According to the molecular docking study results, compound *phytol* had a lower grid score than the other compounds and *diclofenac sodium* (Table 1). Less binding energy is required for the ligand to attach to the target protein, indicating that the ligand can spontaneously bind to the target [38].

Phytol, a diterpene constituent of *chlorophyll*, has been demonstrated to possess a variety of pharmacological properties, particularly in the context of the treatment of agonizing inflammatory diseases. *Phytol* inhibited the release of proinflammatory cytokines in the synovial fluid and the production of Interleukin-6 (IL-6) and COX-2 immunocontent in the spinal cord in a pharmacological study that utilized a rat model of arthritis. *Phytol*'s primary mechanism of action involves the modulation of mediators that are essential for the development of arthritis pain, thereby attenuating inflammatory reactions in the spinal cord and joints [39]. At a dose of 5 mg/kg, *phytol* significantly reduced inflammation in Wistar rats with paw edema by 70.0% [40]. It attenuated the inflammatory response by inhibiting neutrophil migration, which was partially attributed to the reduced levels of TNF-α and IL-1β [41].

The interaction that occurs between *phytol* and COX-2 is the formation of hydrogen bonds in the OH group that binds to the amino acid aspartic acid 485 and two hydrophobic interactions in the carbon chain that binds to the amino acid isoleucine 484 and proline 460 (Fig. 2). Meanwhile, the bonds formed between *diclofenac sodium* and COX-2 are two electrostatic bonds on the amino acid glutamic acid 465 and lysine 478 and two hydrophobic interactions on alanine 475 (Fig. 2).

There are two main mechanisms following drug loading, chemical interactions and physical interactions. These interactions are mediated by several types of bonding, including hydrophobic interactions, electrostatic interactions, hydrogen bonds, Van der Waals interactions, and covalent bonds [42]. Hydrophobic interaction is a characteristic of nonpolar molecules (hydrophobic elements of amphiphiles) that form anhydrous domains in aqueous solutions [43]. Ions that have opposite electric charges, such as cations (positive charge) and anions (negative charge), are said to interact with one another through a phenomenon known as electrostatic interaction [42]. Hydrogen bonds serve an essential function in biological systems. The hydrogen bond is an electrostatic interaction that stabilizes two molecules. It occurs when a proton donor's partly positively charged hydrogen atom forms a connection with the lone pair of a strongly electronegative element in a proton acceptor molecule [44]. Van der Waals interaction is a non-specific and non-directional attraction that occurs owing to charge fluctuations generated by two or more atoms so close to each other that their outer electron clouds barely touch. The distance between the two parties has a significant role in this interaction [45]. A covalent bond forms when the electronegativity difference between two atoms is not significant enough to facilitate electron transfer, leading to ion production. Two elements share one or more pairs of electrons to form a covalent bond [46]. Two atomic orbitals overlap in the interaction [47].

Molecular Dynamics Simulation

Compounds *phytol* and *diclofenac sodium* were continued to the MD stage. MD results for 20 ns showed that there were four hydrophobic interactions between *phytol* and COX-2, namely at the amino acids leucine 65, lysine 64, tyrosine 108, and valine 74 (Fig. 2). These interactions are different from the interactions formed during molecular docking. The *diclofenac sodium* and COX-2 interaction after the MD simulation also

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differ from the docking results. The MD results include an electrostatic interaction with lysine 436, two hydrogen bonds with lysine 344 and tryptophan 85, and two hydrophobic interactions with lysine 344 and tryptophan 85 (Fig. 2).

MD simulations have been widely utilized to study the dynamic behavior of molecular systems and their surroundings throughout time. The simulations enable ligands, proteins, and water to exhibit flexibility within a virtual enclosure. It is utilized for examining the atomic-level mobility of biological molecules because of its high computational demands. Unlike biologically relevant conformational changes, these studies are limited to shorter time scales. Specific force fields regulate the simulated system's behavior according to molecular dynamics. A parameterized mathematical function that constitutes the force field is responsible for defining the system's potential energy [37].

In the RMSD graph of the compound *phytol*, the receptor begins to open, and the ligand starts to search for the binding site or the appropriate coordinate on the protein [48] at 1 ns, at which point the RMSD increases at 1.5-2.3 Å (Fig. 3). The increase in the RMSD value increases with the length of the simulation time. The RMSD value at 14 ns until the end of the RMSD value tends to be stable. This indicates that the maximal conformation of the protein bound to the ligand has begun to be attained and that the protein can maintain its position. Additionally, the interactions between residues tend to preserve the protein's structure. In the *diclofenac sodium* compound, the RMSD value increased significantly at some point (Fig. 3). An increase in the RMSD value, which is relatively high on the graph, indicates that achieving the maximal conformation of the protein bound to the ligand is challenging. It is challenging for the protein to maintain its position.

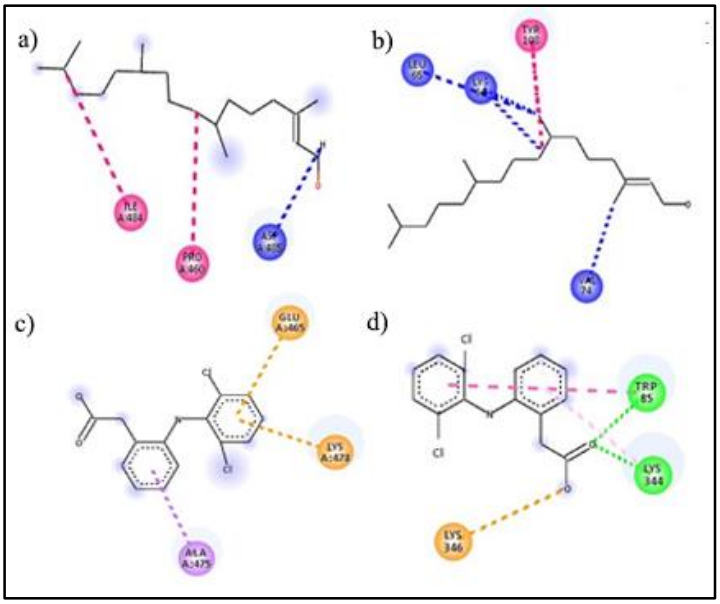

Figure 2. Visualization of the interaction of a) molecular docking results of *phytol* with COX-2; b) MD simulation results of *phytol* with COX-2; c) molecular docking results of *diclofenac sodium* with COX-2; and d) MD simulation results of *diclofenac sodium* with COX-2

The amino acids Pro25, Cys26, Glu31, Asp43, Cys44, Asn53, Asn72, and Thr70 exhibit a high degree of flexibility and instability in the MD simulation of complexes between *phytol* and COX-2 (Fig. 3). The high RMSF graph illustrates the conditions. In the *diclofenac sodium* complex with COX-2, residues Asn19, Cys22, Ser23, Asn24, Pro25, Gln27, Asn28, Thr35, Asp43, and Thr45 have high fluctuation values (Fig. 3). However, the residues that bind *phytol* and *diclofenac sodium* are stable and do not change significantly throughout the simulation [49].

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The MM-PBSA calculation approach is commonly employed to determine the binding free energy of the ligand-receptor system in MD simulations. The test compound exhibits a reduced binding energy compared to the reference compound, with values of -36.259 kcal/mol and 28.001 kcal/mol, respectively. It signifies that the test compound exhibits a higher receptor affinity than the reference substance. The MM-PBSA calculation is derived by summing the Van Der Waals energy, electrostatic energy, polar solvation energy, SASA energy, SAV energy, and WCA energy [50].

Hydrogen bonds appear more frequently during the MD simulation in the interaction of *phytol* with COX-2 than in *diclofenac sodium* with COX-2 (Fig. 4). However, at the end of the 20 ns simulation, *phytol* had no hydrogen bonds with COX-2, and there were two hydrogen bonds between *diclofenac sodium* and COX-2.

Analysis of the radius of gyration can be utilized to characterize the compactness and density of protein molecules. An increase in the value of the radius of gyration in a simulation indicates that the volume of the protein structure has increased geometrically. This increase in volume reflects the decreased density of protein molecules or the structure's diminished cohesion [49]. In the MD simulations for 20 ns, the ligand-receptor complex's gyration radius has reduced for both the test and reference compounds (Fig. 4). However, the structure volume of the reference compound is greater than that of the test compound until the end of the simulation. Therefore, the structure of the protein molecule in the test compound's MD simulation is more compact than that of the reference.

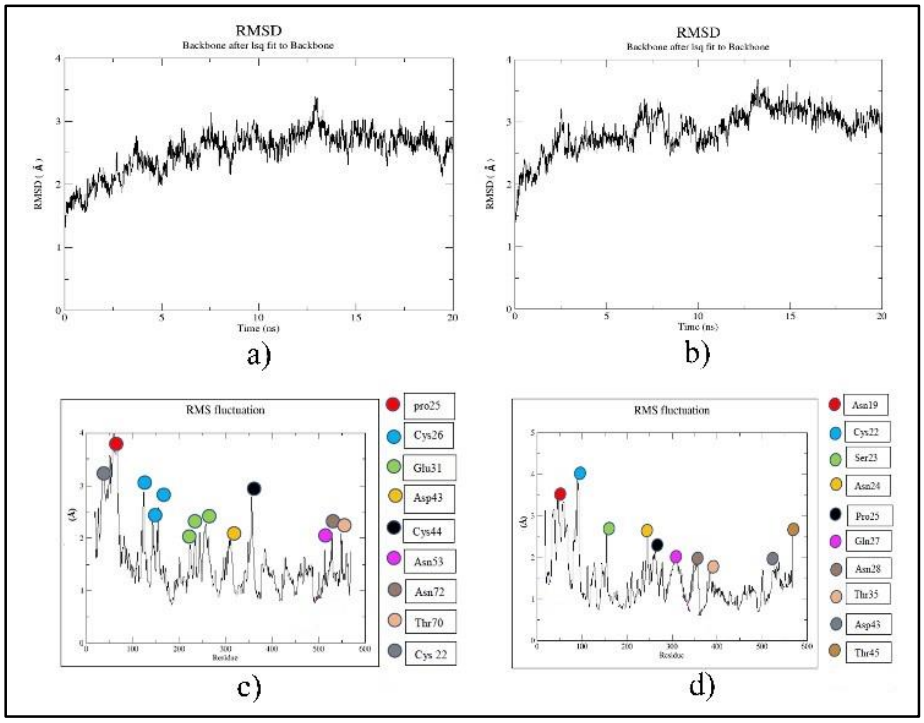

Figure 3. The curves of MD simulation result a) RMSD of *phytol* with COX-2; b) RMSD of *diclofenac sodium* with COX-2; c) RMSF of *phytol* with COX-2; d) RMSF of *diclofenac sodium* with COX-2

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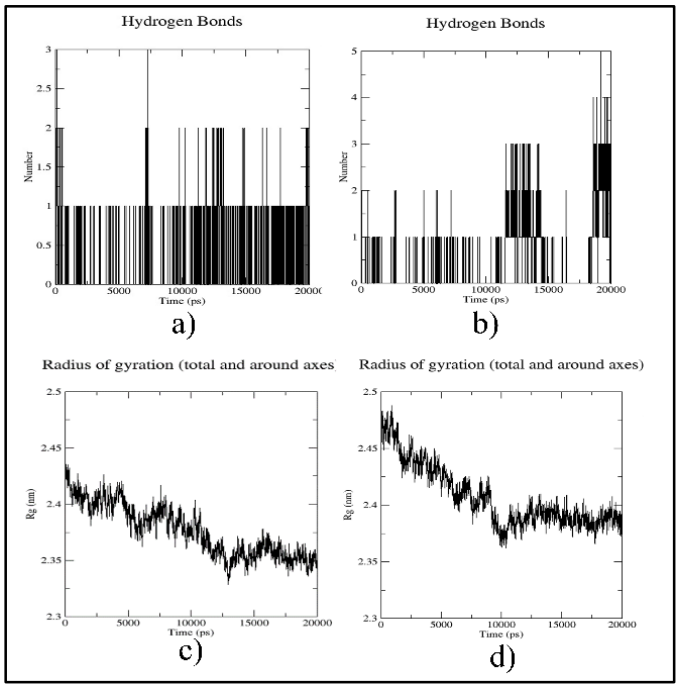

Figure 4. The graph of MD simulation result a) hydrogen bond of *phytol* with COX-2; b) hydrogen bond of diclofenac sodium with COX-2; c) radius of gyration of *phytol* with COX-2; d) radius og gyration of diclofenac sodium with COX-2

In the pharmaceutical and biotechnology sectors, *phytol* is a potential candidate for a variety of applications. It is a *phytanic acid* that has the potential to influence the development of pathophysiological conditions significantly. Numerous investigations have supported *phytol* as a drug candidate. A cohort of healthy rats was administered 100 mg/kg of *phytol* orally once daily for seven consecutive days in a one percent *carboxy methyl cellulose* suspension. The experiment revealed that all animals examined exhibited typical and healthful behaviour [51]. Phytol exhibited genotoxicity on *Allium cepa* at 2-16 mM, while it exhibited toxic and cytotoxic effects at 4-16 mM. However, the damage index and frequency in *Allium cepa* were reduced by *phytol* at 2 mM after 48 and 72 hours, suggesting a potential adaptive response or the ability to prevent DNA damage [52]. In Sprague-Dawley rats, severe pulmonary injury can result from an inhalation dose of *phytol* [53].

The correlation between *phytol* and its toxic and cytotoxic effects is contingent upon the dosage administered under specific experimental settings. Consequently, there remains significant potential for novel research focused on the application of phytol and its metabolites as therapeutic agents [54]. It is crucial to highlight that *phytol* is an insoluble molecule in water. It restricts its use and formulation for human consumption. Nanoencapsulation, liposome, and micelle technologies of essential oils enhance their physicochemical, pharmacokinetic, and pharmacodynamic properties for oral administration while also providing other biotechnological advantages, particularly in reducing toxic, cytotoxic, and genotoxic risks [52].

Conclusion

Phytol has the highest affinity out of all 22 chemicals found in the leaves of *H. alternata* for COX-2. The affinity of this compound is also better than its reference, *diclofenac sodium*. In the MM-PBSA results, *phytol* also showed a better affinity than *diclofenac sodium*. Therefore, *phytol* substances may be advanced into COX-2 inhibitors through additional studies involving in vitro, in vivo, and clinical testing.

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