Received: 15 July 2024 | Revised: 27 August 2024 | Accepted: 25 October 2024 | Published online: 1 November 2024

REVIEW

Medinformatics
2024, Vol. 00(00) 1–11
DOI: 10.47852/bonviewMEDIN42023842

In Silico Studies as Support for Natural Products Research

Fekade Beshah Tessema[1,2,*], Tilahun Belayneh Asfaw[3], Mesfin Getachew Tadesse[2,4], Yilma Hunde Gonfa[5] and Rakesh Kumar Bachheti[2,6]

[1]*Department of Chemistry, College of Natural and Computational Sciences, Woldia University, Ethiopia*

[2]*Department of Industrial Chemistry, College of Natural and Applied Sciences, Addis Ababa Science and Technology University, Ethiopia*

[3]*Department of Chemistry, College of Natural and Computational Sciences, Gondar University, Ethiopia*

[4]*Centre of Excellence in Biotechnology and Bioprocess, Addis Ababa Science and Technology University, Ethiopia*

[5]*Department of Chemistry, College of Natural and Computational Sciences, Ambo University, Ethiopia*

[6]*Department of Allied Sciences, Graphic Era Hill University, India*

Abstract: It can be argued that in silico studies do not receive enough attention despite being a key part of addressing the limitations of our laboratory facilities, the high cost of chemicals, and the equipment required for wet laboratory activities. Natural product studies are demanding higher costs of chemicals, reagents, and varied laboratory facilities. This becomes a serious limitation in getting data from natural product studies. In silico studies use chemical structures as inputs as well as software and online web servers to generate data to support, predict, and validate wet laboratory activities. Interaction studies use computational tools to calculate binding energies and other associated properties. Predictions are based on the structure-activity relationships derived from previously conducted preclinical and clinical studies. As a main component of in silico studies, the physicochemical and pharmacokinetic properties of small molecules can be determined using online web servers such as SwissADME and absorption, distribution, metabolism, excretion, and toxicity web servers. An interaction study uses molecular docking software such as AutoDock, AutoDock Vina, GOLD, and online servers such as SwissDock. Furthermore, the stabilities of complexes considered in interaction studies can be confirmed using molecular dynamics simulation software such as VMD. Prediction of activity spectra for substances (PASS) is widely used to predict biological activities for molecules based on multilevel neighborhoods of atom descriptors. In silico studies have played an important role in medicinal chemistry, pharmacology, and related research for screening, interaction studies, prediction, and other related purposes. Results of in silico predictions will not be far from wet lab activities as in most cases these studies consider previously attempted clinical and preclinical biological activities. Some examples are presented here to encourage the use of in silico studies.

Keywords: binding energy, in silico, molecular docking, natural products, PASS

1. Introduction

Chemistry can be done in the wet lab and on the computer based on the activities managed so far in the wet lab. The three broad categories of experiments are in vitro, in vivo, and in silico studies. In silico studies are those performed on a computer or via simulation on a computer. Such studies consider previously attempted activity results for predicting a certain activity using a computer. Short experimentation performed by computer is referred to as in silico [1]. In silico studies are more than two decades old in the scientific community. It is not that much used or not encouraged to be used here in Ethiopia.

From a given structure as an input, so many properties can be computed and predicted using a lot of alternative software and online servers. The input structure can be generated using dedicated softwares such as Chemdraw, Avogadro, Chemsketch and online servers such as PubChem, Chemspider, and others. Structures of macromolecules can also be retrieved from the Protein Data Bank (PDB) RSC website.

Natural products (NP) and their derivatives are directly or indirectly linked to several current traditional treatments for various ailments, which makes them appropriate platforms for the development of drug prototypes and novel drugs. In the field of drug development based on NPs, computational methods have become more often utilized globally. For example, a great deal of chemical, biological, pharmacological, toxicological, and

*Corresponding author: Fekade Beshah Tessema, Department of Chemistry, College of Natural and Computational Sciences, Woldia University and Department of Industrial Chemistry, College of Natural and Applied Sciences, Addis Ababa Science and Technology University, Ethiopia. Email: fekade.b@wldu.edu.et

structural NP data are available through the creation and application of NP databases [2].

NP studies involve the scientific investigation of substances produced by living organisms, such as plants, animals, and microorganisms. The key areas of focus in NP studies include the discovery and isolation of new NPs from various sources, chemical characterization to determine their chemical structure and investigation of biological activity for the NPs identified. The latter two key areas of focus can be supported by using in silico studies. The advent of in silico tools, which offer superior-quality predictions, has improved the standard of healthcare research [3].

Phytochemicals, which are complex, non-essential compounds present in plants, have a major positive impact on human health and well-being. More than 3000 phytochemicals have been found in nature thus far [4]. NPs are compounds found naturally in plants, animals, and microorganisms [5]. NPs exist in the living nature as a complex mixture which comprises many but in smaller amounts individually. This made the investigation of NPs mainly towards drug discovery laborious, environmentally unfriendly, and expensive due to the use of organic solvents and reagents. Longer time of investigation is also another limitation of the field of study. Studying the biosynthetic pathways for phytochemicals and obtaining complete profiles of crude extracts from modern methods like GC-MS and LC-MS will be enough to start in silico studies. This also supports the NP studies to alleviate the major limitations in the study area. Furthermore, pharmacological properties and physicochemical studies, molecular interaction studies, and predictions will enrich the data obtained from NP investigations. By sharing issues with numerous scientific domains, the study of NPs is interdisciplinary. A quick yet largely effective strategy must be used to combat the illness, such as in the case of the COVID-19 viral pandemic. Sampangi-Ramaiah, Vishwakarma, and Shaanker were among those who applied the strategy, which involved utilizing in silico studies to determine the best substitutes for some of the most widely used natural ingredients in our kitchens [6]. Moreover, these tools have been valuable for designing peptide mimetics, optimizing their structure to improve clinical outcomes, repurposing known therapeutics, studying potential drugs and identifying drug targets, as well as investigating the efficacy of NP components in suppressing COVID-19 infection [3]. Considering the wise use of our limited resources, we need to do in silico studies before running the costly wet lab activities. Screening tasks are highly expensive in the wet lab. It can be done at lower cost using developed softwares and online servers. The cost of reagent standards and facilities to run wet lab activities are now becoming the hardest part of chemical investigations. By using in silico studies, one can manage cost of investigation, lower environmental risks, plan wise directions of analysis [7], and above all get the advantages of it as a preliminary step of investigation. Author noticed the less interest in in silico activities. While considering investigation time, cost, and the use of inputs other than chemical products, in silico studies were found to be preferred as preliminary activities. This initiates to highlight the Why and How of in silico studies with examples from NPs investigation.

2. Components of in Silico Studies and Tools

2.1. Physicochemical and pharmacokinetic properties determination

These days, participation in multidisciplinary activities is required. Biological and pharmacological activities direct chemical research efforts in medicinal chemistry studies. Molecular modeling as a modern medicinal chemistry method has been applied increasingly often by the pharmaceutical business for the study of structure-activity relationships (SAR). Pharmacokinetic properties like absorption, distribution, metabolism, excretion, and toxicity (ADMET) and pharmacodynamics data (e.g., potency, affinity, efficacy, and selectivity) can be generated for medicinal chemistry research activities through the use of these approaches [8]. It is essential for pharmacological research to comprehend the mechanisms via which tiny compounds, or ligands, interact with macromolecules [9].

A substance's physicochemical properties include its aqueous solubility, ionization (pKa), lipophilicity, polar surface area, the number of hydrogen bond donors and acceptors, physical and chemical stability, molecular structure, and weight. These characteristics will affect the pharmacokinetics of NPs, how they interact with human bodies, and their ability to convert physiologically active molecules into medicines that are therapeutically effective [10]. The time-course study of drug absorption, distribution, metabolism, and excretion (ADME) is known as pharmacokinetics. By altering the quantities and rates of drug exposure to tissues, each of these four parameters has an impact on the pharmacologic activity and therapeutic efficacy of the molecule as a pharmaceutical. Toxicology screening and ADME profiling are two of the most important research projects in the drug development and discovery process. New chemical entities' "draggability" is based on their ADME and toxicologic (ADME/Tox) properties [11].

When developing new medications, it is critical to take ADME properties into account. This involves doing a drug-likeness analysis to help determine whether or not to interact with biological systems inhibitors [12]. Furthermore, inhibitors with poor ADME qualities and substantial adverse effects on biological systems are often the primary cause of the majority of failures medicines in the clinical phase of studies [13].

The Rule of Five, also referred to as Lipinski's Rule of Five, was established by Christopher A. Lipinski in 1997 as a general framework for assessing drug similarity and determining if an inhibitor with specific biological and pharmacological features would be an orally active medicine in humans [12]. According to the rule, a molecule or inhibitor meets the requirements for oral absorption and activity if two or more of these requirements – including molecular weight – are met. The criteria for drug-likeness are linked to intestinal permeability and aqueous solubility, which control the initial stage of bioavailability during oral administration [14]. The Egan egg graph, or brain or intestinal estimated permeation predictive model (BOILED-Egg), is a graphical representation of the substances' absorption in the gastrointestinal system and brain that is created using the SwissADME online web server [14]. In the yolk region (yellow), graph molecules are expected to passively flow through the blood-brain barrier. By considering six physicochemical properties – saturation, lipophilicity, polarity, size, solubility, and flexibility – the bioavailability radar offers a rapid evaluation of a molecule's drug-likeness [15]. With the same online web server, synthetic accessibilities for molecules can also be predicted.

2.2. Prediction of activity spectra for substances (PASS)

Organic substances display biological activity as a result of their interactions with a variety of biological entities. The biological object (species, sex, age, etc.), the exposure method (dosage, route of

administration), the properties of the biological assay, and the features of the substance all affect biological activity. The biological activity spectrum of an organic molecule is the variety of biological activities it displays in interactions with different biological entities. It represents the "intrinsic" quality of a compound that is only influenced by its structure [16].

The free online tool PASS Online (http://www.way2drug.com/passonline) can predict biological activity spectra of organic compounds for over 8000 different categories of biological activity with an average accuracy of above 95% using the structural formulas of organic compounds. Based on the study of the SARs in the training set – which contains details on the biological activity and structural makeup of more than 1.5 million organic molecules – the prediction was generated. Molecular structural formulas data either in the Mol or the SDF file format for a structure set are used by the PASS application. "Active" or "inactive" is how biological activity is qualitatively described in PASS [16].

Since this was all that was known when the research was first underway, the two-dimensional structural formula of an organic compound was chosen to characterize its molecular structure. The structure of a chemical molecule can be described by unique molecular descriptors known as multilevel neighborhoods of atoms (MNA) descriptors [17]. These descriptors were developed using methods for the "structure-property" relationship problem [18].

For evaluating biological activity profiles or spectra that describe the intricate interactions between chemical compounds and biological activities, methods based on ligand structure have become far more popular. The following conditions restrict the use of target structure-based approaches for the development of new drugs: the target macromolecule's three-dimensional structure must be known; the dynamics of changes in a protein's three-dimensional structure during function; it is challenging to identify the conformation that is biologically active for conformationally flexible ligands; and the choice of estimation functions is not always clear [19].

Additionally, Filimomov et al [19] included examples of how to prioritize chemical synthesis and biological activity of chemicals based on prediction findings and how to use PASS Online in practice. One of the upcoming trends being considered is the use of PASS Online as a platform for collaborative academic research efforts aimed at discovering and developing new pharmacological medications.

As shown on the help menu of PASS online, the biological activity types predicted by PASS include:

- The **primary pharmacological effects** include hepatoprotective, anti-inflammatory, and antihypertensive effects.
- **Mechanisms of action** include cyclooxygenase 1 inhibitor, adenosine uptake inhibitor, and 5-HT1A agonist.
- **Specific toxicities** include mutagenic, carcinogenic, and teratogenic effects.
- The **interaction with anti-targets** includes HERG channel blockers.
- **Metabolic activities** (such as CYP1A substrate, CYP3A4 inhibitor, CYP2C9 inducer, etc.);
- **Gene expression** influences (such as APOA1 expression enhancer, NOS2 expression inhibitor, etc.)
- **Transporter activity** (such as sodium/bile acid cotransporter inhibitor, dopamine transporter antagonist, etc.).

The major characteristics of PASS are summarized as shown in Table 1.

PASS is a commonly used tool by organic and medicinal chemists, pharmacologists, and others for biological testing and synthesis planning. According to the most recent PASS online usage statistics, in 2023 there are 46 web services, 50,532 users from 106 different countries, 1,648,227 predictions, and more than 3,000 articles (http://www.way2drug.com/passonline).

PASS Prediction is utilized in the SistematX Web Portal (http://sistematx.ufpb.br) to calculate physicochemical (drug- and lead-like) characteristics and biological activity profiles, as well as to generate and visualize 1H and 13C nuclear magnetic resonance spectra [2].

2.3. Molecular docking studies

2.3.1. Preparations of molecules for molecular docking

The receptor is available for download from the Protein Data Bank (rcsb.org/pdb/), and the ligand structure can be obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/), built using ChemDraw, or retrieved from the literature [20]. PROCHECK analysis on PDBsum should be used to verify the structural quality of the receptor proteins [21], PROCHECK online server [22], and QMEAN [23]. The AutoDock suite consists of a set of free and open-source tools for computationally docking small molecules and virtual receptor screening. It currently has a number of supplementary tools: A pre-made computational docking program called AutoDock Vina uses a simple scoring technique along with fast gradient optimization using conformational search. Using a fast-Lamarckian genetic algorithm (GA) search strategy with an empirical free energy force field, AutoDock is a computational docking tool. AutoDock Tools

Table 1. The major characteristics of PASS

Characteristics	Description
Training Set	Include 959,801 drugs, drug candidates, pharmacological, and toxic chemicals. Can be retrained with new data sets
Biological Activity	8,387 biological activities can be predicted as Active vs. Inactive. Predicts many, ideally, all known activities
Ease of access	User-friendly interface, one click to get prediction
Chemical Structure	Multilevel Neighborhoods of Atoms (MNA) descriptors [19] Uses only structural formulas (Structure) as input data
Mathematical Algorithm	The Bayesian methodology was chosen after a thorough assessment of several techniques [19];
Validation	Over the entire training set, the average prediction accuracy in LOO CV is approximately 95% [19]; Using major compounds from the MDDR database, robustness was demonstrated [17]

(ADT) is the graphical user interface for docking, analysis, and coordinate preparation. For docking and virtual screening to be successful, much attention must be paid to the precision of the coordinates utilized for receptors and ligands. The modified PDB file format known as PDBQT, which more simply represents the molecules, is used by both AutoDock and AutoDock Vina [24].

Programs that could be used to prepare and convert the various ligand files and macromolecule complex types are ADT, OpenBabel [25], and Biovia Discovery Studio [26]. The ADT edit menu is used to remove the water molecules from the receptor protein, change its overall structure, and add polar hydrogen atoms as required. Partial atomic charges for the ligand file will be computed using the Amber force field ff99SB, and minimization will be used. After being reduced the structure will be converted to PDB format [20].

Once the appropriate modifications have been done, we must carefully examine the coordinate files to ensure that the protonation state and charges – particularly any metals, if any – are compatible with the system. As ADT does not provide any charges for metal ions it would be crucial to add charges manually to the PDBQT file using a text editor. Receptor coordinate files that are added to the PDB often have a lot of problems that need to be fixed, confirming that the coordinate set has the appropriate biological unit, important residues, or loops and that structural waters and important cofactors are included [24].

Prior to doing molecular docking simulations, active site prediction is yet another stage of preparation. Often, docking ligands to the whole surface of a protein is computationally prohibitively expensive. The metaPocket method, accessible at http://metapocket.eml.org, is one of several computational techniques used to predict ligand-binding sites (Huang, 2009). An online service called PDBsum provides structural information about PDB entries [27]. It is also possible to get information about the active site residues in the receptor protein structure from PDBsum at: http://www.ebi.ac.uk/pdbsum.

Another extensive automated active site detection, docking, and scoring methodology for proteins with known structures is described by Singh, Biswas, and Jayaram. The protein's functional groups that line the cavities are scored according to their physicochemical characteristics by the active site finder, which locates all of the cavities in a protein [28].

2.3.2. Molecular docking simulations

The most widely used structure-based drug design (SBDD) methods for examining molecular identification events such as binding energetics, molecular interactions, and induced conformational changes are molecular docking, structure-based virtual screening, and molecular dynamics (MD) [29]. Molecular docking is a widely used technique in SBDD due to its high degree of precision in predicting the conformation of small-molecule ligands inside the proper target binding site [30]. After the development of the first algorithms in the 1980s, molecular docking emerged as a key instrument in the drug discovery process. It is simple to perform research on important molecular processes, such as ligand-binding modalities and the accompanying intermolecular interactions that preserve the stability of the ligand-receptor complex [31]. Molecular docking methods give docked compound rankings based on the binding affinity of ligand-receptor complexes through quantitative estimates of binding energetics. Molecular docking techniques use a cyclical procedure to evaluate the ligand conformation using specific scoring functions in order to discover the most likely binding conformations [32].

Two main types of approaches are used in molecular docking. One approach estimates energy profiles for docked conformers of ligand targets using computer simulations. In contrast, the second tactic makes use of a technique to ascertain whether the surfaces of the ligand and the target are complementary [33]. Conformational search strategies progressively alter the ligands' structural characteristics, including their torsional (dihedral), translational, and rotational degrees of freedom, using systematic and stochastic search techniques [34]. While stochastic search strategies perform the conformational search by randomly changing the ligands' structural properties, systematic search strategies promote tiny changes in the structural parameters, progressively changing the ligands' conformation. Docking methods are widely used and employ a variety of search methodologies, including genetic, fragment-based, Monte Carlo, and MD algorithms [33]. Depending on the purpose of docking simulations, there are several types of molecular docking processes that involve either flexible or stiff ligand/target combinations.

Stochastic search has been successfully handled by GA in molecular docking programs like AutoDock and Gold [35]. These applications are primarily utilized for high-throughput docking simulations [33]. By applying ideas from natural selection and evolution theory, the GA method lowers the high processing cost associated with stochastic techniques.

In molecular docking systems, binding energies of predicted ligand-receptor complexes are computed using scoring functions. As per Agarwal et al [36], the final anticipated binding-free energy (ΔGbind) is comprised of the following factors: torsional free energy (ΔGtor), dispersion and repulsion (ΔGvdw), hydrogen bond (ΔGhbond), desolvation (ΔGdesolv), electrostatic (ΔGelec), and unbound system's energy (ΔGunb). According to Folappe and Hubbard [22], the energy fluctuation arising from the development of the ligand-receptor complex is determined by the Gibbs free energy (ΔG) and the binding constant (Kd). The binding energy is anticipated from an analysis of the major physical-chemical processes, such as entropic effects, desolvation, and intermolecular interactions, that are involved in ligand-receptor binding [37]. Additional parameters to assess the quality and dependability of the 3D structural model [38] and the stability of the complex formed between the ligands and receptors [39] are root mean square deviation (RMSD) values. A dependable model and stable complexes are indicated by lower RMSD values [32, 35, 40].

2.3.3. Visualization of docking results

The analysis of binding conformations, the identification of significant intermolecular interactions, the characterization of potential binding sites, mechanistic studies, and the clarification of ligand-induced conformational changes are all made possible by structural descriptions of ligand-receptor complexes. Once a ligand-receptor complex has been identified, biological activity and structural information are linked [32]. Analyzing important factors, like the presence of certain intermolecular interactions, is made easier by visualizing the predicted ligand-receptor complexes. UCSF Chimera [41], VMD [42], Pymol [43], LigPlot + [44], and Biovia Discovery studio [26, 45] visualizers are some of the molecular modeling.

Molecular docking, an essential tool for contemporary research, can demonstrate a task's viability if it gets done prior to the experimental phase of any study. Molecular docking has revolutionized discoveries in a number of domains. One way to predict whether an enzyme will be activated or inhibited is to

observe how small chemicals, known as ligands, interact with protein targets, which may include enzymes. This kind of information could serve as a foundation for the development of sensible drugs [33].

A successful NP docking investigation should consider a number of crucial factors, including the following: (1) Workflow optimization and validation using cross-docking, redocking, and test sets from literature; (2) High-quality structural data on ligands and targets; (3) Biological test system with direct target-ligand interaction; (4) Scoring function evaluation and rescoring; and (5) Molecular filters to remove PAINS and select molecules with desired ADMET properties [46].

2.4. Molecular dynamic simulations

A theoretical method based on resolving Newton's equation of motion for an atomic system is called MD simulation. To gain a deeper understanding of the behavior and stability of receptor-ligand complexes, this kind of computation is highly helpful. Based on a high negative docking score, the optimal binding poses of complexes discovered through molecular docking will be chosen. To verify the stabilities of the complexes (receptor-ligand) with high negative score energies produced using molecular docking, MD simulation can be used [47].

An initial coordinate file with the ligand-receptor complex's atomic coordinates is needed for MD simulations. Among the programs available for MD simulations of biomolecules are AMBER, CHARMM, GROMACS, and NAMD. Tools like those included in GROMACS packages, Amber Tools, and VMD plug-ins can be used for trajectory analysis [48]. It is challenging to accurately identify, using computational approaches, the correct position of a ligand within a receptor binding. Predicting the binding mode using static methods like docking may be affected by phenomena like polarizability effects, induced fit, which is the adaptation of nearby residues to the ligand's presence as well as the presence of ions, cofactors, or water molecules. The combination of molecular docking and MD simulations helps to rectify these issues by giving a more realistic picture, even though it does not completely eliminate the ambiguity. Although MD simulations are expensive, new elements of the binding behavior of ligands can be reached with the use of high-throughput MD and technology advancements [49].

Future applications and potentials include the combined use of docking with ligand-based MD, binding-free energy approaches, artificial intelligence (AI), and statistical techniques. Based on the information at hand, docking can be used in conjunction with various in silico techniques to develop integrated workflows that yield better prediction performances. Additionally, other methods can be coupled to integrate docking (e.g., docking can be integrated with binding-free energy calculations and MD to improve the results of virtual screening). Likewise, many techniques can be applied at different phases of the screening procedure to improve docking projections. To identify the appropriate receptor, for example, MD and AI-based methods could be combined [50].

Currently, like when it was initially established, molecular docking is used to help rationalize ligand interaction with a target of interest and to carry out virtual screening activities based on the structure. In addition to these uses, it can be employed in target fishing and target profiling to find a range of targets that the ligands show high complementarity with; some of these targets may be accountable for unanticipated adverse drug reactions (off-targets prediction). Additionally, docking is being used to find ligands that bind to a variety of chosen targets of interest concurrently (poly-pharmacology) and to find new applications for substances that already have enhanced safety profiles (drug repositioning) (Figure 1) [50]. With potential uses in therapeutic repositioning and drug rescue, reverse docking is also a helpful method for investigating possible targets of small-molecule ligands [51].

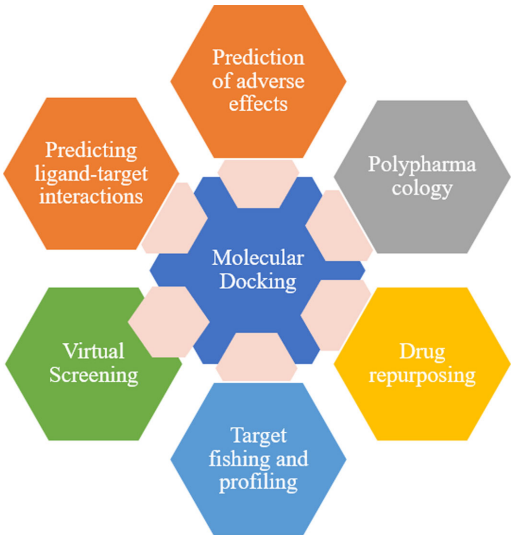

Figure 1. Main applications of molecular docking

3. Examples on What to Do

Molecular docking studies are the most frequent component of in silico studies. In silico molecular docking-related articles have been published much more often over the last 20 years [52, 53]. In the past few decades, molecular docking has been widely used in both academic and industry settings as a quick and low-cost procedure. The number of published papers is increasing at a rapid rate, and new approaches are constantly being developed [54]. Some of these and other components of in silico studies are reviewed as follows.

3.1. Prediction of biological activities

Application of dehydrocostus lactone (DHCL) as antineoplastic (breast cancer) predicted by PASS web server. A frequently used reference drug in hormonal therapy is tamoxifen, and DHCL has been used as a ligand. The selection of estrogen receptor alpha (ER-α) protein (PDB ID: 3ERT) was based on its function in the growth of breast cancer cells. The optimal conformational pose for the anticipated receptor-ligand complex to develop was determined by the application of molecular docking and DHCL was found to inhibit the ER-α protein. A binding interaction investigation demonstrates DHCL's appropriateness for hormone therapy in the treatment of breast cancer. The use of DHCL as a medication was validated using the human intestinal absorption test and the Lipinski rule of five for drug-likeness [55].

After the confirmation of the presence of flavonoids and phenolic acids using HPLC analysis, PASS online server was used to predict antioxidant and related activities for the flower methanol extract of Dodonaea angustifolia. Out of all the polyphenols that were found and quantified, the results of the biological activity prediction indicated that the identified

flavonoids had higher antioxidant activities [56]. The spectrum can be used for proposing mechanism of action for molecules towards a certain inhibitor. It also helps for developing procedure for wet lab activities

3.2. Screening major responsible components of an extract/EO for a certain bio/pharmacological activity

A study was conducted to analyze the pharmacokinetics data of NPs using the in silico method in order to identify the most effective drugs [57]. Cai et al. presented a pharmacology-based method to identify NPs with potential anti-cancer properties that can affect the immune microenvironment. This network-based strategy provides a valuable tool for identifying leads for tumor immunotherapy. It could be a useful and complementary approach to expedite and improve the efficiency of finding promising candidates for anti-tumor immunotherapy. Moreover, it may have applications in other complex diseases when combined with thorough experimental validation [58].

NPs with polypharmacological characteristics have demonstrated potential as innovative treatments for various complex illnesses, including cancer. Currently, there is a lack of understanding regarding the interactions between compounds and their targets, and it is impractical to experimentally examine every potential interaction. Recent advancements in systems pharmacology and computational (in silico) methods have provided powerful tools for analyzing the polypharmacological profiles of NPs [59].

AutoDock 4.2.6 software was used for the in silico molecular docking analysis which is performed to screen out natural anti-inflammatory compounds among 412 lichen compounds, Chain A of cyclooxygenase-2 complexes (PDB ID: 5IKQ) was used for anti-inflammatory activity. The components of the lichen were identified as ligands. ADMET and drug-likeness analysis follow the prediction. Six of the highest-scoring compounds were then subjected to molecular dynamics simulation (MDS) in order to evaluate conformational changes and fluctuations during protein-ligand interaction, as well as the stability of the docked protein-ligand complex. After 30 ns of MDS, the results of RMSD, Rg, and interaction energy showed that these Lichen compounds were quite stable in the Cox-2 active site pocket when compared to the reference [60]. Binding energies, RMSD and Ki values, interaction type, and distance are taken into consideration in order to determine the most likely responsible component for the treatment goal and observed activity.

Similar to this, Auto Dock Vina 1.2 was used to virtually screen the key components. The molecular docking target for Dm AChE (PDB ID: 6XYY) was used to compare the insecticidal activity [61] of the essential oil components from the aerial part of Chrysanthemum parthenium, with Malathion and Pirimiphos-methyl acting as references. In a study by Alshahrani and colleagues, biogenic compounds were screened using an in silico approach against the MCM7 protein in order to identify potential MCM7 inhibitors [62].

3.3. Helps to confirm results from wet labs

An in silico analysis was used to evaluate A. integrifolia's antihypertensive properties [63]. Flavonoids identified by HPLC analysis, iridoid glycosides identified from A. integrifolia in the course of the investigation, and those obtained from synonyms (A. remota and A. bractosa) were taken into consideration in the molecular docking study. AutoDock Vina in PyRx 0.8 was used to study interactions. Using internet servers, the estimated binding energies and activities, such as vasoprotection and drug-likeness features, were predicted. LigPlot v.1.4.5 was used for the 2D visualization of the docking results, while Biovia Discovery Studio was used for the interactive 3D visualization. In comparison to enalapril (reference drug: -5.9 kcal/mol), the binding energies of all 13 candidates evaluated in this study range from -10.2 kcal/mol to -7.5 kcal/mol. For the most part, hydrogen bonds are formed by the binding energies. In comparison with iridoid glycosides, flavonoids were discovered to have higher substantial binding affinities. Flavonoids have a higher probability of biological activity than iridoid glycosides, according to PASS test biological activity prediction. The majority of candidate compounds' Drug-likeness characteristics indicated that there were few exceptions to the Lipinski rule of five.

The traditional use of the aerial part of the concerned medicinal plant is supported by lower binding energies involving hydrogen bonding and predicted impacts on hypertension. Rutin, myricetin, quercetin, and kaempferol are the primary flavonoids that give A. integrifolia its antihypertensive properties. The investigated iridoid glycosides exhibit essentially equal effects on their antihypertensive activity, although being superior to the reference drug [63].

Traditional Chinese Medicine (TCM) was computationally screened for biogenic compounds against the OmpU protein in order to find potential OmpU inhibitors to treat cholera [64]. Potential anti-shigellosis action was investigated as the aerial portion of A. integrifolia was traditionally used and shown to be active against Shigella spp. Because of its part in the growth of dangerous microorganisms, VcDHO was chosen as a target for medication. Both the traditional use and the wet lab results confirmed based on the result of the in silico study [65]. For the more mature models (such as those based on pharmacological therapies), their use in supplementing clinical trials can be considered [66]. A key part of the verification process involves validating the model by comparing its predictions with real-world data obtained in in vitro or in vivo. The accuracy and agreement of this comparison are combined into a single scale based on the level of risk that could result from incorrect decisions and undesirable outcomes when using the model. Specifically, the acceptable difference between computational results and experimental data ranges from less than 20% when the model carries a low risk, to less than 5% when the risk associated with the model is high [66].

3.4. Others

In silico assays' pivotal role in the global fight against COVID-19 and some of their applications in biology and medicine are among the recent reported activities [3]. After noting the promising action of structurally related anthraquinone compounds against biofilm-forming marine bacteria, Pereet and colleagues set out to investigate the anti-fouling potential of other anthraquinone-related molecules. From the COCONUT NPs database, 2194 chemicals were examined [67]. Six distinct pharmacophore features were identified in the study, which may aid future research in locating, evaluating, and creating anthraquinone structures from the extensive compound library or in the synthetic synthesis of those that may function independently or in concert with other additives for anti-fouling targets.

Eruca sativa extract's in silico ADME/Tox analysis reveals that the phytochemical compounds found using HR-LC/MS in the plant

have a notable pharmacokinetic and safety profile. It also demonstrates that E. sativa's crude ethanolic extract is a potential medication option for the management and treatment of a variety of illnesses as well as for therapeutic uses [68].

Using the Schrödinger suite 2021-4, in silico studies were conducted to examine the binding mechanisms of four major analogues: benzothiophene, heteroaromatic chalcones, marine sesquiterpene, and sesquiterpene lactone – against ER-α targeting for breast cancer. The ADMET screening was managed by the QikProp module, the ligand-binding energy was determined by the Prime MM-GB/SA module, and the molecular docking studies were completed using the Glide module. Comparing benzothiophene analogue BT_ER_15f (G-score −15.922 Kcal/mol) to the standard medication tamoxifen (Docking score −13.560 Kcal/mol), the former exhibited the greatest binding activity against the target protein [69].

TCM database was screened using a docking-based approach to find potential IL-1β inhibitors that target the IL-1β/IL-1R interface and have ideal pharmacological properties. In order to extract the candidate compounds, the docking-based screening was primarily carried out by picking the important residues of the IL-1β interface. Subsequently, the compounds were reduced further based on their binding scores and significant interactions with the important residues of IL-1β [70].

A database containing 3128 phytocomponents from 268 medicinal plants that are listed in the Russian Pharmacopoeia has been produced by Ionov and his colleagues. The PASS software was utilized to estimate the biological activity profiles and physicochemical features of the compounds, adding to the existing information about them [71].

An in silico activity prediction and docking study of various flavanol derivatives as anti-prostate cancer drugs was based on Monte Carlo optimization. The study was conducted to investigate the mechanistic interpretation of natural flavanols docked into the active site of Human Cytochrome P450 CYP17A1 (PDB: 3RUK) [72].

Evaluating the relationship between possible inhibitors and their viral targets, creating new preventive and therapeutic drugs, evaluating previously unidentified molecular structures, and classifying COVID-19-related data using AI and deep learning techniques have all benefited greatly from the use of in silico approaches [57]. Aghajani and colleagues reported on the possibility of designing novel inhibitor drugs without resistance through the use of molecular docking and molecular dynamic simulations [73].

According to a recent study, certain FDA-approved medications have anti-CHIKV effect that may be further used to combat CHIKV. A prioritization process can be used to determine which medications have the greatest potential for in vivo validation studies in small animals and subsequent trials aimed at repurposing them against both DenV and ChikV [74]. A summary of interventions of in silico studies towards NP studies is shown in Figure 2.

4. Advantages and Limitations of in Silico Studies

Ethics approval or specialist equipment are usually not needed for in silico investigations since they do not involve the use of human subjects, animal models, or cell culture. A well-established medication's suitability for a novel condition can be promptly determined by molecular docking studies, which can also be used to investigate possible off-target interactions and their possible negative effects. All you need is a computer and the required software to easily and affordably execute analyses. In comparison with other research methods, results are obtained rather quickly.

Limitations of in silico studies

Wet lab tests are still necessary to validate the interactions between tiny molecules and proteins in the actual world. The results of molecular docking studies investigating a drug's possible effectiveness in treating a disease typically do not transfer well to use in humans, animals, or cells. Because docking approaches use basic scoring algorithms, they are not very accurate in estimating binding energies, despite their undeniable benefits. Typical molecular docking techniques are unable to reliably predict enantiomer pairings [75].

The reliability and relevance of computer-based models, such as quantitative SARs, depend greatly on quality assurance during both the model development and prediction stages. If we begin with highly reliable and relevant experimental input data, there is a high probability that non-test methods can produce useful results

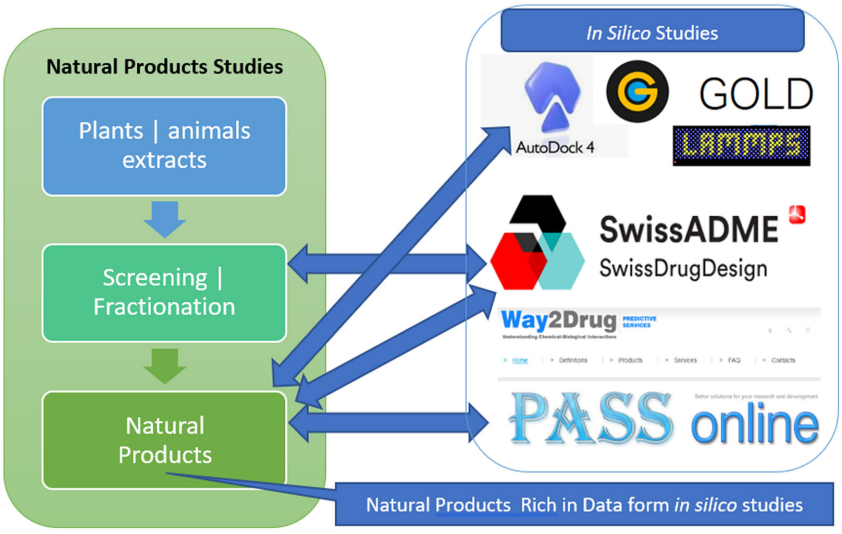

Figure 2. Summary of the uses of in silico studies in the natural product studies

and that the model's outputs will be acceptable to end-users, such as industry and regulators [76].

Despite the unanimous recognition of the added value of in silico models for drug development, including systems medicine/pharmacology models and clinical trial simulation tools, there is currently an unmet growing need for specific guidance documents related to these models [77]. Among the fewer attempts a scheme for model verification that can be applied across a variety of model types (e.g., structural alerts, QSAR, molecular interactions) has been developed by Hewitt et al. [78].

5. Conclusion

Considering the limitations of our lab facilities, it would be better to use in silico studies to plan, support as well as validate wet lab activities. In silico studies can support NPs studies through screening the most significant components and adding more important data for the NPs in concern. In silico activities are not as such difficult to use. Starting with simple structures as an input and uses user-friendly software's to generate useful data for the purpose aforementioned. In silico studies are found to be fast and effective and can be managed repeatedly with low cost. For further considerations, in silico studies can be integrated with computational studies for calculating chemical descriptors for a compound, energy minimization, and computing scoring functions. Programming skills are not mandatory for ordinary use, and if programming skills are added, a lot can be done towards solving the existing problems/limitations found while running in silico studies.

Ethical Statement

This study does not contain any studies with human or animal subjects performed by any of the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest to this work.

Data Availability Statement

The data that support this work are available upon reasonable request to the corresponding author.

Author Contribution Statement

Fekade Beshah Tessema: Conceptualization, Methodology, Writing – original draft, Visualization. **Tilahun Belayneh Asfaw:** Validation, Writing – review & editing. **Mesfin Getachew Tadesse:** Resources, Visualization, Supervision. **Yilma Hunde Gonfa:** Validation, Writing – review & editing. **Rakesh Kumar Bachheti:** Resources, Visualization, Supervision.

References

[1] Ekins, S., Mestres, J., & Testa, B. (2007). In silico pharmacology for drug discovery: Methods for virtual ligand screening and profiling. *British Journal of Pharmacology*, *152*(1), 9–20. https://doi.org/10.1038/sj.bjp.0707306

[2] Costa, R. P., Lucena, L. F., Silva, L. M. A., Zocolo, G. J., Herrera-Acevedo, C., Scotti, L., . . . , & Scotti, M. T. (2021). The SistematX web portal of natural products: An update. *Journal of Chemical Information and Modeling*, *61*(6), 2516–2522. https://doi.org/10.1021/acs.jcim.1c00083

[3] Moradi, M., Golmohammadi, R., Najafi, A., Moghaddam, M. M., Fasihi-Ramandi, M., & Mirnejad, R. (2022). A contemporary review on the important role of in silico approaches for managing different aspects of COVID-19 crisis. *Informatics in Medicine Unlocked*, *28*, 100862. https://doi.org/10.1016/j.imu.2022.100862

[4] Essa, M. M., Akbar, M., & Guillemin, G. (2016). *The benefits of natural products for neurodegenerative diseases*. Switzerland: Springer.

[5] Cordell, G. A., & Colvard, M. D. (2012). Natural products and traditional medicine: Turning on a paradigm. *Journal of Natural Products*, *75*(3), 514–525. https://doi.org/10.1021/np200803m

[6] Sampangi-Ramaiah, M. H., Vishwakarma, R., & Shaanker, R. U. (2020). Molecular docking analysis of selected natural products from plants for inhibition of SARS-CoV-2 main protease. *Current Science*, *118*(7), 1087–1092. https://www.jstor.org/stable/27226415

[7] Patra, J., Singh, D., Jain, S., & Mahindroo, N. (2021). Application of docking for lead optimization. In M. S. Coumar (Ed.), *Molecular docking for computer-aided drug design* (pp. 271–294). https://doi.org/10.1016/B978-0-12-822312-3.00012-6

[8] Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (2012). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, *64*, 4–17. https://doi.org/10.1016/j.addr.2012.09.019

[9] Valasani, K. R., Vangavaragu, J. R., Day, V. W., & Yan, S. S. (2014). Structure based design, synthesis, pharmacophore modeling, virtual screening, and molecular docking studies for identification of novel cyclophilin D inhibitors. *Journal of Chemical Information and Modeling*, *54*(3), 902–912. https://doi.org/10.1021/ci5000196

[10] Loftsson, T. (2015). Physicochemical properties and pharmacokinetics. In Loftsson, T. (Ed.), *Essential pharmacokinetics: A primer for pharmaceutical scientists* (pp. 85–104). https://doi.org/10.1016/B978-0-12-801411-0.00003-2

[11] Loftsson, T. (2015). Introduction. In Loftsson, T. (Ed.), *Essential pharmacokinetics: A primer for pharmaceutical scientists* (pp. 1–8). https://doi.org/10.1016/B978-0-12-801411-0.00001-9

[12] Attique, S. A., Hassan, M., Usman, M., Atif, R. M., Mahboob, S., Al-Ghanim, K. A., . . . , & Nawaz, M. Z. (2019). A molecular docking approach to evaluate the pharmacological properties of natural and synthetic treatment candidates for use against hypertension. *International Journal of Environmental Research and Public Health*, *16*(6), 923. https://doi.org/10.3390/ijerph16060923

[13] Abdullahi, M., & Adeniji, S. E. (2020). In-silico molecular docking and ADME/pharmacokinetic prediction studies of some novel carboxamide derivatives as anti-tubercular agents. *Chemistry Africa*, *3*(4), 989–1000. https://doi.org/10.1007/s42250-020-00162-3

[14] Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, *7*(1), 42717. https://doi.org/10.1038/srep42717

[15] Zoete, V., Daina, A., Bovigny, C., & Michielin, O. (2016). SwissSimilarity: A web tool for low to ultra high throughput ligand-based virtual screening. *Journal of Chemical Information and Modeling*, *56*(8), 1399–1404. https://doi.org/10.1021/acs.jcim.6b00174

[16] Singh, A., & Vellapandian, C. (2024). In silico and pharmacokinetic assessment of echinocystic acid effectiveness in Alzheimer's disease like pathology. *Future Science OA*, *10*(1). https://doi.org/10.2144/fsoa-2023-0150

[17] Lagunin, A., Stepanchikova, A., Filimonov, D., & Poroikov, V. (2000). PASS: Prediction of activity spectra for biologically active substances. *Bioinformatics*, *16*(8), 747–748. https://doi.org/10.1093/bioinformatics/16.8.747

[18] Abdou, W. M., Kamel, A. A., Khidre, R. E., Geronikaki, A., & Ekonomopoulou, M. T. (2012). Synthesis of 5-and 6-N-heterocyclic methylenebisphosphonate derivatives and evaluation of their cytogenetic activity in normal human lymphocyte cultures. *Chemical Biology & Drug Design*, *79*(5), 719–730. https://doi.org/10.1111/j.1747-0285.2012.01327.x

[19] Filimonov, D. A., Lagunin, A. A., Gloriozova, T. A., Rudik, A. V., Druzhilovskii, D. S., Pogodin, P. V., & Poroikov, V. V. (2014). Prediction of the biological activity spectra of organic compounds using the PASS online web resource. *Chemistry of Heterocyclic Compounds*, *50*, 444–457. https://doi.org/10.1007/s10593-014-1496-1

[20] Ahmad, I., Azminah, A., Mulia, K., Yanuar, A., & Munim, A. (2019). Angiotensin-converting enzyme inhibitory activity of polyphenolic compounds from Peperomia pellucida (L) Kunth: An in silico molecular docking study. *Journal of Applied Pharmaceutical Science*, *9*(8), 25–31. http://dx.doi.org/10.7324/JAPS.2019.90804.1

[21] Laskowski, R. A., & Thornton, J. M. (2022). PDBsum extras: SARS-Cov-2 and AlphaFold models. *Protein Science*, *31*(1), 283–289. https://doi.org/10.1002/pro.4238

[22] Rabbi, M. F., Akter, S. A., Hasan, M. J., & Amin, A. (2021). In silico characterization of a hypothetical protein from Shigella dysenteriae ATCC 12039 reveals a pathogenesis-related protein of the type-VI secretion system. *Bioinformatics and Biology Insights*, 15. https://doi.org/10.1177/11779322211011140

[23] Benkert, P., Biasini, M., & Schwede, T. (2011). Toward the estimation of the absolute quality of individual protein structure models. *Bioinformatics*, *27*(3), 343–350. https://doi.org/10.1093/bioinformatics/btq662

[24] Forli, S., Huey, R., Pique, M. E., Sanner, M. F., Goodsell, D. S., & Olson, A. J. (2016). Computational protein–ligand docking and virtual drug screening with the AutoDock suite. *Nature Protocols*, *11*(5), 905–919. https://doi.org/10.1038/nprot.2016.051

[25] O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. *Journal of Cheminformatics*, *3*, 1–14. https://doi.org/10.1186/1758-2946-3-33

[26] Raman, E. P., Paul, T. J., Hayes, R. L., & Brooks III, C. L. (2020). Automated, accurate, and scalable relative protein–ligand binding free-energy calculations using lambda dynamics. *Journal of Chemical Theory and Computation*, *16*(12), 7895–7914. https://doi.org/10.1021/acs.jctc.0c00830

[27] Laskowski, R. A., Jabłońska, J., Pravda, L., Vařeková, R. S., & Thornton, J. M. (2018). PDBsum: Structural summaries of PDB entries. *Protein Science*, *27*(1), 129–134. https://doi.org/10.1002/pro.3289

[28] Singh, T., Biswas, D., & Jayaram, B. (2011). AADS-An automated active site identification, docking, and scoring protocol for protein targets based on physicochemical descriptors. *Journal of Chemical Information and Modeling*, *51*(10), 2515–2527. https://doi.org/10.1021/ci200193z

[29] Batool, M., Ahmad, B., & Choi, S. (2019). A structure-based drug discovery paradigm. *International Journal of Molecular Sciences*, *20*(11), 2783. https://doi.org/10.3390/ijms20112783

[30] Meng, X. Y., Zhang, H. X., Mezei, M., & Cui, M. (2011). Molecular docking: A powerful approach for structure-based drug discovery. *Current Computer-Aided Drug Design*, *7*(2), 146–157. https://doi.org/10.2174/157340911795677602

[31] Huang, S. Y., & Zou, X. (2010). Advances and challenges in protein-ligand docking. *International Journal of Molecular Sciences*, *11*(8), 3016–3034. https://doi.org/10.3390/ijms11083016

[32] Ferreira, L. G., Dos Santos, R. N., Oliva, G., & Andricopulo, A. D. (2015). Molecular docking and structure-based drug design strategies. *Molecules*, *20*(7), 13384–13421. https://doi.org/10.3390/molecules200713384

[33] Agarwal, S., & Mehrotra, R. (2016). An overview of molecular genetics. *JSM Chemistry*, *4*(2), 1024. Retrieved from: https://www.jscimedcentral.com/public/assets/articles/chemistry-4-1024.pdf

[34] Agrafiotis, D. K., Gibbs, A. C., Zhu, F., Izrailev, S., & Martin, E. (2007). Conformational sampling of bioactive molecules: A comparative study. *Journal of Chemical Information and Modeling*, *47*(3), 1067–1086. https://doi.org/10.1021/ci6005454

[35] Krovat, E. M., Steindl, T., & Langer, T. (2005). Recent advances in docking and scoring. *Current Computer-Aided Drug Design*, *1*(1), 93–102. https://doi.org/10.2174/1573409052952314

[36] Agarwal, S., Chadha, D., & Mehrotra, R. (2015). Molecular modeling and spectroscopic studies of semustine binding with DNA and its comparison with lomustine–DNA adduct formation. *Journal of Biomolecular Structure and Dynamics*, *33*(8), 1653–1668. https://doi.org/10.1080/07391102.2014.968874

[37] Foloppe, N., & Hubbard, R. (2006). Towards predictive ligand design with free-energy based computational methods? *Current Medicinal Chemistry*, *13*(29), 3583–3608. https://doi.org/10.2174/092986706779026165

[38] Li, J., Fu, A., & Zhang, L. (2019). An overview of scoring functions used for protein–ligand interactions in molecular docking. *Interdisciplinary Sciences: Computational Life Sciences*, *11*, 320–328. https://doi.org/10.1007/s12539-019-00327-w

[39] Iman, M., Saadabadi, A., & Davood, A. (2015). Molecular docking analysis and molecular dynamics simulation study of ameltolide analogous as a sodium channel blocker. *Turkish Journal of Chemistry*, *39*(2), 306–316. https://doi.org/10.3906/kim-1402-37

[40] Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, *31*(2), 455–461. https://doi.org/10.1002/jcc.21334

[41] Pettersen, E. F., Goddard, T. D., Huang, C. C., Meng, E. C., Couch, G. S., Croll, T. I., . . . , & Ferrin, T. E. (2021). UCSF ChimeraX: Structure visualization for researchers, educators, and developers. *Protein Science*, *30*(1), 70–82. https://doi.org/10.1002/pro.3943

[42] Wu, C., Liu, S., Zhang, S., & Yang, Z. (2020). Molcontroller: A VMD graphical user interface featuring molecule manipulation. *Journal of Chemical Information and Modeling*, *60*(10), 5126–5131. https://doi.org/10.1021/acs.jcim.0c00754

[43] Lill, M. A., & Danielson, M. L. (2011). Computer-aided drug design platform using PyMOL. *Journal of Computer-Aided*

Molecular Design, *25*, 13–19. https://doi.org/10.1007/s10822-010-9395-8

[44] Laskowski, R. A., & Swindells, M. B. (2011). LigPlot+: Multiple ligand–protein interaction diagrams for drug discovery. *Journal of Chemical Information and Modeling*, *51*(10), 2778–2786. https://doi.org/10.1021/ci200227u

[45] Dassault Systèmes. (2021) Free download: BIOVIA discovery studio visualizer. Retrieved from: https://discover.3ds.com/discovery-studio-visualizer-download

[46] Temml, V., & Schuster, D. (2021). Molecular docking for natural product investigations: Pitfalls and ways to overcome them. In M. S. Coumar (Ed.), *Molecular docking for computer-aided drug design* (pp. 391–405). https://doi.org/10.1016/B978-0-12-822312-3.00027-8

[47] Saghiri, K., Daoud, I., Melkemi, N., & Mesli, F. (2022). QSAR study, molecular docking/dynamics simulations and ADME prediction of 2-phenyl-1H-indole derivatives as potential breast cancer inhibitors. *Biointerface Research in Applied Chemistry*, *13*(2), 154. https://doi.org/10.33263/BRIAC132.154

[48] Bitencourt-Ferreira, G., & de Azevedo, W. F. (2019). Molecular docking simulations with ArgusLab. In W. F. de Azevedo Jr. (Ed.), *Docking screens for drug discovery* (pp. 203–220). https://doi.org/10.1007/978-1-4939-9752-7_13

[49] Santos, L. H., Ferreira, R. S., & Caffarena, E. R. (2019). Integrating molecular docking and molecular dynamics simulations. In W. F. de Azevedo Jr. (Ed.), *Docking screens for drug discovery* (pp. 13–34). https://doi.org/10.1007/978-1-4939-9752-7_2

[50] Pinzi, L., & Rastelli, G. (2019). Molecular docking: Shifting paradigms in drug discovery. *International Journal of Molecular Sciences*, *20*(18), 4331. https://doi.org/10.3390/ijms20184331

[51] Kharkar, P. S., Warrier, S., & Gaud, R. S. (2014). Reverse docking: A powerful tool for drug repositioning and drug rescue. *Future Medicinal Chemistry*, *6*(3), 333–342. https://doi.org/10.4155/fmc.13.207

[52] Fan, J., Fu, A., & Zhang, L. (2019). Progress in molecular docking. *Quantitative Biology*, *7*, 83–89. https://doi.org/10.1007/s40484-019-0172-y

[53] Prieto-Martínez, F. D., Arciniega, M., & Medina-Franco, J. L. (2018). Molecular docking: Current advances and challenges. *TIP. Revista especializada en ciencias químico-biológicas*, *21*, 65–87. https://doi.org/10.22201/fesz.23958723e.2018.0.143

[54] Torres, P. H., Sodero, A. C., Jofily, P., & Silva-Jr, F. P. (2019). Key topics in molecular docking for drug design. *International Journal of Molecular Sciences*, *20*(18), 4574. https://doi.org/10.3390/ijms20184574

[55] Tessema, F. B., Gonfa, Y. H., Asfaw, T. B., Tadesse, M. G., Bachheti, A. J., Singab, A. N., & Bachheti, R. K. (2024). Dehydrocostus lactone from the root of Ajuga integrifolia (Buch.-Ham. Ex D. Don): Quantitative determination and in-silico study for anti-breast cancer activity. *Plant Science Today*, *11*(1), 34–44. https://doi.org/10.14719/pst.2344

[56] Tessema, F. B., Gonfa, Y. H., Asfaw, T. B., Tadesse, T. G., Tadesse, M. G., Bachheti, A., …, & Bachheti, R. K. (2023). Flavonoids and phenolic acids from aerial part of Ajuga integrifolia (Buch.-Ham. Ex D. Don): Anti-shigellosis activity and in silico molecular docking studies. *Molecules*, *28*(3), 1111. https://doi.org/10.3390/molecules28031111

[57] Rahman, M. M., Islam, M. R., Akash, S., Mim, S. A., Rahaman, M. S., Emran, T. B., …, & Wilairatana, P. (2022). In silico investigation and potential therapeutic approaches of natural products for COVID-19: Computer-aided drug design perspective. *Frontiers in Cellular and Infection Microbiology*, *12*, 929430. https://doi.org/10.3389/fcimb.2022.929430

[58] Cai, C., Wu, Q., Hong, H., He, L., Liu, Z., Gu, Y., …, & Fang, J. (2021). In silico identification of natural products from Traditional Chinese Medicine for cancer immunotherapy. *Scientific Reports*, *11*(1), 3332. https://doi.org/10.1038/s41598-021-82857-2

[59] Fang, J., Liu, C., Wang, Q., Lin, P., & Cheng, F. (2018). In silico polypharmacology of natural products. *Briefings in Bioinformatics*, *19*(6), 1153–1171. https://doi.org/10.1093/bib/bbx045

[60] Joshi, T., Sharma, P., Joshi, T., & Chandra, S. (2020). In silico screening of anti-inflammatory compounds from Lichen by targeting cyclooxygenase-2. *Journal of Biomolecular Structure and Dynamics*, *38*(12), 3544–3562. https://doi.org/10.1080/07391102.2019.1664328

[61] Tessema, F. B., Belachew, A. M., Gonfa, Y. H., Asfaw, T. B., Admassie, Z. G., Bachheti, A., …, & Tadesse, M. G. (2024). Efficacy of fumigant compounds from essential oil of feverfew (*Chrysanthemum parthenium L.*) against maize weevil (Sitophilus zeamais Mots.): Fumigant toxicity test and in-silico study. *Bulletin of the Chemical Society of Ethiopia*, *38*(2), 457–472. https://doi.org/10.4314/bcse.v38i2.13

[62] Alshahrani, M. Y., Alshahrani, K. M., Tasleem, M., Akeel, A., Almeleebia, T. M., Ahmad, I., …, & Saeed, M. (2021). Computational screening of natural compounds for identification of potential anti-cancer agents targeting MCM7 protein. *Molecules*, *26*(19), 5878. https://doi.org/10.3390/molecules26195878

[63] Tessema, F. B., Gonfa, Y. H., Asfaw, T. B., Tadesse, M. G., & Bachheti, R. K. (2024). In silico molecular docking approach to identify potential antihypertensive compounds from Ajuga integrifolia Buch.-Ham. Ex D. Don (Armagusa). *Advances and Applications in Bioinformatics and Chemistry*, *17*, 47–59. https://doi.org/10.2147/AABC.S392878

[64] Rahaman, A., Almalki, A. A., Rafeeq, M. M., Akhtar, O., Anjum, F., Mashraqi, M. M., …, & Jamal, Q. M. S. (2021). Identification of potent natural resource small molecule inhibitor to control Vibrio cholera by targeting its outer membrane protein U: An in silico approach. *Molecules*, *26*(21), 6517. https://doi.org/10.3390/molecules26216517

[65] Tessema, F. B., Gonfa, Y. H., Asfaw, T. B., Tadesse, M. G., Tadesse, T. G., Bachheti, A., …, & Bachheti, R. K. (2023). Targeted HPTLC profile, quantification of flavonoids and phenolic acids, and antimicrobial activity of Dodonaea angustifolia (Lf) leaves and flowers. *Molecules*, *28*(6), 2870. https://doi.org/10.3390/molecules28062870

[66] Petrini, L., Pennati, G., & Fotiadis, D. I. (2022). Editorial: Verification and validation of in silico models for biomedical implantable devices. *Frontiers in Medical Technology*, *4*, 856067. https://doi.org/10.3389/fmedt.2022.856067

[67] Preet, G., Astakala, R. V., Gomez-Banderas, J., Rajakulendran, J. E., Hasan, A. H., Ebel, R., & Jaspars, M. (2023). Virtual screening of a library of naturally occurring anthraquinones for potential anti-fouling agents. *Molecules*, *28*(3), 995. https://doi.org/10.3390/molecules28030995

[68] Awadelkareem, A. M., Al-Shammari, E., Elkhalifa, A. E. O., Adnan, M., Siddiqui, A. J., Snoussi, M., …, & Ashraf, S. A. (2022). Phytochemical and in silico ADME/Tox analysis of Eruca sativa extract with antioxidant, antibacterial and

anticancer potential against Caco-2 and HCT-116 colorectal carcinoma cell lines. *Molecules*, *27*(4), 1409. https://doi.org/10.3390/molecules27041409

[69] Rajagopal, K., Kalusalingam, A., Bharathidasan, A. R., Sivaprakash, A., Shanmugam, K., Sundaramoorthy, M., & Byran, G. (2023). In silico drug design of anti-breast cancer agents. *Molecules*, *28*(10), 4175. https://doi.org/10.3390/molecules28104175

[70] Liu, T. T., Chen, Y. K., Adil, M., Almehmadi, M., Alshabrmi, F. M., Allahyani, M., . . . , & Peng, Q. (2023). In Silico Identification of Natural Product-Based Inhibitors Targeting IL-1β/IL-1R Protein–Protein Interface. *Molecules*, *28*(13), 4885. https://doi.org/10.3390/molecules28134885

[71] Ionov, N., Druzhilovskiy, D., Filimonov, D., & Poroikov, V. (2023). Phyto4Health: Database of phytocomponents from russian pharmacopoeia plants. *Journal of Chemical Information and Modeling*, *63*(7), 1847–1851. https://doi.org/10.1021/acs.jcim.2c01567

[72] Tajiani, F., Ahmadi, S., Lotfi, S., Kumar, P., & Almasirad, A. (2023). In-silico activity prediction and docking studies of some flavonol derivatives as anti-prostate cancer agents based on Monte Carlo optimization. *BMC Chemistry*, *17*(1), 87. https://doi.org/10.1186/s13065-023-00999-y

[73] Aghajani, J., Farnia, P., Farnia, P., Ghanavi, J., & Velayati, A. A. (2022). Molecular dynamic simulations and molecular docking as a potential way for designed new inhibitor drug without resistance. *Tanaffos*, *21*(1), 1–14. https://www.tanaffosjournal.ir/article_254222.html

[74] Kasabe, B., Ahire, G., Patil, P., Punekar, M., Davuluri, K. S., Kakade, M., . . . , & Cherian, S. (2023). Drug repurposing approach against chikungunya virus: An in vitro and in silico study. *Frontiers in Cellular and Infection Microbiology*, *13*, 1132538. https://doi.org/10.3389/fcimb.2023.1132538

[75] Ramírez, D., & Caballero, J. (2016). Is it reliable to use common molecular docking methods for comparing the binding affinities of enantiomer pairs for their protein target?. *International Journal of Molecular Sciences*, *17*(4), 525. https://doi.org/10.3390/ijms17040525

[76] Nendza, M., Aldenberg, T., Benfenati, E., Benigni, R., Cronin, M. T. D., Escher, S., . . . , & Vermeire, T. (2010). Data quality assessment for in silico methods: A survey of approaches and needs. In M. Cronin, & J. Madden (Eds.), *In silico toxicology* (pp. 59–117). https://doi.org/10.1039/9781849732093-00059

[77] Musuamba, F. T., Bursi, R., Manolis, E., Karlsson, K., Kulesza, A., Courcelles, E., . . . , & Geris, L. (2020). Verifying and validating quantitative systems pharmacology and in silico models in drug development: Current needs, gaps, and challenges. *CPT: Pharmacometrics and Systems Pharmacology*, *9*(4), 195–197. https://doi.org/10.1002/psp4.12504

[78] Hewitt, M., Ellison, C. M., Cronin, M. T., Pastor, M., Steger-Hartmann, T., Munoz-Muriendas, J., . . . , & Madden, J. C. (2015). Ensuring confidence in predictions: A scheme to assess the scientific validity of in silico models. *Advanced Drug Delivery Reviews*, *86*, 101–111. https://doi.org/10.1016/j.addr.2015.03.005

How to Cite: Tessema, F. B., Asfaw, T. B., Tadesse, M. G., Gonfa, Y. H., & Bachheti, R. K. (2024). In Silico Studies as Support for Natural Products Research. *Medinformatics*. https://doi.org/10.47852/bonviewMEDIN42023842