Chapter 10

Computational screening of phytochemicals for anti-bacterial drug discovery

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List of abbreviations

ADMET	absorption, distribution, metabolism, excretion and toxicity
CADD	computer-aided drug designing
MD	molecular dynamics
PASS	prediction of activity spectra for substances
QSAR	quantitative structure-activity relationship

10.1 Introduction

Many plants have been recognized to have healing powers against human illnesses from ancient times due to their secondary metabolite content [1]. The study of phytochemicals from traditional medicine is becoming more popular in recent literature, and this is proving to be a great resource for modern medicine in identifying novel bioactive compounds with potential medical applications [2,3]. A lot of attention has been received in the last 10 years for the antibacterial activity of phytochemicals [1]. Phytochemicals can be defined as a vast collection of naturally occurring chemical substances that give plants their color, flavor, scent, and texture [1]. Over the years, these molecules have become crucial metabolites that help plants survive momentary or long-term dangers in their environment against free radicals, viruses, bacteria, and fungus along with controlling vital development and reproduction functions [3,4]. Plants produce a wide range of phytochemicals. Chemical structures, botanical sources, biosynthetic processes, and biological features can all be used to

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classify phytochemicals into several categories [5]. Chemical structures are used to classify the majority of phytochemicals [1,5]. Therefore, based on their chemical structure, phytochemicals can be divided into Alkaloids, sulfur-containing phytochemicals, terpenoids, and polyphenols. Depending on their varying chemical structures and molecular functions, the different classes of phytochemicals exhibit their numerous medicinal properties [6].

The extraordinary progress in computer science and its wide-ranging applications has facilitated research to many folds [7]. In silico approaches could be used to find the effects of unexplored phytochemicals by identifying their molecular targets with the help of an algorithm. The combination of chemoinformatics and bioinformatics, as well as systems biology techniques, aids the process of drug discovery. These methods combine the use of publicly available databases and various software tools to screen candidate compounds, recognize patterns, and analyze their binding interactions with their potential targets. The functions of omics tools, such as descriptor and pharmacophore development, molecular docking, biological activity prediction, and quantitative or qualitative structure-activity relationships (QSAR) modeling, are well known [8,9]. Therefore, these approaches can be used for computational screening of phytochemicals for anti-bacterial drug discovery.

Over the last few decades, there has been a considerable increase in the applications of computational approaches, artificial intelligence, and mathematical modeling in phytochemical research, particularly in plant metabolomics and screening plant metabolites, pharmacological and toxicological property prediction (virtual screening or in silico studies), chemical fingerprinting, chemical taxonomy, biosynthetic and phylogenetic research [7]. All these approaches help us to delineate the properties of phytochemicals. One such property is to act as an anti-bacterial agent. Research in recent years has revealed that phytochemicals exhibit anti-bacterial activity through various mechanisms, including the destruction of bacterial membranes, and suppression of virulence factors, such as enzyme and toxin inhibition, and bacterial biofilm formation [1]. The anti-bacterial activity can, therefore, be identified by computer-aided approaches, which can further lead to drug discovery.

10.2 Phytochemicals as anti-bacterial agents

Phytochemicals have shown potential in combating bacterial infections and overcoming the challenge of bacterial resistance development. Their anti-bacterial activities are linked to chemical influence on the function or biosynthesis of essential constituents, as well as overcoming anti-bacterial host defenses [10]. The various anti-bacterial properties of phytochemicals have been identified, including interference with bacterial cell wall biosynthesis and cell membrane disintegration, prevention of bacterial protein production, DNA replication and damage response, and cellular metabolism [11,12]. Phytochemicals may have anti-bacterial effects through differing mechanisms, depending upon their

classification, discussed in the previous section [13]. This section elaborates on the anti-bacterial properties of the different classes of phytochemicals.

10.2.1 Anti-bacterial activity of alkaloids

Alkaloids are a broad and structurally diverse category of plant-derived phytocompounds. Alkaloids rapidly establish hydrogen bonding with receptors, proteins, and enzymes since they have a basic nitrogen atom and also have one or more acidic amine hydrogen atoms [14]. Further, the presence of functional groups having proton-acceptors and donors such as phenolic hydroxyl and polycyclic groups indicates their remarkable functional properties [15]. Alkaloids show much potential as effective anti-bacterial drugs due to their broad antibacterial spectrum and their ability to act against drug-tolerant variants. Various studies, based on the mechanism of action of the anti-bacterial activity of alkaloids, suggest that they act by damaging the cytoplasmic membrane [16], impacting DNA activity [17], and restricting protein production [18] in bacterial cells. Isoquinoline alkaloids are a subclass of characteristic alkaloids that have been proposed to exhibit strong anti-bacterial action [19]. Sanguinarine and berberine are types of isoquinoline alkaloids possessing anti-bacterial properties. The mode of mechanism includes arresting the cell cycle process [14], disrupting the plasma membrane leading to leakage of its contents, and suppressing protein synthesis [20]. Various alkaloids like sanguinarine [19] and agelasines [14] are known to impair the cell wall and cell membrane of bacterial cells by affecting their permeability potential [14]. They do so by preventing the formation of the biofilm, which is a substance surrounding the bacteria proving to be beneficial against adverse conditions [21]. Further, alkaloids also affect the efflux pump system, which is an integral part of the bacterial plasma membrane [20]. These pumps enable the bacterium to show resistance to anti-bacterial agents, preventing their entry into the cell [19].

Therefore, through the various mechanisms and pathways mentioned previously, alkaloids hold a lot of promise to be studied further as potential anti-bacterial drugs, and their modes of action specifically can be explored and identified by applying computational screening methodologies.

10.2.2 Anti-bacterial activity of sulfur-containing phytochemicals

Sulfur-containing phytochemicals or organosulfur phytochemicals are another class of phytochemicals; they are organic compounds with at least one carbon-sulfur bond present. Some of the organosulfur phytochemicals found are thiosulfinates, glucosinolates, allicin, isothiocyanate, and many others [1]. Organosulfur phytochemicals have exhibited anti-bacterial activity against both Gram-negative and -positive bacteria since 1965 [22]. Glucosinolates, a subclass of organosulfur compounds, have been identified to act against bacteria by modifying cellular protein structures, leading to the suppression of their

growth [23]. They have also been found to damage the bacterial plasma membrane structure leading to the outflow of constituents of the cell and causing a decrease in the enzymatic activity [24]. Isothiocyanates, the smaller components of glucosinolates, have been found to exhibit bactericidal activity by blocking the activity of the enzyme urease in bacterial cells [1]. Further, they have been found to suppress the growth of bacterium species like *Staphylococcus aureus* [25]. Thiosulfinates have been identified to possess bacteriostatic properties [24]. Allicin, a thiosulfinate extracted from garlic [26], is well known for its anti-bacterial properties, including the total obstruction of RNA production and a substantial inhibition of DNA and protein synthesis [27].

Organosulfur compounds have been discovered to reduce biofilm formation and cause their destruction [28], preventing them from successfully protecting the bacterial cell. Lastly, these compounds may also cause suppression of genes which leads to bacterial mobility, flagellum formation, respiratory, and various biomolecule synthesis pathways [29].

10.2.3 Anti-bacterial activity of terpenoids

Terpenoids are the largest class of phytochemicals that are derived from mevalonic acid and characteristically have more than one isoprene unit present in their structure. They have been identified to exhibit compelling action against various bacterial strains [30]. The general mechanism of terpenoids against bacteria is due to the presence of an aromatic structure with a polar functional group. The anti-bacterial action of terpenes depends on their chemical and structural properties [31]. Terpenoids such as thymol and carvacrol possess hydroxyl groups that enable them to be involved in hydrogen bonding with enzymatic active sites, preventing their standard functionality [30]. Due to their highly reactive nature, the hydroxyl groups function as proton exchangers with the membrane of bacterial cells, allowing the terpenoid molecules to be attached to the lipid bilayer of the plasma membrane, altering its conformational functional properties [32,33]. Artemisinin, a sesquiterpene phytochemical drug, has shown effective anti-bacterial activity against various types of bacteria like facultative, aerobic, and anaerobic bacteria [30]. Andrographolide, a diterpene lactone, also exhibited anti-bacterial properties in combination with azithromycin drug, and it acted by preventing the formation of biofilm [32].

10.2.4 Anti-bacterial activity of polyphenols

Polyphenols have shown great potential as anti-bacterial agents due to their ability to inhibit various pathogenic mechanisms adopted by bacteria [34]. One of the anti-bacterial mechanisms of action adopted by polyphenols is the obstruction of biosynthesis of nucleic acids, which in turn hinders the formation of DNA and RNA within the bacterial cell [35]. Another mechanism identified is that the polyphenols assimilate on the surface of the bacterial cell

wall and exhibit bactericidal activity through various pathways, including the production of hydrogen peroxide by oxidative polyphenols [36]. They also act in a similar manner to destroy the plasma membrane by altering its properties [37]. A significant property of polyphenols that has been identified is their ability to cause bacterial cell aggregation [37]. This clumping of cells can lead to improper or insufficient functioning. Due to the accumulation of the cells, the bacteria are unable to receive an adequate amount of oxygen, which disturbs the respiratory chain within the cells [37]. Moreover, the reduced area may also lead to the insufficient uptake of essential nutrients, providing another explanation for the cause of inhibition of nucleic acid synthesis. Like the other phytochemicals, polyphenols interfere with biofilm formation and quorum signaling by preventing the association of signal molecules with their receptors in bacterial cells [37]. Additionally, they have also been found to block bacterial cells from attaching to the host substratum [35].

Therefore, it is evident that all classes of phytochemicals have innumerable anti-bacterial attributes. A few prominent examples of phytochemicals and their anti-bacterial mechanisms of action have been highlighted in Table 10.1. However, to determine their exact functionality and extent of use as potential antibacterial drugs, it is essential to study their structure-activity relations, stability of their complex with receptors, interactions with specific biological targets, and other characteristics. It is possible to do so by utilizing various computational screening methodologies that can aid in drug discovery.

10.3 High-performance computational drug discovery

The exponential advancement in technology has brought forward its innumerable advantages and applications in the field of drug design and discovery. Computational approaches such as different facets of artificial intelligence like predictive analysis and deep neural networking and mathematical programming models can be implemented in different aspects of research in phytochemicals [7]. These can aid in screening various phytochemical databases to identify potential drug candidates, analyze and study their structure-activity relationships, and discover their properties associated with toxicity and therapeutic properties, among many others.

Drug development in the pharmaceutical sector can be a very tedious and costly process wherein there is still uncertainty about whether the final drug will be approved. The virtual screening of potential phytochemicals as drugs may bring forward more promising candidates, which may further be taken into consideration for experimental screening and can significantly increase efficiency and help cut down costs. Sarker and Nahar [7] defined the term Computational Phytochemistry as a field where various computational tools, methods, and models are incorporated into the analysis and study of phytochemicals to discover valuable plant metabolites and study compounds exhibiting bioactive properties [7]. Therefore integrating the process of computer-aided drug design

TABLE 10.1 Phytochemicals, their sources, and anti-bacterial mechanism(s) of action.

S/N	Phytochemical class	Phytochemical name	Sources	Anti-bacterial mechanism(s) of action	Targets	Reference
1	Alkaloids	Sanguinarine	Roots of *Macleaya cordata* and *Macleaya microcarpa*	Destroys the integrity of the cell membrane by reductions in intracellular ATP concentration, pH, and cell membrane potential, as well as severe changes in cellular shape. Arrests cell cycle and inhibits protein synthesis	*E. coli*, *S. epidermidis*, *Streptococcus pyogenes*, *K. pneumoniae*, *Acinetobacter baumannii*, *S. aureus*, and *B. subtilis*	[10,38,39]
2	Alkaloids	Berberine	Roots, barks, and stems of *Coptidisrhizome* and Barberry plants	Blocks the activity of the bacterial division protein FtsZ. Arrests cell cycle and inhibits protein synthesis	*Aeromonas hydrophila*, *Bifidobacterium adolescentis*, *Edwardsiella ictaluri*, *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, and *Vibrio vulnificus*	[10,40]
3	Alkaloids	Ajmalicine	Hairy roots of *Catharanthus roseus*	Targets the MurA enzyme in bacterial species, which is responsible for the peptidoglycan formation; and the enzyme shikimate dehydrogenase, a key enzyme in amino acid synthesis	Multi-drug-resistant *Acinetobacter baumannii* and *Escherichia coli*	[41,42]

4	Alkaloids	Tomatidine	Stems, roots, leaves, and whole-plant fractions of tomato plant	Hydroxyl groups hinder microorganisms, and these groups can interact with bacteria's cell membranes, destroying membrane composition and causing the loss of cellular components	*E. coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Listeria ivanovii*	[43]
5	Sulfur-containing phytochemicals	Iso-thiocyanates	Present in cruciferous vegetables	Prevents biofilm formation, quorum signaling, inhibits urease enzyme, toxin production, and destroys bacterial cell membrane	*S. mutans*, *L. casei*, *S. aureus*, *E. faecalis*, *A. actinomycetemcomitans*, *F. nucleatum*, *P. nigrescens*, *C. perfringens*, *C. albicans*, *E. coli*, *E. faecalis*, *L. monocytogenes*	[1,44,45]
6	Sulfur-containing phytochemicals	Allicin	Garlic (*Allium sativum* L.)	Inhibits bacterial enzymatic activity by reacting with their functional groups, inhibiting toxin formation, quorum signaling and alters membrane permeability	Species of *Escherichia*, *Staphylococcus*, *Salmonella*, and methicillin-resistant *Staphylococcus aureus*	[28,46,47]
7	Sulfur-containing phytochemicals	Glucosinolates	Brassicaceae, Capparidaceae, Moringaceae, and Resedaceae families	Hydrolysis products of glucosinolates cause bacterial growth inhibition, plasma membrane destruction, and	*S. aureus*, *E. faecalis*, and *S. saprophyticus*	[24,48]

Continued

TABLE 10.1 Phytochemicals, their sources, and anti-bacterial mechanism(s) of action—cont'd

S/N	Phytochemical class	Phytochemical name	Sources	Anti-bacterial mechanism(s) of action	Targets	Reference
				reduction in enzyme activity		
8	Terpenoids	Thymol	Thymus, Oregano, Satureja, Coridithymus, Thymbra and Lippia	Causes bacterial membrane breakdown, bacterial lysis, and intracellular content release, culminating in death. Inhibition of efflux pumps, bacterial motility, and membrane ATPases, prevention of formation and disruption of preformed biofilms	*E. coli* O157:H7, *S. typhimurium*, *L. monocytogenes*	[1,49]
9	Terpenoids	Carvacrol	Found in *Origanum vulgare*, *Thymus vulgaris* and wild bergamot	Effects on the structural and functional features of the cytoplasmic membrane. Inhibits enzymatic activity within bacterial cells	*E. coli* and *L. monocytogenes*	[30,50–52]

10	Terpenoids	Limonin	*Citrus grandis*, residues after juice extraction of *Citrus reticulata* and peels, and residues after juice extraction of *Citrus hystrix*	Exhibits bacteriostatic effect, and inhibits bacterial cell communication and biofilm formation	*Escherichia coli, Staphylococcus aureus, Bacillus thuringiensis, Salmonella,* and *Shigella* spp.	[53,54]
11	Polyphenols	Galangin	Found in honey, *Alpinia officinarum, Helichrysum aureonitens* and in propolis	Directly inhibits the activity of β-lactamase and exhibits intrinsic anti-bacterial activity	*K. pneumoniae*	[1,55,56]

with traditional experimental approaches can prove to be extremely valuable and productive in the efficient process of drug design and development. Even though the diverse properties of phytochemicals have been known for ages, in-depth research on their biological effects and their efficacy as drugs for various diseases has only recently begun.

This section focuses on the various tools and technologies currently available that can be utilized to identify and study the anti-bacterial potential of phytochemicals. These methods combine the use of publicly available datasets and various software applications to evaluate candidate compounds, recognize patterns, and analyze their interaction affinity with their likely targets. The various computational screening methods discussed in this section are enlisted in Table 10.2, along with current tools that can be utilized to conduct them.

Further, the outline of the process of computational screening of phytochemicals for anti-bacterial drug discovery has been represented in Fig. 10.1.

10.3.1 Phytochemical database: Analysis and validation

The first step in the computational screening of anti-bacterial drug discovery would be data mining. Data mining involves analyzing vast amounts of data to find correlations and develop links to solve issues through various computer-based analytical tools. For computational phytochemical screening, an integrative and comprehensive strategy for data processing is required, beginning with the identification of plant products and ending with the separation and determination of potential drug candidates. Several publicly accessible databases have accumulated and presented data on various plants and their products, which may be helpful from a medicinal point of view. These databases can be utilized to retrieve information about phytochemicals whose structural and functional properties may lead to their use as anti-bacterial agents. This section discusses the various phytochemical-based databases present, which can be useful.

NAturalPRoducts ALERT (NAPRALERT) is a highly regarded natural product relational database that includes information on the biochemical, pharmacological, and medicinal properties of extracts from in vitro, in vivo, in situ, and humans and clinical studies [7]. It contains data on the medicinal properties of more than 200,000 plant species. Indian medicinal plants, phytochemistry, and therapeutics (IMPPAT) database is another electronic database containing information on more than 9000 phytochemicals along with their two- and three-dimensional chemical structures. Further, via computational tools, IMPPAT has incorporated the ADMET properties of the phytochemicals, enabling users to determine their structural and functional properties using the IMPPAT database [73]. Another database commonly used to retrieve information regarding plants and their products is Dr. Duke's Phytochemical and Ethnobotanical Database, which enables the convenient search for specific plants, chemicals, bioactivity, and ethnobotany using their scientific or common names [74]. Medicinal plant

TABLE 10.2 Computational screening methods used for drug discovery from phytochemicals.

S/N	Computational screening method	Application	Software/ Tools	References
1	Database screening	To identify the potential phytochemicals from plants known to have anti-bacterial properties	ebDB GRIN TCM-ID Flora Europea HIT	[7]
2	1D-QSAR	1D structure of the ligand is used to correlate their affinity with the ligand's properties such as lipophilicity and solubility	AutoQSAR	[57,58]
3	2D-QSAR	Determines correlation between the physicochemical properties of the atoms and functional groups present in a ligand with its bioactivity	QSAR-Co	[57,59]
4	3D-QSAR	Determines the correlation based on the 3D spatial arrangement of atoms in a ligand molecule with its biological activity caused due to various interactions such as electrostatic and steric	HASL AutoGPA	[60–62]
5	4D-QSAR	Compares the ligands' bioactivity with its conformational flexibility, rotational bonds and its various configurations	LQTAgrid	[63,64]
6	Molecular docking	Predicts interaction between the phytochemical ligand and bacterial target molecule	AutoDOCK MOE-Dock Glide MCDock Surflex	[65,66]

Continued

TABLE 10.2 Computational screening methods used for drug discovery from phytochemicals—cont'd

S/N	Computational screening method	Application	Software/ Tools	References
7	Molecular dynamics	Analyses the movement of atoms in a ligand's molecular structure to predict its involvement in biomolecular processes	AMBER CHARMM GROMACS Enlighten2 YASARA	[67–71]
8	ADMET profiling	Identification of ADMET properties to determine the potential of phytochemicals as anti-bacterial drugs	VolSurf QikProp MetaDrug PK-Sim DDDPlus	[72]

database for drug designing (MPD3) is a web-based tool that can provide data on numerous phytochemicals from various plants having medicine-like properties [75]. The proposed database can be particularly valuable for computer-aided drug discovery (CADD), as it is simple to use and efficient.

Similarly, there are various other plant databases listed in Table 10.2, which can be utilized to discover and acquire information regarding phytochemicals having properties, which may make them useful in anti-bacterial drug discovery.

10.3.2 Structure-based virtual screening

Once the phytochemicals showing evidence of anti-bacterial properties are identified through data mining, it is essential to evaluate their interaction and affinity toward the target molecules, which will enable their anti-bacterial mechanism of action.

Virtual screening is an effective in silico approach in the drug development process. The presence of a 3D model of the protein of interest is a requirement for performing virtual *screening* [76]. Virtual screening is frequently used in the research of new pharmaceuticals, and it has significantly led to the production

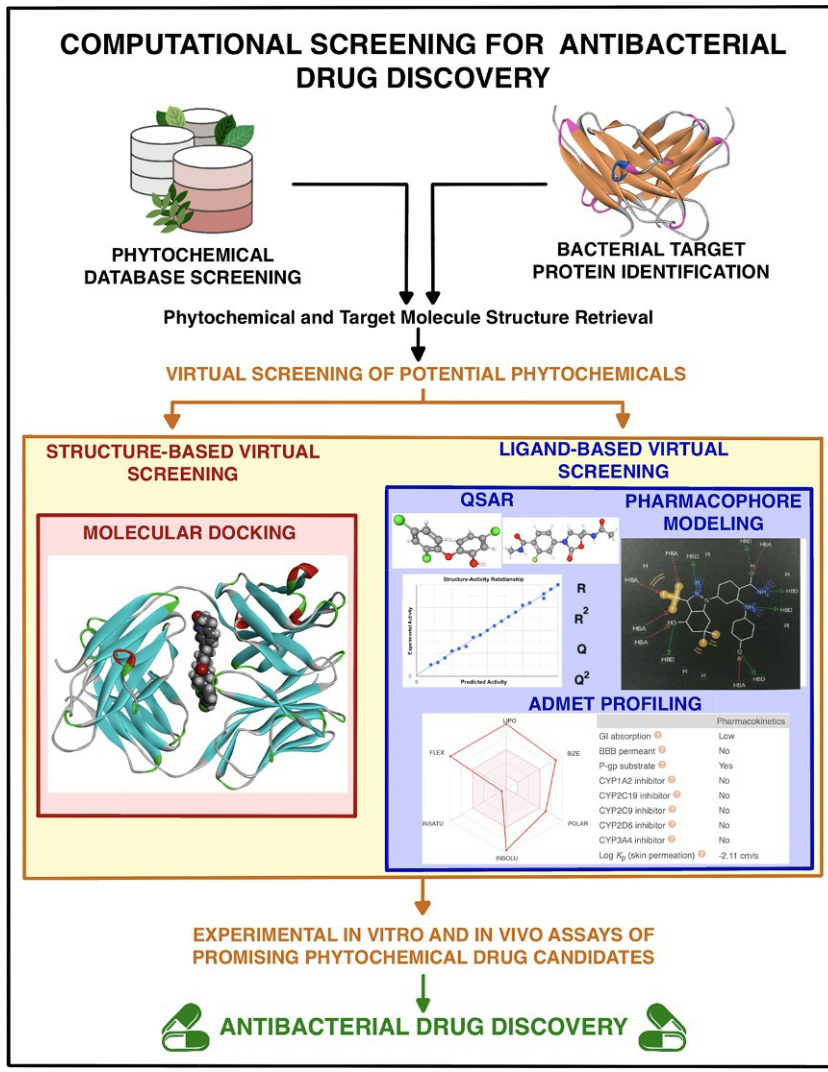

FIG. 10.1 Outline of computational anti-bacterial drug discovery from phytochemicals, using methods such as molecular docking, QSAR, pharmacophore modeling, and ADMET profiling.

of commercially available drugs. Virtual screening based on structure or target-based virtual screening interprets optimum association among phytochemicals and a bacterial target to build complexes. Thus, the phytochemicals are arranged based on their affinity for the bacterial protein, with the phytochemical having the most potent interaction appearing first [77]. This computational screening approach requires knowledge of the targeted protein's three-dimensional structure to predict the associations involving the target and specific chemical

component [77]. Phytochemicals are selected and categorized based on their affinity for the target site in this technique. As a result, the phytochemicals that are more likely to have pharmacological interaction with the bacterial target can be identified. Score functions are being applied to check the probability of a binding domain characterizing the ligand-target attraction [78]. Structure-based virtual screening is a commonly used technique in drug discovery and development due to its time and cost efficiency [77]. Further, this technique enables the molecules in question to be tested entirely using computational tools. Therefore, the potential phytochemicals whose three-dimensional structures are available can be used to conduct structure-based virtual screening against their known targets that result in their anti-bacterial activity.

10.3.2.1 Molecular docking

Molecular docking is extensively employed to conduct structure-based virtual screening technology, which takes advantage of the structural and chemical changes that occur when a drug-like compound interacts with its targeted site, trying to predict the favorable positioning of ligands in the binding site using scoring functions [65]. Molecular docking is a valuable tool to predict the interaction of phytochemical compounds with the anti-bacterial target sites and their affinity toward each other. Docking is accomplished in two phases: firstly, sampling ligand conformations in allosteric protein regions and rating the structural arrangements using a scoring function [79]. Sampling algorithms replicate hypothetical means of interaction, whereas the scoring function would give the molecule the highest score out of all created conformations in a perfect interaction.

In rigid docking, the sampling algorithm uses translational and rotational degrees of freedom to explore alternative positions of ligands at the active binding site, whereas, in flexible docking, degrees of freedom based on spatial arrangement are included with translations and rotations of the ligands. Search algorithms use various strategies to anticipate the suitable conformation of ligands, including evaluating the chemical and geometrical properties of the participating atoms [79]. Molecular docking software applies mathematical models to assess the strength of non-covalent reactions involving a ligand and a protein target. They can be utilized to figure out the attachment region of the ligand and the structure of the complex formed [78]. This strategy can be used to find allosteric sites. Further, it can also estimate the binding ability of a receptor molecule to a ligand and aid in the identification of drug candidates [78].

Sharavanan et al. conducted a molecular docking study with phytochemicals derived from the plant *Leucas aspera* against bacterial subcellular protein targets such as MreB, a cytoskeleton protein present in bacteria like *E. coli*, and FtsZ regulatory proteins [65]. The docking simulation was carried out using the MolDock sampling algorithm and Molegro Virtual Docker [80]. The potential

phytochemicals such as Leucosperone B were docked against protein targets found in *E. coli* and *B. subtilis*. The docking scores obtained from the algorithm were compared to those of an existing control drug, penicillin [80]. Ultimately, the low-energy docking position was considered to be a significant interaction between protein and ligand molecules, as the possibility of them occurring naturally is greater. Even though the penicillin showed better affinity toward the targets like FtsZ, Leucosperone B's docking scores were good, and the phytocompound showed a high affinity toward the protein targets [80]. The compound was found to participate in four hydrogen bonds with the target receptor MreC [80], indicating its strong reaction with bacterial components. Thus, this study signified that the phytochemical Leucosperone B could be considered for anti-bacterial drug discovery.

Similarly, various other molecular docking studies [81–84] have been conducted to identify the interaction of phytochemical ligands with different bacterial protein targets to understand the affinity, activity, and strength of phyto inhibitors against bacterial action.

10.3.3 Ligand-based virtual screening

Unlike structure-based virtual screening, ligand-based virtual screening does not examine small molecule repositories. Instead, it focuses on preliminary information on identified compounds that bind to the desired protein target. A pharmacophore framework that outlines the minimum structural properties a ligand must exhibit, enabling it to attach to the targeted molecule, can be generated using these recognized molecules [85]. To determine the activity of novel analogs, this method utilizes QSAR, formed from an association between estimated attributes of molecules and their biological activity found empirically [85]. This section focuses on two commonly used ligand-based virtual screening methodologies, which can be adapted to identify phytochemicals for antibacterial drug discovery.

10.3.3.1 Quantitative structure-activity relationships

The QSAR connects biological activities to physicochemical qualities in a quantitative way. The molecular activity is mathematically linked to one or more target proteins using QSAR analysis. The statistical patterns developed as a result are used to estimate the bioactivity of novel molecules that have yet to be evaluated in the lab [8]. QSAR is used in molecular design, assessment of various biological functions, lead compound refinement and virtual screening, categorization, detection, and comprehension of pharmacological action mechanisms, and analysis of the toxicity of drug candidates [86]. QSAR is a multi-step procedure that starts with the shortlisting of a collection of training compounds depending on experimental functions, followed by the construction of a statistical connection to explain the compounds' properties based on their

structure and physical chemistry [72]. A model is created that shows a link between the molecular targets and the biological features of the ligands. Following that, the model is used to predict the activity of the test set in a similar manner that the training compounds were predicted [72]. The various types of QSAR, their uses and tools to perform QSAR analysis have been discussed in Table 10.2. A plethora of research has been conducted to evaluate the structure-activity relationship between phytochemicals and their target receptor protein, which enables their anti-bacterial action [84,87–89].

For instance, Araya-Cloutier et al. (2018) performed a QSAR analysis between isoflavonoids and protein targets on *L. monocytogenes* and *E. coli* to determine their anti-bacterial properties [90]. In the QSAR study, a genetic algorithm was applied to correlate the physicochemical property of the phytocompounds to their anti-bacterial activity by implementing the ordinary least square regression model [90]. In the *L. monocytogenes* models, the descriptors were found to be the hydrophilic nature of the isoflavonoid, the presence of reacting hydrophilic groups on the molecules, globularity, and the branching of the molecules [90]. Similarly, in the case of *E. coli* models, the properties corresponding to their action against bacteria were the number of hydrophobic groups, hydrogen bond-forming groups, globularity, and molecular flexibility [90]. Thus, these results indicated that the circular shape and molecular flexibility were the two most contributing factors to the anti-bacterial mechanism of action of isoflavonoids.

Another QSAR study between polyphenols and gram-positive and negative bacteria that are commonly seen to contaminate food was conducted to identify the action of polyphenol phytocompounds against them [91]. In MATLAB software, the multi-linear regression QSAR was applied with the help of the enhancement replacement method. By using the Kubinyi Function, the properties to be analyzed were shortlisted and incorporated into the model. Based on the Kubinyi Function scores, the best molecular descriptors were identified (high scores) and were further confirmed by performing the t-test [91]. The results found that the hydrophobicity, lipophilicity, electrostatic forces, molecular flexibility, and other properties all cumulatively contributed to the anti-bacterial mode of action of the polyphenolic phytocompounds.

10.3.3.2 Pharmacophore-based virtual screening

A pharmacophore is a conceptual representation of molecular properties required for ligand molecule identification by a biological structure, describing how different ligands varying in structure can bind to the same receptor site [85]. Pharmacophore properties of a compound correspond to those atomic or structural arrangements of molecules that contribute to their specific activity, upon a change which can lead to modification in their activity as well. Further, it has also been noted that different compounds having similar atomic configurations at their active site also exhibit similar functionality [85]. With the

advancement of chemical databases and computer applications in recent years, virtual database screening utilizing the pharmacophore approach has become one of the most common methods for finding potential drug candidates for drug discovery.

Active ligands and the active site of the receptor protein are required for pharmacophore modeling. A pharmacophore can be constructed by several methods. Shared feature evaluation can be used to identify physicochemical characteristics that are common in a group of potent drugs that appear to be relevant for binding association [92]. Furthermore, different chemical configurations for specific training set compounds, along with the matching inhibitory activity or dissociation constant values, can be utilized to link the 3D layout of their chemical properties with training set molecules' bioactivity [92].

Two general steps have been identified to conduct ligand-based pharmacophore modeling successfully. The first step involves designing the structural layout of phytochemical ligand molecules in a training set to depict the conformation-based activity of the compounds [93]. Secondly, comparing the structural features of the various ligands in the training set contributes to their similar action to construct the model [93].

Nyawai et al. [94] performed pharmacophore modeling on phytochemicals from the plant species *Clinacanthus nutans* to discover their conformation-based properties leading to their anti-bacterial action. They did so by creating a three-dimensional pharmacophore structure of the ligand phytochemicals with minimal energy due to their positioning, and then the ligands were clustered for their accurate alignment [94]. The model was developed by overlapping and aligning the phytochemical ligand test set with the training set, composed of currently available anti-bacterial drugs such as ampicillin, amoxicillin, and cefixime. The common structural features identified between the drugs and the phytochemicals were four groups participating in donating and one in accepting hydrogen bonds, a single hydrophobic group, and the presence of an aromatic structure within the hydrophobic region [94]. Thus, confirming that these structural properties impart their anti-bacterial activity by participating in bacterial cell binding, similar to those of broad-spectrum antibiotics.

10.3.3.3 ADMET profiling

Another crucial step in the process of drug discovery is the analysis of the pharmacokinetics of the potential drug candidates. The absorption, distribution, metabolism, excretion, and toxicity (ADMET) are crucial parameters that need to be considered and analyzed in drug discovery. Even though phytochemicals have proven to be highly beneficial in the medicinal sector, certain phytochemicals can be toxic. Further, the dosage and means of administration of the phytochemicals are essential aspects that need to be taken into consideration while designing phytochemical drugs.

In drug discovery and development, significant progress has been achieved in high-throughput scanning for these attributes. ADMET forecasting is now possible using in silico approaches, allowing for more appropriate pharmacological lead identification. Various ADMET analysis software has been developed to aid the process of computational drug discovery. KnowItAll ADME/Tox Edition, ADMET Predictor, MedChem Studio, and IMPACT-F are among the few which are currently being used to perform the analysis [88].

Table 10.2 mentions other ADMET web tools currently available as well.

10.4 Computer-aided anti-bacterial drug discovery of phytochemicals

The different high-performance computational drug discovery methods have been discussed in this chapter. This section discusses the need for computational screening of phytochemicals for anti-bacterial drug discovery and various studies, which identified potential phytochemical drug candidates.

10.4.1 Importance of computational studies of phytochemicals for anti-bacterial drug discovery

The different properties of phytochemicals and their application for the development of the desired drug have been discussed. Phytochemicals have various biological activities and are employed as anti-oxidants, immunomodulators, anti-microbials, cardiovascular medicines, and anti-cancer medications [95]. Multiple studies demonstrate that several phytochemical compounds isolated from medicinal plants have effective anti-bacterial potential against multi-drug-resistant infections and that these compounds could be used as anti-bacterial medications, according to the scientific literature [96]. Their identification, on the other hand, is still limited. The complete reliance on time-consuming in vitro and in vivo screening technologies is a key roadblock in the discovery of effective phytocompounds [95]. Isolation, purification, and screening of drug candidates are currently the most important phases in natural product drug development. The use of industrial-scale extraction and biotechnology is required for the successful conversion of lead compounds into clinically useful medications [97]. The drug discovery process is lengthy, complicated, and costly. It frequently leads to more failures than triumphs. Alternatively, employing an online database and bioinformatics tools to perform computational drug discovery might be a cost-effective and time-saving method. These advanced bioinformatics tools and methods can, therefore, be used for designing, optimization, and high-throughput screening of phytocompounds [95].

Plants have undoubtedly proven to be a good source of novel anti-bacterial medications in several types of research over the last few decades. As a result, anti-bacterial medicinal plants hold a lot of promise due to their biodiversity,

and most of them have not been well studied yet. To assure the selection of bioactive and non-toxic or probable side effects of the nominated anti-bacterial phytochemicals, more extensive investigations connected to the isolation of anti-bacterial phytochemical components from medicinal plants must be carried out [96]. Therefore, extensive computational studies of phytochemicals are required to facilitate anti-bacterial drug discovery.

10.4.2 *In silico* aided anti-bacterial drug discovery from phytochemicals

Various research have proven that phytochemicals can be used for anti-bacterial drug discovery. This has been achieved by studying the different computational techniques along with in silico studies. The following case studies give us a detailed idea of how these phytochemicals act as anti-bacterial agents using computer-aided approaches.

10.4.2.1 Identification of anti-bacterial and anti-diarrheal activities of Colocasia gigantea *Hook F. leaves based on computational approaches*

The anti-bacterial and anti-oxidant properties of the methanol soluble extract of *Colocasia gigantea* have been investigated [98]. Phytochemical extraction and structure elucidation of *Colocasia* leaves produce chemical substances such as isoorientin, orientin, Lut-6-C-Hex-8-C-Pent, vicenin, alpha-amyrin, beta-amyrin, monoglycerol stearic acid, apigenin, vitexin, and isovitexin [99]. For the experiment, plant materials were collected, cleaned, sun-dried, and then ground into a coarse powder [99,100]. The in vitro anti-microbial investigation was performed using the disc diffusion method followed by a molecular docking analysis of secondary metabolites [98]. After obtaining the anti-bacterial activity against certain pathogens, the molecular docking analysis was performed. The first step in docking analysis is the selection of compounds for computational studies. PubChem was used to retrieve the chemical structures of isolated molecules (https://pubchem.ncbi.nlm.nih.gov/). PubChem is a database of chemical compounds and their biological roles [101]. It includes data on tiny molecules, lipids, carbohydrates, and (chemically modified) amino acid and nucleic acid sequences, among other chemical entities (including siRNA and miRNA) [102]. To assess the potential utility of PubChem for drug development, the targets for proteins in PubChem are systematically summarized by their function, 3D structure, and biological route [103]. Through chemical analysis, alpha-amyrin, beta-amyrin, monoglyceryl stearic acid, and penduletin were chosen as major chemical compounds [100].

The next step is ligand preparation. From the PubChem compound database, the synthetic structures of *C. gigantea*'s four compounds (alpha-amyrin, beta-amyrin, monoglyceryl stearic acid, and penduletin) were obtained [98].

The ligand was created using the LigPrep, which was provided in Schrödinger-suite-Maestro v 11.1, with the following parameters: neutralized at pH 7.0 ± 2.0 using Epik 2.2, and minimized using the OPLS 2003 force field [98].

Followed by this, the enzyme/receptor preparation was done. The Protein Data Bank (RCSB PDB) of the Study Collaboratory for Structural Bioinformatics (RCSB) offers tools and resources for research and teaching that provide a structural picture of life. The RCSB PDB website (http://www.rcsb.org) makes use of the PDB archive's curated 3D macromolecular data to provide new ways to access, report, and explore data [104]. This RCSB PDB has been used to obtain 3D structures of macromolecules such as kappa-opioid receptor and human delta-opioid receptor for anti-diarrheal docking studies, beta-ketoacyl-ACP synthase 3 receptor for anti-microbial docking studies, glutathione reductase for anti-oxidant docking study. The enzyme/receptor was prepared for a docking experiment using the Protein Preparation Wizard, which was included in Schrödinger suite-Maestro v11.1 [98]. The glide standard precision docking is the final phase. To evaluate the potential mechanism of action of the selected compounds in relation to the respective enzymes/receptors for anti-diarrheal and anti-bacterial activity in *C. gigantea*, a molecular docking analysis was performed. For docking studies, glide standard precision docking, which is included in Schrödingersuite-Maestro v 11.1, was utilized [98]. After molecular docking, the determination of pharmacokinetic parameters by the SwissADME web tool was carried out. SwissADME performs validation to predict the pharmacokinetic parameters of drug compounds [105]. Based on Lipinski's rule, it calculates the total molecular weight of the compounds, lipophilicity (LogP), the number of hydrogen bond acceptors, and the number of hydrogen bond donors [98].

Furthermore, admetSAR was used to predict toxicological characteristics. The toxicological properties of the selected compounds were determined using the admetSAR online program [98]. Using the PASS online tool, the anti-bacterial effects of the four major phytoconstituents alpha-amyrin, beta-amyrin, monoglyceryl stearic acid, and penduletin were examined [98]. The prediction of activity spectra for substances (PASS) software predicts pharmacological effects and biochemical mechanisms based on a substance's structural formula. It can be used to find new targets (mechanisms) for some ligands and, conversely, new ligands for some biological targets [106]. In the anti-microbial study, molecular docking revealed that monoglyceryl stearic acid and alpha-amyrin had the highest and lowest binding affinity against the beta-ketoacyl-ACP synthase 3 receptor, respectively [4]. Asn247, gly209, asn274, gly305, leu205, leu191, thr190, ala111, thr81, ile156, phe213, met207, and val212 were found to interact owing to Van der Waals forces [98].

SwissADME, an online application, was used to determine the pharmacokinetic properties of the substances chosen by Lipinski. The study found that all of the substances followed Lipinski's principles, indicating that they have a high oral bioavailability. The admetSAR web server also predicted the

toxicological features of the four chemicals. The selected compounds were found to be non-Ames poisonous, non-carcinogenic, and have low toxicity levels [4]. Finally, the anti-bacterial properties of four main *C. gigantea* compounds were investigated using the PASS online program. The potency displayed a higher Pa value than Pi [98].

According to the findings of this study, the methanol extract of *C. gigantea* leaves can be a rich source of anti-oxidants as well as a promising option for anti-diarrheal and mild anti-bacterial treatment. Furthermore, some bioactive potential compounds showed promising binding affinity to specific proteins in molecular docking analysis, and the ADMET investigation revealed their drug-like characteristics. The experimental results for bioactive components were consistent with PASS predictions [98].

10.4.2.2 Molecular docking, molecular dynamic simulations, and in vitro assays to screen potential lead molecules against prioritized targets of multi-drug-resistant (MDR) Acinetobacter baumanni

The goal of this study was to use metabolic pathway analysis and database search approaches to identify potential drug targets for *A. baumannii*, and to compare the binding affinities of three conventional pharmaceuticals and their known targets to the binding potential of selected ideal herbal leads against drug targets utilizing molecular modeling, molecular dynamic (MD) simulations, and in vitro experiments [42].

The identification of the drug targets was carried out using the KEGG pathway. The three-dimensional structures of the five stated targets that were not present in their original forms were estimated using homology modeling. The computational screening and selection of ligands were further carried out. The molecules were screened using the PreADMET web server [42]. PubChem and ChemSpider were used to find the 3D structures of these phyto ligands. The drug-likeness characteristics of the phyto ligands were predicted using PreADMET's, Lipinski's rule of five, CMC-like rule, Lead-like rule, MDDR-like rule, and WDI-like rule [42]. To predict toxicity, the compounds that qualified for the ADME features were further chosen. For molecular docking, the lead molecules that qualified these predictions were chosen [42]. Molecular docking studies were used to predict receptor-ligand interactions. AutoDock Vina v1.1.2 was used for flexible body docking. AutoDock Vina delivers a two-order-of-magnitude speedup over the molecular docking program, while also greatly enhancing the accuracy of binding mode predictions. It generates grid maps automatically and organizes the results in an unobtrusive fashion for the user [105]. Three commonly used antibiotics were employed in the molecular docking experiments. The selected antibiotics were clinafloxacin, imipenem, and polymyxin-E [42]. These medications' three-dimensional structures were obtained from the PubChem and Chemspider databases. ChemSpider

is a free online chemical database that gives users access to physical and chemical properties, molecular structure, spectral data, synthesis methods, safety information, and nomenclature for almost 25 million different chemical compounds from about 400 different data sources [107]. The selected antibodies were docked and flexible body docking was carried out. The ideal docked poses for each antibiotic with their specific targets were screened using minimum binding energy (kcal/mol), cluster RMS, quantity of hydrogen bonds, and other interacting residues. The interaction of antibiotics and their specific targets was compared to the binding capacity of herbal-based ligands [42].

MD simulations are used to optimize the structure of the protein receptor before docking and account for protein flexibility; to refine docked complexes and account for induced fit; to calculate binding free energies and provide an accurate ranking of potential ligands; and, more recently, to find the binding site and correctly dock the ligand during the docking process itself [108]. To study the stability of docked conformation and priorities the binding potentiality of the selected herbal leads, the best-docked complexes of limonin and diaminopimelate epimerase, and a homology model of aspartate semi-aldehyde dehydrogenase, strictamin, and UDP-N-acetylglucosamine 1-carboxyvinyltransferase, were chosen for the MD simulation [42]. The Swiss Param topology generator was used to create the topology for the ligands. The force field Charmm 27 was used to create the topology for the proteins and the complex. GROMACS v 5.0.5 was used to execute an explicit solvent MDs simulation in a dodecahedron box using the extended simple point charge Spc/e water model [42]. Finally, the gmx rms command was used to calculate the root-mean-square displacement (RMSD) of all heavy atoms in the initial structures to determine the structure's stability and whether the complex is stable and close to the experimental structure [42].

Computation prediction revealed the drug-likeness properties of the selected lead molecules. According to Lipinski's rule of five, 97 compounds qualified for drug-like characteristics. Because of the computational prediction, compounds including ajmalicine, strictamin, and limonin were qualified for drug-likeness, and ADMET was chosen as an ideal lead for molecular docking studies [42]. The docking studies revealed limonin to have the best binding energy [42]. In the MD simulation, the herbal lead limonin showed stable binding to the receptor diaminopimelate epimerase, implying that the lead-receptor association is stable. The RMSD ranges were found to be 1.4–1.47 nm for 250 ns MD simulation. According to MD simulations, the docked complex appeared to be stable, and the herbal lead strictamin exhibited stable binding to the receptor UDP-N-acetylglucosamine 1-carboxyvinyltransferase [42].

According to the results of the computational screening, the herbal-based lead compounds had greater binding potential with lower binding energy, cluster RMSD, and stabilizing interactions than three traditional antibiotics toward their known targets. The MD simulation research suggests that docked complexes are stable in terms of binding energy, number of hydrogen bonds, and conformation during the MD simulation [42]. In contrast to interactions

between anti-bacterials like imipenem (carbapenam), clinafloxacin (fluoroquinolones), and polymyxin-E (colistin), phyto ligands like ajmalicine, limonin, and strictamin have good binding potential, low binding energy, and stabilizing interactions toward the chosen targets, according to computational modeling and MD simulations. As a result, the current study suggests that these phyto ligands, all derived from medicinal plants, could be employed as alternate lead compounds against multi-drug-resistant *A. baumannii*'s prioritized drug targets [42].

10.4.2.3 Computational screening of terpenoids for anti-bacterial drug discovery against Staphylococcus aureus

The following study uses an in silico approach for target identification and molecular docking analysis to investigate the anti-microbial properties of terpenoids from *Bacopa monnieri* and *Andrographis paniculata* against *Staphylococcus aureus*, as well as the interactions of phytochemicals involved in antibacterial activity [109].

In this study, CADD is employed to introduce terpenoid-based therapeutics against MRSA. In the pharmaceutical industry, computational drug design has been frequently utilized to either uncover new compounds or improve lead compounds that exhibit considerable inhibitory activity against a target biological receptor [110]. The researchers are looking for new ligands in the terpenoids from *B. monnieri* and *Andropgraphis paniculata*. The novel target identification against MRSA was carried out by choosing four ligands and collecting them PubChem [109]. Ligands were produced with the maestro Schrödinger software suite's 2D sketcher tool, which turned them into 3D structures. To elucidate the proposed docking mode to explain the binding interaction with herbal extracts produced from the two plants, three-dimensional structures of novel target proteins were retrieved in .pdb format from RCSB and prepared for docking [109]. The molecular docking studies are performed against novel targets. The in silico investigation of new and potent drugs requires MD simulation. The MD studies were carried out using the maestro suite of Schrödinger software [109].

In terms of docking score and gliding energy, the results were obtained. The hydrogen bond interaction and ligand interaction show how the target and ligand molecules interact. Andrographin got the best docking score (highest negative) among the other phytocompounds examined and would be the best ligand to inhibit the target protein 2X4K of *S. aureus* [109]. Bacoside had no interaction with the target protein 2X4K [109]. The amino acids in the docking pocket surround the drug molecule at the docking site and participate in hydrogen bond production as hydrogen bond donors or acceptors [109]. During docking between selected target protein 2IHY and plant extracts of *A. paniculata*, andrographin obtained the highest negative docking score and glide energy. As a result, andrographin may be a better ligand for inhibiting the target protein

2IHY and so preventing methicillin-resistant *Staphylococcus aureus* (MRSA). Even docking of bacoside with target protein 21HY generated a high negative docking score [109].

Therefore, it was concluded that *B. monnieri* and *A. paniculata* phytochemicals possessed a significant anti-bacterial activity against MRSA. The conclusion obtained from the in silico analysis revealed that andrographis and bacoside have a higher selectivity for the target proteins 2X4K and 2IHY, and could be an effective anti-microbial drugs.

10.5 Conclusion and future perspectives

With the increasing number of pathogens along with the upsurge of bacterial drug resistance, anti-bacterial drug discovery is gaining more importance in today's world. In the current century, one of the challenges faced in the healthcare industry globally is the severe illnesses induced by bacteria resistant to regularly used anti-bacterial drugs. As a result, there has been a large increase in incidence and death, as well as longer duration of hospital stays and higher costs of healthcare. Therefore, the need to discover and utilize new drugs, which can effectively tackle this issue, is of great importance.

Phytochemicals obtained from plant sources serve as a great medium in the drug discovery process. Their various properties, as highlighted in this chapter, can be exploited to obtain desired anti-bacterial drugs that can fight against specific bacterial species. These bioactive compounds are being used in various fields and increase the chances of more discoveries in the future. The different classes of phytochemicals provide a wide range of applications because of their specific properties. For these bioactive compounds to serve as anti-bacterial agents, their computational screening needs to be carried out.

CADD is gaining popularity as it uses the ability of the phytochemicals to serve as an anti-bacterial agent, which can then be applied for drug discovery. The creation of high-quality databases and resources that can be tuned for structural variety or commonality has resulted from the quest for novel pharmaceuticals. Further, advancements in molecular docking approaches, paired with developments in computing architecture, are allowing for rapid strides in the process of drug design and discovery. The different high-performance computational drug discovery methods that can be used according to the requirement of the target molecules and the drug discovery process have been elaborated in this chapter. Since the drug discovery process is tedious and time-consuming, different in silico tools must be employed to make the process of drug discovery and design simpler. The different computational drug discovery methods like molecular docking, QSAR, pharmacore-based virtual screening, and in silico ADMET analysis can, therefore, be used for designing, optimization, and high-throughput screening of phytochemicals. CADD is now universally perceived as a promising supplement to high-throughput screening and a massive timesaver.

The drug development approach, involving ligand-based and structure-based methodologies, is seen to have its own set of benefits and drawbacks. The combination of the two methodologies, which complement one another, has demonstrated remarkable advantages in terms of speed and efficiency in the virtual screening process for generating prospective leads. There is still an opportunity for improvement and new features to enhance and authenticate high-throughput virtual screening models, which are becoming increasingly significant in the area of novel drug development studies.

Even though the significance and potential of plant products are widely known, the research conducted on them to identify and effectively benefit from their properties, such as anti-bacterial action, is still relatively limited. Thus, augmenting the existing knowledge of phytochemicals with computational screening methodologies can facilitate and expedite the process by which drug discovery and development are viewed and implemented. With the rapid progress happening in the field of science and technology, these computational methods will pave the way for even greater opportunities in the near future in the field of computational phytochemistry.

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