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4 Extraction of Phenolic Compounds from Some Ayurvedic Botanicals (*Nigella sativa, Andrographis paniculata, and Phyllanthus amarus*) and Evaluation of Their Antibacterial and Antiviral Properties Using Bioinformatics Approaches

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ABBREVIATIONS

CADD	computer-aided drug design
PDB	protein data bank
RCSB	Research Collaboratory for Structural Bioinformatics
SMILES	simplified molecular-input line-entry system
MD	molecular dynamics
DFT	density functional theory
HM	homology model
ADMET	absorption, distribution, metabolism, excretion and toxicity

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RMSD	root means square deviation
MMGB/SA	molecular mechanics-generalized Born surface area
MMPB/SA	molecular mechanics Poisson-Boltzmann surface area
2D	2-dimensional
3D	3-dimensional
OPLS	Optimized Potentials for Liquid Simulations
RNA	ribonucleic acid
RNAp	RNA polymerase
SARS-CoV2	severe acute respiratory syndrome coronavirus 2
COVID-19	coronavirus 2019
S	spike
E	envelope
M	membrane
N	nucleocapsid
ORF	open reading frame
Nsp	non-structural protein
Mpro	main protease
CLpro	chymotrypsin-like protease
PLpro	Papain-like protease
TMPRSS2	transmembrane protease serine 2
ACE2	angiotensin-converting enzyme
RBD	RNA-binding domain
RdRp	RNA-dependent RNA polymerase
SCP	steroy carrier protein
CHIKV	Chikungunya virus
DENV	dengue virus
HIV	immunodeficiency virus
AP	*Andrographis paniculata*
NS	*Nigella sativa*
PA	*Phyllanthus amarus*
BASA	biaryl succinic acid
HCQ	hydroxychloroquine
CF	cystic fibrosis
TB	tuberculosis
RP-HPLC	reversed-phase high-performance liquid chromatography
UV	ultra violet
LC-ESI-QTOF/MS	liquid chromatography coupled with electrospray ionization-quadrupole-time of flight-mass spectrometry
UHPLC	ultra-high-performance liquid chromatography
UPLC	ultra-performance liquid chromatography
GC-MS	gas chromatography-mass spectrometry
AR	antibiotic resistance
NDM	New Delhi metallo-β-lactamase
IMP	Imipenemase
VIM	Verona integron-encoded metallo-β-lactamase

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4.1 INTRODUCTION

Introducing a new drug to the market generally requires a lengthy time of at least 13 years with capitalized cost, exorbitant resource and expertise demands, and high investment risks. The process is oftentimes complicated by adherence to certain regulations and policies, making it a complex operation (Wooller et al. 2017; Reddy 2017). The science of computer-aided drug design (CADD) enhances the discovery of promising drug candidates from large resources in a faster, more economical, and environmentally friendly process. It extensively incorporates the applications of bioinformatics, cheminformatics, molecular modelling and simulations, and quantum techniques for scanning chemical libraries via de novo fragment- or structure-based designs. It represents an essential core of drug discovery (the discovery phase) in modern days, involving the study of the mechanistic biological events regarding the plausible molecular targets and interactivity with drug molecules. The potentials discovered in this phase including the targets, identified hits, and lead-like candidates are optimized for the next stage, i.e., the development phase. In this phase, they are validated through pre-clinical and clinical I-III *in vitro* and *in vivo* experimental models. The third stage is the registry where the distribution, marketing, and clinical usage of the newly discovered drug are established. These earmark the cumbersomeness of the entire traditional drug discovery process. Interestingly, the applications of the CADD significantly ameliorate most of the limitations including lengthy time, high cost, and resource implications (including animal and human subjects) (Prieto-Martínez et al. 2019; Sliwoski et al. 2014; Schaduangrat et al. 2020; Brogi et al. 2020; Wooller et al. 2017).

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Bacteria and viruses are mostly implicated in the emergence and spread of infectious diseases and are responsible for most of the global pandemics including cholera, flu, and the ravaging coronaviruses (Piret and Boivin 2021). Bacterial and viral pathogens constitute the mainsprings of human disease-–elated death globally (Lewis et al. 2022). Therefore, the search for effective antibacterial and antiviral agents to mitigate their effects and maintain human wellness remains a global scientific exploration. Moreover, since the inception of humanity, plants have been recognized as the main sources of traditional medicine. In their various forms, they are naturally enriched with bioactive phytochemicals of different classes, amenable for controlling viral and microbial pathogens and their devastating effects in man (Joshi 2023; Stuper-Szablewska et al. 2023). Notably, Ayurveda is a historical traditional system of medicine predominantly traceable to India thousands of years ago. In the system, medicinal plants in various parts and constituents have been formulated in different forms, including extracts, into medicines for preventing and treating human diseases including those induced by pathogenic bacteria and viruses (Kuralkar and Kuralkar 2021). In particular, *Andrographis paniculata* (AP), *Nigella sativa* (NS), and *Phyllanthus amarus* (PA) are among the renowned botanical constituents in extract formulations of Ayurveda for treating various diseases and ailments. The phytochemicals from these plants have been reported with interesting activities against several pathogenic infections (Gyawali et al. 2021; Kumar, Dobos, and Rampp 2017; Chattopadhyay et al. 2022).

Therefore, in this chapter, the phenolic phytochemicals of the aforementioned Ayurvedic botanicals have been showcased for antibacterial and antiviral potential

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using bioinformatics approaches. The chapter significantly provides first-hand information on the medicinal potency of some notable phenolic compounds extractable from the highlighted plants based on several biomodelling studies from the bioinformatics perspective. In addition to the impacts of bioinformatics in drug and vaccine development, the traditional medicinal system of Ayurveda, extraction methods for the phenolic constituents of three selected plants, and interesting antibacterial and antiviral potential of some notable phenolic phytochemicals of the plants are further explored.

4.1.1 Bioinformatics in Drug Discovery

Bioinformatics typically involves the application of computational methods and software tools to analyse a large variety of biological data for research reproducibility, distinctively supporting biomedical research. Creation of large databases and high-throughput biodata processing and analysis via relevant scientific applications such as omics (citromics, genomics, proteomics, transcriptomics), epigenetics, genomic architecture, and ribosome profiling have been extensively applied for mechanism-based drug discovery. Other relevant aspects such as homology protein/receptor modelling, structural analysis of protein and ribonucleic acids (RNAs), and identification of distinct drug targets/biomarkers have aided modern drug discovery through robust protein-ligand docking, virtual screening, and molecular simulations. (Schaduangrat et al. 2020; Xia 2017; Wooller et al. 2017). Recently, artificial intelligence, machine learning, and other modern bioinformatics applications have been successfully employed for the modelling of biomedical datasets and network-based prediction of drug-target and/or drug-drug interactions (Abbas et al. 2021). In addition to the accelerated identification and validation of drug targets, drug screening, and drug optimization, bioinformatics also aids the characterization of side effects and prediction of drug resistance (Xia 2017; Reddy 2017). As such, bioinformatics has become essentially indispensable in translational drug discovery within research institutions and pharmaceutical industries (Wooller et al. 2017), although applications and pipelines of bioinformatics essentially require capital investments in sophisticated computing and data processing resources and expertise and skill for protocol configuration (Schaduangrat et al. 2020; Wooller et al. 2017). Moreover, its successful application in therapeutic designs depends largely on the nature of the disease and aetiologies. As such, an extensive understanding of the diseases' biochemistry is crucial for selecting appropriate bioinformatics tools for minimizing failure in the processes (Wooller et al. 2017).

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4.1.2 Bioinformatics in Vaccine Development for Bacterial and Viral Diseases

The sudden emergence of new and/or re-emergence of mutated strains of pathogenic bacterial and viral organisms usually constitute a pandemic. Thus, due to the almost unbearable time and resources required for the discovery of effective therapeutics to meet the urgent global demands by healthcare systems during the episodes, the

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development of vaccines remains essential. For instance, the unexpected outbreak of coronavirus 2019 (COVID-19) has overwhelmingly affected human wellness and the global economic systems in an unpredictable fashion, causing morbidity and mortality now and then. Up-to-date, effective therapeutics for its treatment remain arduous despite the ceaseless concerted scientific efforts expanded in this direction globally. However, the timely development of various vaccines has drastically reduced the infection rates and subsequently improved the COVID-19–related health challenges worldwide (Yusuf Oloruntoyin Ayipo, Ahmad et al. 2022; Yusuf Oloruntoyin Ayipo, Bakare et al. 2022). In particular, the enormous applications of bioinformatics in the development of these vaccines and others for pre-existing bacterial and viral diseases cannot be overlooked. Similar to other pathogenic diseases, the viral genomic data of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), antigenic epitopes, antibody structural and protein-protein docking analyses, and simulation of the antigen-antibody reactions have been employed as bioinformatics approaches towards vaccine development for COVID-19 (Chukwudozie et al. 2021).

4.2 AYURVEDIC MEDICINE

Ayurveda, translated in English as "knowledge of life", is a traditional system of medicine of Hindu extraction. It represents one of the oldest medicinal systems, dating back to the Vedic era (around 3000 years ago) in India. The science of Ayurveda is believed to be centred around three main texts: "Ashtanga Hridaya", "Charaka Samhita", and "Sushruta Samhita" and other minor texts detailing the natures of various diseases, diagnostic interventions, and therapeutic recommendations. It covers contextual disease management with medicinal applications of over 700 herbs in about 6000 formulations (single and combination) including decoctions, extracts, juices, powders, and tablets (Gyawali et al. 2021). Interestingly, AP, NS, and PA are among the common plant constituents of Ayurveda formulations for medicinal purposes (Gyawali et al. 2021; Kumar, Dobos, and Rampp 2017; Chattopadhyay et al. 2022).

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4.3 ANTIBACTERIAL AND ANTIVIRAL POTENCIES OF EXTRACTS OF SELECTED AYURVEDIC BOTANICALS

4.3.1 *ANDROGRAPHIS PANICULATA*

AP is also known as "create" or "king of bitter" in the English language because of its extremely bitter taste. It is widely used in traditional medicines including Ayurveda. Phytoconstituent evaluations of AP showed active compounds including flavonoids and andrographolides with multi-faceted therapeutic properties such as anticancer, antibacterial, and antiviral activities (Atu et al. 2022; Mohmmed Arifullah et al. 2013). The aqueous extract of the herb was evaluated against *Staphylococcus aureus, Porphyromonas gingivalis, Actinomyces viscosus, Streptococcus mutans,* and *Streptococcus sobrinus*. Moderate inhibition was recorded with the concentration of 1.0 g/mL across the pathogens, while the dilution of 1:2 and 1:4 gave significantly higher inhibition on all the pathogenic organisms tested (Mohamad et al. 2021). Some constituents of AP were also examined for antibacterial activity on *E. coli, Pseudomonas aeruginosa, S. aureus, Klebsiella pneumonia,* and

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Streptococcus thermophilus. They mostly demonstrated significant inhibition against all the tested bacterial strains (Mohmmed Arifullah et al. 2013). Methanol and chloroform extracts of AP have been studied for inhibitory effects on quorum sensing for fighting *P. aeruginosa*. Both extracts showed good inhibition, especially the chloroform fraction, suggesting that most of the effective phytoconstituents must have been partitioned into the chloroform portion (Banerjee et al. 2017). The biochemical studies conducted on the extract of AP showed that the extract is rich in terpenes. Further screening suggested that the terpenoids are non-toxic, and it was therefore applied in the antibacterial evaluation on TEM-1 β-lactamase. The result shows significant inhibition of the enzyme, confirming its efficacy in fighting bacteria (Hajong and Sarma 2021). Leaf extracts of AP were tested at different concentrations (5 μL, 10 μL, 20 μL) for antibacterial activities against some gram-positive and gram-negative bacteria strains. They showed significant and similar inhibition in the tested organisms except for *S. aureus*, having a very low inhibition at low to moderate concentrations (Nayak, Pavithera, and Nanda 2015). The bactericidal effects of aqueous, ethanol, and acetone fractions of AP extracts also showed potent inhibitory activity on *K. pneumonia, P. aeruginosa,* and *Bacillus subtilis*, though the acetone fraction showed better activity than the aqueous and ethanol extracts, possibly due to the type of phytoconstituents of the extract (Rajeshkumar 2015).

4.3.2 Phyllanthus amarus

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PA is an Indian medicinal herb belonging to the Euphorbiaceae family. It is widespread across the tropical countries of the world. This plant is commonly used in Ayurvedic medicine to treat diseases such as intestinal infections, kidney disorders, hepatitis, and stomach and liver problems. Some phytoconstituents of this plant are alkaloids, lignans, flavonoids, triterpenes, and tannins. These have conferred pharmacological properties on PA extracts such as anticancer, anti-inflammatory, analgesic, hepatoprotective, antibacterial, and antiviral agents (Sundaram et al. 2016; Sousa et al. 2017). Using the disc diffusion method, the aqueous extract of the leaf and root of PA were screened for antibacterial activities and were found to be bactericidal at concentrations of 10 and 20 μg/mL. Appreciable effects were recorded against gram-positive bacterial strains such as *B. subtilis, S. aureus, Streptococcus faecalis*, and *Staphylococcus albus* and gram-negative strains including *Proteus vulgaris, E. coli, K. pneumonia,* and *P. aeruginosa*. The preliminary phytochemical examinations showed the presence of saponins, tannins, polysterols, amino acids, alkaloids, lignins, and most importantly, the phenolic compounds which may be responsible for the interesting antibacterial activities of these extracts (Dhandapania, Balakrishnanb, and Anandhakumar 2007). Another extract of PA was tested for antibacterial activities against *S. aureus* and *P. aeruginosa*. The results indicated that the extract moderately inhibited *S. aureus* but showed no activity against *P. aeruginosa* (Corciovă et al. 2018). The PA extract as well as phyllanthin, a phytoconstituent isolated from PA, were both employed in the evaluation of their antibacterial potentials against bacterial strains. The extract demonstrated significant antibacterial activity against all gram-negative bacterial strains but was inactive against the gram-positives (Maria et al. 2019).

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4.3.3 Nigella sativa

NS, commonly called black seed in English, is of the *Nigella* genus belonging to the family Ranunculaceae. This genus is widespread in Asia, Europe, and North Africa. The black seed is an important plant that has been utilized over the years in traditional medicine to treat several ailments. Notably, its usefulness includes as a diuretic, stomach problems, liver tonic, and diaphoretic diseases in cultures such as those of the Chinese and the Arabs. Reports have also shown that extracts of NS possess anticarcinogenic, anti-inflammatory, antioxidant, memory enhancement, and antibacterial activities, facilitated by the bioactive phytoconstituents (Alshwyeh et al. 2022) available in all parts of the plant, including the root (Maity et al. 2013). The plant houses many phytoconstituents including non-volatile compounds and phenolic compounds. Apigenin, chlorogenic acid, rutin, gallic acid, catechin, and vanillic acid are phenolic compounds isolated from NS (Dalli et al. 2022). In evaluating the antibacterial efficacy of *NS,* researchers have often partitioned the extract into fractions. Thus, Dalli and colleagues reportedly evaluated the antibacterial potentials of extracts of NS against some bacteria strains. The essential oil from the plant was also investigated alongside the extracts. The results showed generally moderate to high inhibitory activities against the gram-positive and gram-negative bacteria strains tested (Dalli et al. 2022). Several other studies have also demonstrated the interesting antibacterial activities of various extracts and essential oils of NS against many bacterial strains including multi-drug-resistant isolates (Muhammad Torequl Islam, Khan, and Kumar 2019; Benlafya et al. 2014; Shafodino, Lusilao, and Id 2022).

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These pieces of experimental evidence have validated the claim of antibacterial phytochemicals in Ayurvedic botanicals. Polyphenols are renowned potent antimicrobial agents, as shown by a recent review of the literature (Manso, Lores, and de Miguel 2022). As such, the study of extraction and antibacterial and antiviral properties of phenolic compounds in Ayurvedic botanicals for identifying promising future drugs remains essential. In this regard, the application of bioinformatics tools at the preliminary stages of such worthy investigation could enhance the efficiency of the processes by saving time, being more cost-effective, and being less hazardous.

4.4 EXTRACTION OF PHENOLIC COMPOUNDS FROM SELECTED AYURVEDIC BOTANICALS

Various methods have been employed to extract phenolic phytoconstituents from several parts of the selected Ayurvedic botanicals AP, PA, and NS. These include the conventional methods such as decoction, digestion, and infusion (Matou et al. 2021); maceration (Hameed et al. 2019; Bourgou et al. 2008); maceration-homogenization (Feng, Dunshea, and Suleria 2020); maceration-rotary shaker (Muthusamy et al. 2018); percolation, serial exhaustive, and temperature-modified Soxhlet extraction (Guha et al. 2010); and the non-conventional modern ultrasound method (Gueffai et al. 2022) and sonication (Rafi et al. 2020). These and other commonly employed methods are summarized in Table 4.1. The chemical structures of the extracted phenolic compounds are presented in Figures 4.1 to 4.3.

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TABLE 4.1
Summary of Extraction Methods for Phenolic Phytoconstituents in Selected Ayurvedic Botanicals

Part of the Plant	Extraction Methods	Methods for Identifying the Phenolic Contents	Phenolic Compounds	References
Nigella sativa				
Shoots	Maceration	RP-HPLC-UV-vis	Gallic acid (NS1), (—)-*p*-hydroxybenzoic acid (NS2), chlorogenic acid (NS3), vanillic acid (NS4), *trans* 2-hydroxycinnamic acid (NS5), epicatechin/(+)-catechin (NS6), quercetin (AP8/NS7), apigenin (NS8), amentoflavone (NS9)	(Bourgou et al. 2008)
Roots	Maceration	RP-HPLC-UV-vis	NS1, NS2, NS3, NS4, NS5, NS6, NS7, NS8, *p*-coumaric (NS10), ferulic acid (NS11)	(Bourgou et al. 2008)
Seeds	Maceration-homogenize	LC-ESI-QTOF/MS	Kaempferol-3-glucoside (NS12), diosmin (NS13), NS7, kaempferol (AP12/NS14), protocatechuic acid (NS15), NS2–3	(Feng, Dunshea, and Suleria 2020)
Seeds	Maceration	HPLC-UV	NS3, NS14, caffeic acid (NS16)	(Hameed et al. 2019)
Seeds	Conventional method	HPLC-UV	NS6, NS16, rutin (NS17)	(Gueffai et al. 2022)
Seeds	Optimized ultrasound-assisted	HPLC-UV	NS6, AP8/NS7, NS16, NS17	(Gueffai et al. 2022)
Andrographis paniculata				
Leaves and stem	Sonication	LC-MS/MS UHPLC-Orbitrap-MS/MS	NS16	(Rafi et al. 2020)
Leaves	Conventional	UPLC	NS1, NS3, NS15, salicylic acid (AP18), veratric acid (AP19)	(Praveen, Poornananda, and Nayeem 2014)

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TABLE 4.1 (*Continued*)
Summary of Extraction Methods for Phenolic Phytoconstituents in Selected Ayurvedic Botanicals

Part of the Plant	Extraction Methods	Methods for Identifying the Phenolic Contents	Phenolic Compounds	References
Nigella sativa				
Phyllanthus amarus				
Aerial	Ultrasonication	HPLC-UV-MS	NS3, NS10, NS11, NS16, quercitrin (PA2), gentisic acids (PA12), isoquercitrin (PA13) and rutoside (PA14)	(Corciovă et al. 2018)
Whole plant	Hot Soxhlet extraction	HPLC-analysis	NS3, NS10, NS16, NS17, PA13	(Guha et al. 2010)
Aerial	Conventional (infusion and decoction)	UHPLC-HRMS	NS1, corilagin (PA6), geranilin (PA15), brevifolin carboxylic acid (PA16), phyllanthusiin C (PA17), amariinic acid (PA19)	(Matou et al. 2021)
Whole plant	Maceration-rotary shaker	HPLC/LCMS	Ellagic acid (PA8)	(Muthusamy et al. 2018)
roots	maceration	HPLC/LCMS	NS1, NS6, PA12, galloyl methoxycinnamic acid hexoside (PA18), quercetin 3-malonylglucoside (PA20), naringin (PA21), epicatechin gallate (PA22)	(Maity et al. 2013)
Aerial	Ultrasound-assisted and pressurized liquid extraction	UPLC-ESI-QTOF-MS/MS	NS1, PA6, PA8, PA16, PA17, PA19	(Sousa et al. 2016)

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4.5 EVALUATION OF ANTIBACTERIAL ACTIVITIES OF AYURVEDIC BOTANICALS USING BIOINFORMATICS APPROACHES

4.5.1 Andrographis paniculata

Cystic fibrosis (CF) is a biofilm-mediated chronic infection of the lung usually caused by *P. aeruginosa*. A methanolic extract of AP experimentally demonstrated effective inhibition of the biofilm and growth of clinical isolates of *P. aeruginosa*. The major (32) phytochemicals in the extract identified by gas chromatography–mass spectrometry (GC-MS) were evaluated for quorum sensing on the organism using molecular docking. Notably, three phenolic phytochemicals, 6H-dibenzo(b,d) pyran-1-ol (AP1), 2-methoxy-4-(1-phenylpropan-2-ylamino)methylphenol (AP2), and 2-methyl-5-(6-methylhept-en-2-yl)phenol (AP3) (Figure 4.1) were among the compounds with the strongest binding affinities for possibly inducing antibiofilm- and quorum quenching–like effects. These were indicated by the high docking scores and interactive poses with relevant biological structures of *P. aeruginosa* including the RhIG (PDB entry 2B4Q), pseudaminidase (PDB entry 2W38), and LasR (PDB entry 3JPU). The selected compounds displayed interesting drug-like properties, promoting them as promising future antibiotics for CF upon further study (Murugan et al. 2013).

4.5.2 Phyllanthus amarus

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Leptospirosis is a bacterial disease caused by acute or chronic infections of the pathogenic *Leptospira* species. Its ability to evade immune systems and colonize within renal tubules constitutes challenges to treatment. An investigation of phytochemicals isolated from PA including flavonoids and phenolic 4-(3-(3,4dimethoxybenzyl)-4-methoxy-2-(methoxymethyl)butyl)-3,6-dimethoxybenzene-1,2-diol (PA23) (Figure 4.2)

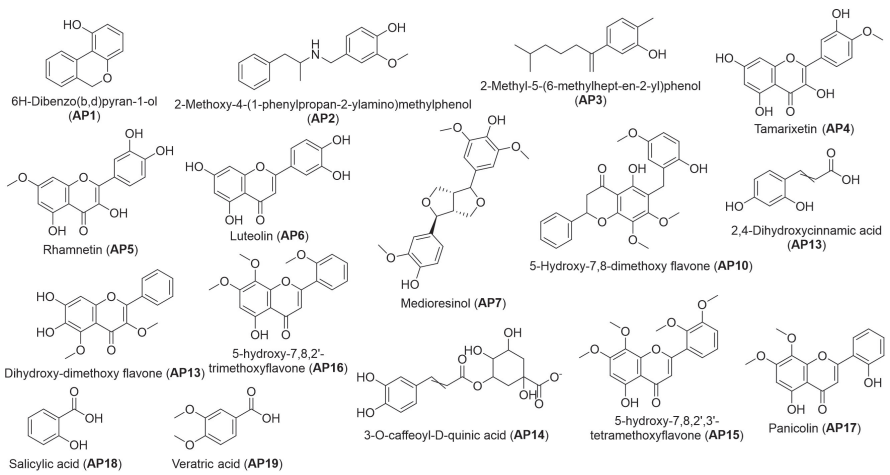


FIGURE 4.1 Chemical structures of some phenolic compounds in *Andrographis paniculate*.

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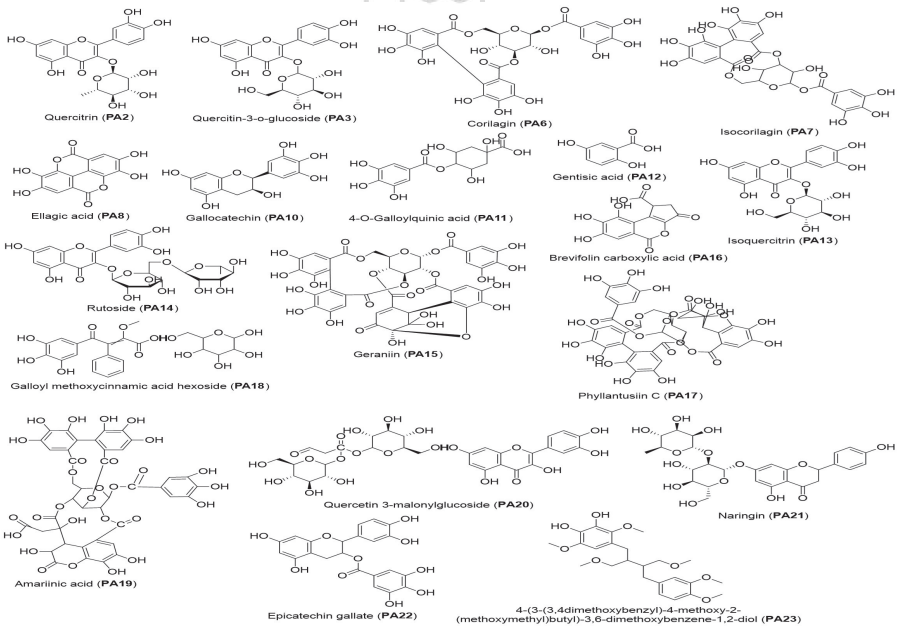


FIGURE 4.2 Chemical structures of some phenolic compounds in *Phyllanthus amarus*.

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showed interesting anti-leptospiral performance of the compounds. Specifically, bioinformatic *in silico* studies involving homology modelling of the *Leptospira* protein (*L. interrogans* serovar Lai str. 56601) from the relevant FASTA sequence were obtained from NCBI using RaptorX software. Molecular docking of PA23 onto the active binding pocket of the structure displayed potent binding to amino acid residues essential for inhibition, including Lys 30, Thr 40, Val 47, Lys 48, Leu 50, Arg 83, Asp 168, and Thr 169. Further validation through *in vitro* and *in vivo* analyses confirmed the excellent anti-leptospiral activities of the phenolic compound, making it worthy of therapeutic consideration (Chandan et al. 2022).

4.5.3 Nigella sativa

Tuberculosis (TB) remains notorious among bacterial infections. It is caused by *Mycobacterium tuberculosis* with its RNA polymerase (RNAp) essentially aiding replications, as such recognized as a plausible therapeutic target. In exploration for potential inhibitors of the target, some major phytoconstituents of NS were screened for prospective candidates using the bioinformatics method of molecular docking against the RNAp of *M. tuberculosis* (PDB entry 5UHB). In particular, carvacrol (NS18), nigellidine (NS19), thymol (NS20), and thymohydroquinone (NS21) (Figure 4.3) were recorded with docking scores of −5.0, −6.4, −4.6, and −4.6 kcal/mol, respectively, although the binding affinities were significantly lower than those of α-hederin, a non-phenolic content, and rifampicin, a positive control which

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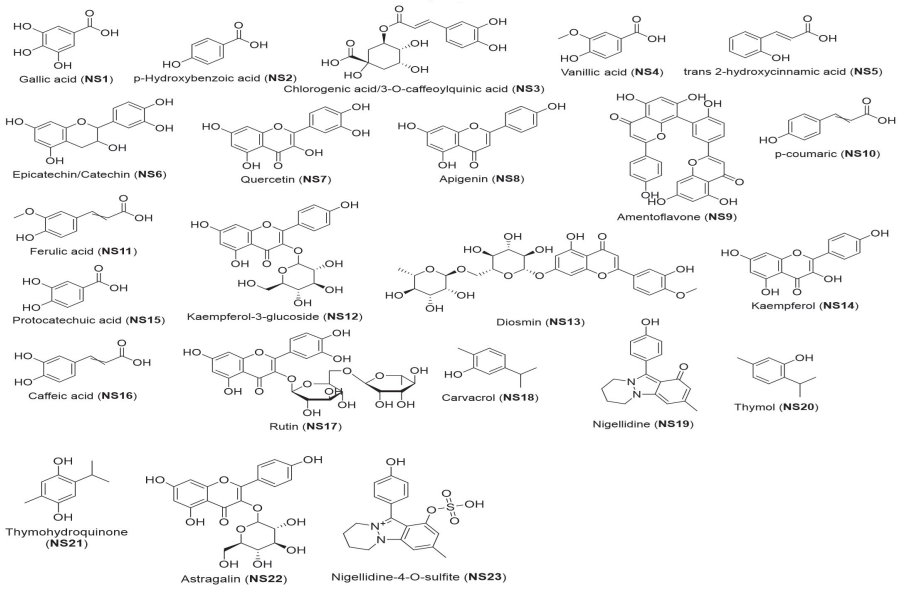


FIGURE 4.3 Chemical structures of some major phenolic compounds in *Nigella sativa*.

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was recorded with −8.9 and −10.5 kcal/mol, respectively (Ahmad Mir et al. 2022). However, the synergistic effects of the compounds could cover therapeutic effects on the NS extract amenable for effective prevention and treatment of TB upon further evaluation. The summary of antibacterial activities of some phenolic compounds from the selected Ayurvedic botanicals evaluated using bioinformatics approaches is represented by Table 4.2.

4.6 ANTIBIOTIC RESISTANCE BY METALLO-B-LACTAMASES AND INHIBITORY INTERACTIONS OF SOME PHENOLIC COMPOUNDS FROM THE SELECTED BOTANICALS USING BIOINFORMATICS APPROACHES

Antibiotic resistance (AR) remains a major public health challenge of the 21st century and is currently among the leading causes of death worldwide. The burden of its rapid spread globally has become critical and could trigger the emergence of much more deadly pathogenic organisms than the existing ones if left unchecked (CDC 2019; Murray et al. 2022). Metallo-β-lactamases are notorious chromosomal AR-inclined enzymes produced by carbapenem-resistant gram-negative bacteria (Tan et al. 2021; Kar et al. 2021). They are predominantly implicated in nosocomial infections mediated by deadly bacterial superbugs: the *Enterobacterales* including *E. coli, K. pneumonia, P. aeruginosa, Acinetobacter baumannii,* and *S. aureus*, making the organisms among the leading causes of AR-attributed deaths (Murray et al. 2022). The New Delhi metallo-β-lactamase (NDM-1), imipenemase (IMP-1), and Verona

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TABLE 4.2
Summary of Antibacterial Investigation of Polyphenols and Some Other Metabolites from Selected Ayurvedic Botanicals Using a Bioinformatics Approach

Ayurvedic Botanical	Phenolic Phytochemicals	Target Organism	Mechanisms of Antimicrobial Action	Bioinformatics Tool	Interactive Structure	PDB Entry	Reference
AP	6H-dibenzo(b,d)pyran-1-ol, 2-methoxy-4-[(1-phenylpropan-2-ylamino)methylphenol and 2-methyl-5-(6-methylhept-en-2-yl)phenol	*P. aeruginosa*	Induction of antibiofilm and quorum quenching effects	Molecular docking	RhIG LasR Pseudaminidase	2B4Q 3JPU 2W38	(Murugan et al. 2013)
NS	Carvacrol, nigellidine, thymol and thymohydroquinone	*M. tuberculosis*	Inhibition of bacterial polymerase activities for replication	Molecular docking; MD	RNAp	5UHB	(Ahmad Mir et al. 2022)
PA	4-(3-(3,4dimethoxybenzyl)-4-methoxy-2-(methoxymethyl) butyl)-3,6-dimethoxybenzene-1,2-diol	*Leptospira* sp.	Inhibition of *Leptospira* protein	Molecular docking	HM of *Leptospira* protein		(Chandan et al. 2022)

Hint: AP = *Andrographis paniculata;* PA = *Phyllanthus amarus;* NS = *Nigella sativa;* MD = molecular dynamics; HM = homology model

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integron-encoded metallo-β-lactamase (VIM-1) represent the most relevant MBLs. They were first discovered in 1988, 1997, and 2008, respectively, and currently have over 1000 variants overwhelming the global healthcare systems through incessant bacterial epidemic diseases. More worrisomely, they evade almost all labelled β-lactam antibiotics even in combinations despite their therapeutic strength, limiting successful inhibitors for clinical applications up to the present day (Boyd et al. 2020; J. Chen, Wang, and Zhu 2017; Kar et al. 2021; Tan et al. 2021). Some recent scientific opinions detailed the insufficiency in the explorations for identifying potent inhibitors of MBLs, making the expansion of the scientific quest for therapeutic interventions to overturn the menace a critical need (CDC 2019; Mojica et al. 2022).

Mechanisms of MBL-mediated AR include the covalent binding of the enzymes to β-lactam antibiotics at the Zn(II) coordination site, thereby inducing the catalytic hydrolysis and eventual cleavage of the β-lactam ring, an active pharmacophore in their structures (Yusuf Oloruntoyin Ayipo, Osunniran et al. 2022; Lima et al. 2020). Thus, effective inhibition of MBLs by covalent inhibitors, especially with structural affinities to the Zn cofactor for coordination-inclined sequestration, could weaken the catalytic functions of the enzymes and promote the therapeutic integrity of antibiotics. Recently, some putative inhibitors of the MBLs, including NDM-1, were identified from natural product phytochemicals using a bioinformatics approach (Kar et al. 2021; Salari-jazi et al. 2021). However, little is known about the propensity of phenolic compounds from Ayurvedic botanicals for inhibiting the notorious enzymes.

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In this chapter, inhibitory potentials of the identified phenolic constituents of NS were investigated against the renowned MBLs, NDM-1, IMP-1, and VIM-1 using molecular docking as a bioinformatic tool. The representative compounds (Figure 4.3) were drawn as 2D molecular structures and converted to the simplified molecular-input line-entry system (SMILES) using the cheminformatics tool ChemDraw 20.1.1 version. Then ligands were prepared using Maestro 12.2 (LigPrep, Schrodinger, LCC, New York, NY, 2019) embedded with Optimized Potentials for Liquid Simulations-3e (OPLS-3e) force fields (Harder et al. 2016). The crystal structures for NDM-1, IMP-1, and VIM-1 were retrieved from the open-access Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) with respective annotations, PDB entry 5YPL, PDB entry 1JT, and PDB entry 5N5H (Burley et al. 2021). The structures were then prepared in the workspace of Maestro 12.2 using Protein Preparation Wizard (Protein Preparation, Schrodinger, LCC, New York, NY, 2019). These were followed by molecular ligand-receptor Glide docking available in Maestro 12.2 (Ligand Docking, Schrodinger, LCC, New York, NY, 2019). The interactive potentials of the phenolic compounds were assessed in terms of scoring algorithms and binding poses for bonding and non-bonding interactions in comparison with imipenem and meropenem, standard reference inhibitors. Being theoretical procedures, the docking protocols were validated by redocking a co-crystallized inhibitor of IMP-1, biaryl succinic acid (BASA), onto the active pocket of the crystal structure (PDB entry 1JJT). Both the co-crystallized and redocked structures were then superimposed and the root means square deviation (RMSD) between them was estimated. The structures showed a good alignment at the active pocket of IMP-1 with an RMSD value of 0.6013 Å (Figure 4.4), a value much less than 2.0 Å, validating the docking procedures as precise and reliable (Castro-Alvarez,

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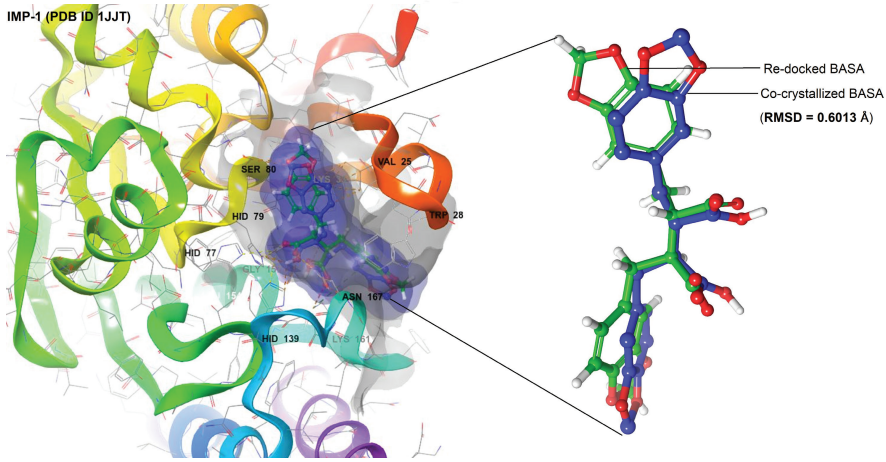


FIGURE 4.4 Validation of docking procedures. Both co-crystallized and redocked biaryl succinic acid superimposed at the active pocket of IMP-1 produced an aligned structure with an RMSD value of 0.6013 Å.

Costa, and Vilarrasa 2017; Ramírez and Caballero 2018; Yusuf Oloruntoyin Ayipo, Alananzeh et al. 2022).

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The binding affinities of the phytochemicals to the probed enzymes are represented by the docking scores, arranged in descending order of negative values. The more negative the number, the higher the affinity. From the results against the enzymes (Tables 4.3–4.5), imipenem and meropenem are the clinical inhibitors of the enzymes, even though they are susceptible to their hydrolytic deactivation. Among the three enzymes, imipenem showed the highest affinity, indicated by the consistent top-ranked docking scores. Among the phenolic compounds of NS, compounds 3, 12, 17, and 22 displayed consistently high docking scores across the probed enzymes competitively with imipenem and higher than meropenem in all cases. The scores are functions of their binding interactions with amino acid residues and Zn co-factor at the active pockets of the enzymes. The highlighted phenolic compounds bonded to various active site residues via H-bonding, π-cation, and the salt bridge as hydrophilic interactions. They showed more hydrophobic π-π and non-bonding van der Waals interactions with other amino acid residues, as well as coordinate covalent bonds to Zn(II) ions. These synergistically confer high binding affinities on them and promote them as potential inhibitors for further study. In addition, their coordinate covalent bond formations to the cationic Zn(II) ions indicate propensities for Zn-chelation effects mostly favoured by electron-rich ligands such as carbonate, sulphate, and acetate, along with hydroxide and succinate groups. In particular, this Zn-sequestration has been recognised as one of the most potent strategies for deactivating the enzymes to conserve the antibiotic actions of drugs and defeat AR (Legru et al. 2021; Yusuf Oloruntoyin Ayipo, Osunniran et al. 2022).

The 2D and 3D diagrams of bonding and non-bonding interactions of the selected compounds 3, 12, 17, and 22 at the active pockets of the enzymes under probe are

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TABLE 4.3
Docking Results of Representative Phenolic Compounds with VIM-1 (PDB Entry 5N5H)

Compound	Docking Score (kcal/mol)	H-bond Interactions	Other Bonding Interactions
Imipenem	−12.960	Asp 118, Glu 146, Asp 213(2)	Zn 301, Asp 213, Glu 146
NS17	−12.163	Asp 117, Asp 118	His 201
NS22	−11.323	Asp 118, Glu 146, Asn 210, Asp 213	Phe 62, His 116
NS12	−11.301	Asp 118, Glu 146, Asn 210, Asp 213	Phe 62, His 116
NS3	−10.796	Glu 201, Asn 210, His 240	Zn 301, His 201, His 240
Meropenem	−10.699	Asp 117, Asn 148	Zn 301, Phe 62
NS13	−10.337	Ser 61, Asp 81, Tyr 87, Asp 118	Phe 62(2)
NS6	−8.291	Asp 17	Tyr 67, His 116, His 240
NS14	−7.836	Asp 117	Zn 302
NS7	−7.543	Asp 117, Glu 146	His 116
NS1	−6.704	Asp 118, Asn 210	His 240
NS16	−6.702	Asp 118, Asn 210	
NS8	−6.301	Glu 146	Phe 62, His 116(2)
NS9	−6.238	Glu 146	Phe 62(2), His 116
NS10	−6.236	–	Zn 301, Zn 302, Phe 62, His 116
NS11	−6.117	Asp 118, Asn 210	–
NS15	−6.054	Asp 118, Asn 210	His 240
NS4	−5.879	Asn 210	Zn 301
NS21	−5.544	Asp 118, Asn 210	His 240
NS23	−5.363	Asn 210	His 240
NS19	−5.316	Asp 117	Zn 302
NS2	−5.149	–	Zn 301
NS20	−4.981	Asp 118	His 240
NS18	−4.715	Asp 118	His 240
NS5	−3.574	Trp 87, Asp 117, Asp 118	His 240

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TABLE 4.4
Docking Results of Representative Phenolic Compounds with IMP-1 (PDB Entry 1JJT)

Compound	Docking Score (kcal/mol)	H-bond Interactions	Other Bonding Interactions
Imipenem	−10.169	Lys 161, Asn 167	Zn 251
NS17	−11.082	Asp 81, Asp 109, Asn 167, Asp 170	–
NS22	−10.774	Ile 160, Lys 161, Gly 164, His 197	His 197
NS12	−8.170	Gly 164, Asn 167	Zn 252, His 197

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(*Continued*)

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TABLE 4.4 (*Continued*)
Docking Results of Representative Phenolic Compounds with IMP-1 (PDB Entry 1JJT)

Compound	Docking Score (kcal/mol)	H-bond Interactions	Other Bonding Interactions
NS3	−12.082	Asn 167	Zn 251, Trp 28
Meropenem	−11.193	Ser 80. Asn 167	Zn 251, His 79
NS13	−9.287	Tyr 163, Gly 166	–
NS6	−7.898	Gly 164(2)	–
NS14	−7.318	Asp 81	Trp 28(2)
NS7	−7.820	Trp 28, Lys 161	Trp 28
NS1	−9.268	Asn 167	Zn 251, His 197
NS16	−9.529	Asn 167	Zn 251
NS8	−6.718	Lys 161	Trp 28
NS9	−10.033	Val 30, Asp 81	Trp 28(2), His 197
NS10	−8.522	Asn 167	Zn 251
NS11	−9.183	Asn 167	Zn 251
NS15	−8.930	Asn 167	Zn 251, His 197
NS4	−8.610	Asn 167	Zn 251
NS21	−4.769	–	–
NS23	−6.722	Asn 167, Asp 170	Zn 251(2)
NS19	−5.154	–	–
NS2	−7.900	Asn 167	Zn 251
NS20	−4.872	–	–
NS18	−4.863	–	–
NS5	−9.062	Asn 167	Zn 251

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TABLE 4.5
Docking Results of Selected Phenolic Compounds with NDM-1 (PDB Entry 5YPL)

Compound	Docking Score (kcal/mol)	H-bond Interactions	Others Bonding Interactions
Imipenem	−12.165	Glu 152, Asn 220, Asp 223	Zn 301, Asp 223
NS17	−9.650	Asp 124, Glu 152, Asp 223	His 250
NS22	−8.653	Gln 123(2), Lys 211	–
NS12	−8.527	Gln 123(2), Asp 124, Lys 211	His 250
NS3	−10.157	Asp 212(2), Asn 220	Zn 301
Meropenem	−11.523	Gln 123, Asn 220, Lys 211	Zn 301
NS13	−10.177	Glu 152(2), Ser 217(2)	–
NS6	−7.848	Gln 123, Asp 124, Glu 152	–

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TABLE 4.5 (*Continued*)
Docking Results of Selected Phenolic Compounds with NDM-1 (PDB Entry 5YPL)

Compound	Docking Score (kcal/mol)	H-bond Interactions	Others Bonding Interactions
NS14	–8.248	Gln 123, Lys 211	–
NS7	–7.927	Gln 123, Asp 124	–
NS1	–7.498	Lys 211, Asn 220	Zn 301
NS16	–8.674	Lys 211, Asn 220	Zn 301, His 250
NS8	–6.202	Gln 123, Lys 211	–
NS9	–7.278	Phe 70, Asp 124, Asp 212	Lys 211
NS10	–8.320	Gln 123	Zn 301, His 122, Lys 211
NS11	–8.526	Gln 123	Zn 301(2), His 122
NS15	–7.035	Lys 211, Asn 220	Zn 301
NS4	–6.709	Lys 211, Asn 220	Zn 301
NS21	–4.884	Asp 124	–
NS23	–5.412	Gln 123, Asn 220	Zn 301(2)
NS19	–5.563	Gln 123	Zn 301
NS2	–6.570	Asn 220	Zn 301
NS20	–4.792	Asp 124	–
NS18	–4.649	Asp 124	–
NS5	–7.189	Asn 220	Zn 301

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presented in Figures 4.5–4.7. Accordingly, Zn1 and Zn2 are renowned metal co-factors in the active substrate-binding pockets of binuclear MBLs in addition to relevant amino acid residues such as Tyr 67, Trp 87, His 201, Glu 202, His 116, Asp 117, Asp 118, Ala 208, Asn 210, and His 240 (Tooke et al. 2019; Kar et al. 2021). These are consistent with the catalytic active site of VIM-1 (PDB entry 5N5H) herein defined (Figure 4.5).

The selected phenolic compounds displayed bonding and non-bonding interactions with the active site residues comparably to the reference inhibitors, indicating similar functional binding affinities. The hydrophobic pocket of IMP-1 (PDB entry 1JJT) inclusively contains Glu 23, Val 25, and Phe 51 in the proximity of the Zn(II) ion, while Trp 28 on the flexible loop 1 enhances the stabilization of the hydrophobic binding of ligands (Yamaguchi et al. 2021). The key amino acid residues for substrate sensitivity of the enzymes include Trp 28, Lys 161, Asn 167, and Zn ions for catalytic coordination (Toney et al. 2001; Arjomandi, Kavoosi, and Adibi 2019; Yamaguchi et al. 2021). As shown in Table 4.4 and Figure 4.6, the selected phenolic compounds 3, 12, 17, and 22 interacted with a good number of the enlisted residues via the hydrophilic H-bonding, π-cation, salt-bridge formation, and coordinate covalent bonds to the metal site. In addition, they displayed hydrophobic properties to some of the enlisted amino acid residues through π-π stacking and non-bonding van der Waals forces. These confer strong binding affinities on them, as shown in docking scores and support their inhibitory potentials against the enzymes.

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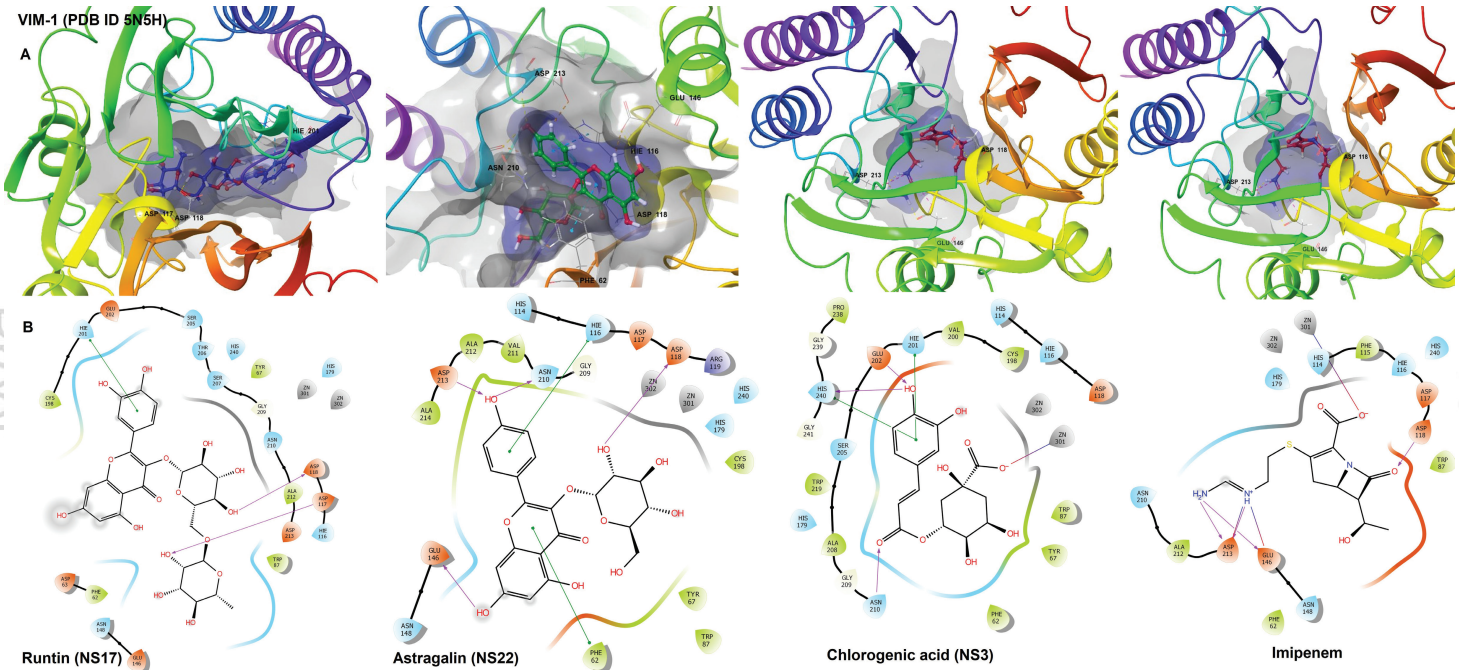


FIGURE 4.5 2D and 3D binding pose showing bonding and non-bonding interactions of rutin, astragalin, and chlorogenic acid with active site residues of VIM-1 (PDB entry 5N5H). Amino acid colours: Red—charged negative; deep blue—charged positive; pale blue—polar; yellow-green—hydrophobic. Bonds: Purple arrow—H-bond; blue-red line—salt bridge; Red line—π-cation; green line—π-π stacking.

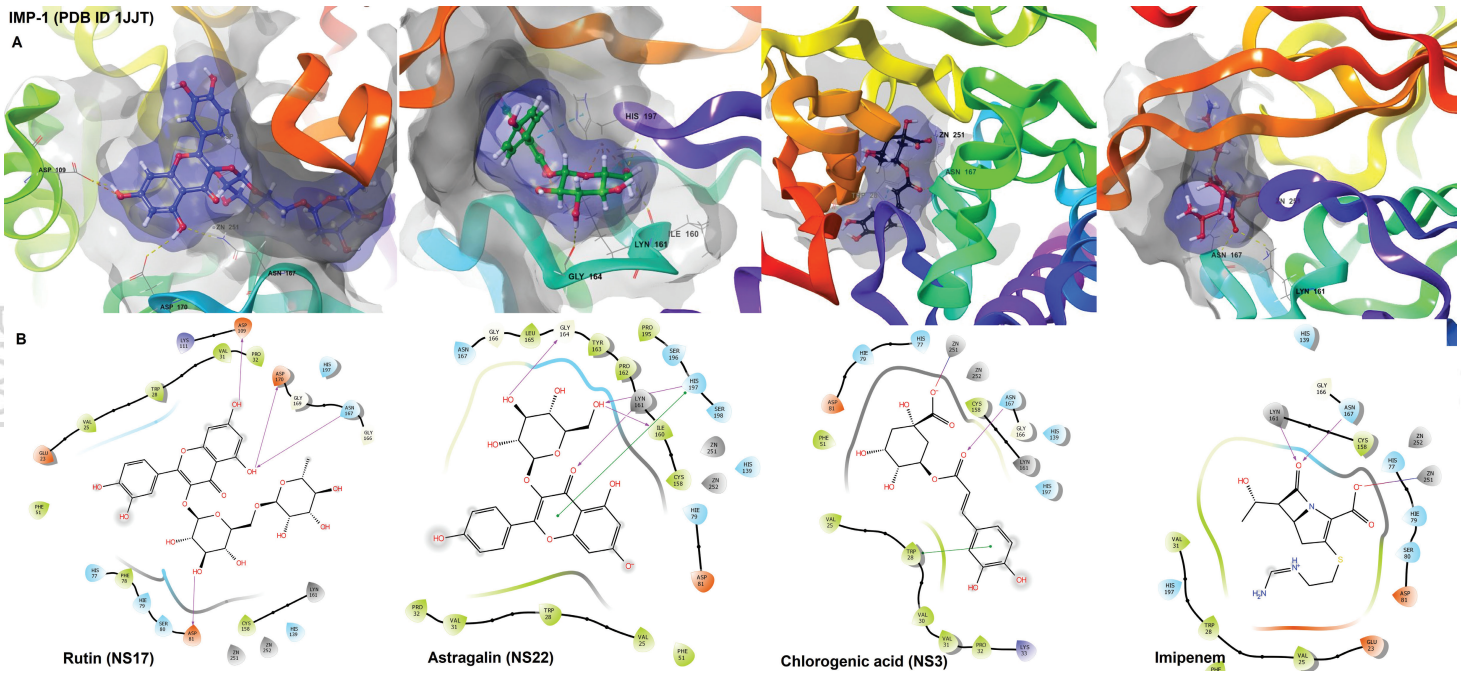


FIGURE 4.6 2D and 3D binding pose showing bonding and non-bonding interactions of rutin, astragalin, and chlorogenic acid with active site residues of IMP-1 (PDB entry 1JJT). Amino acid colours: Red—charged negative; deep blue—charged positive; pale blue—polar; yellow-green—hydrophobic. Bonds: Purple arrow—H-bond; blue-red line—salt bridge; red line—π-cation; green line—π-π stacking.

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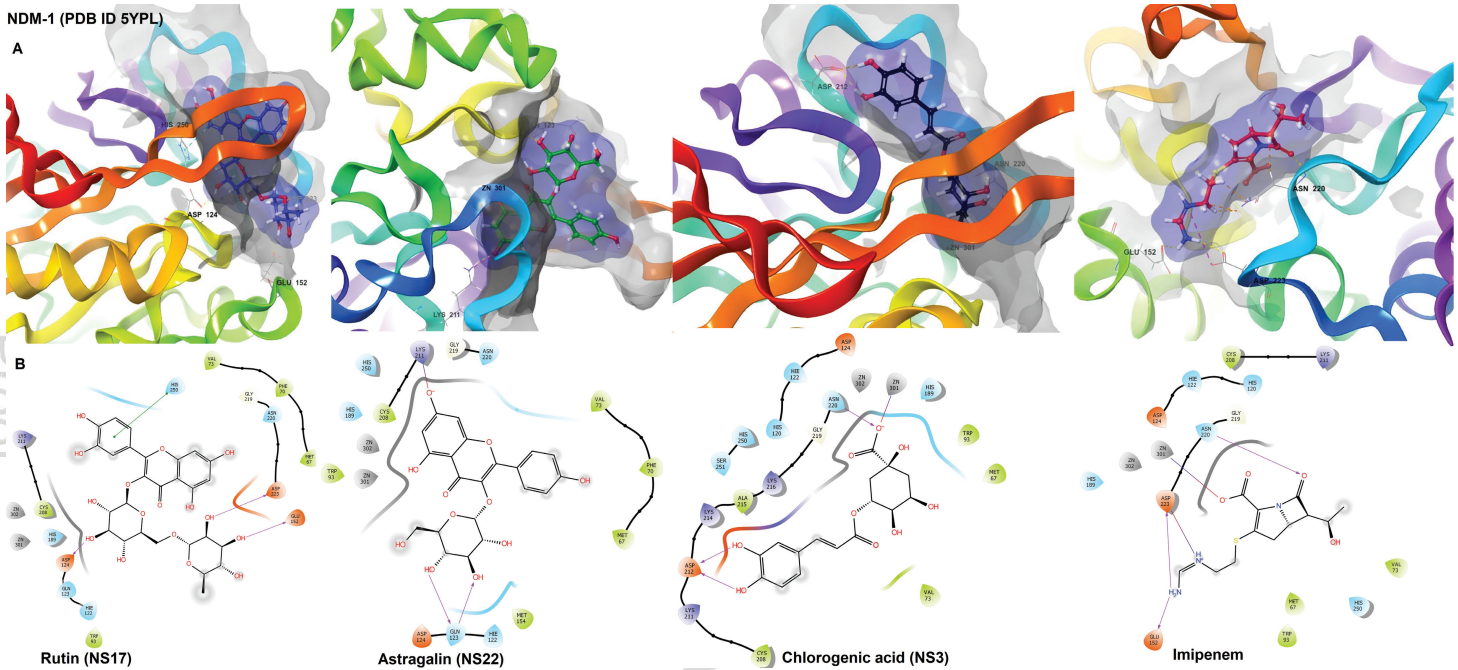


FIGURE 4.7 2D and 3D binding pose showing bonding and non-bonding interactions of rutin, astragalin, and chlorogenic acid with active site residues of NDM-1 (PDB entry 5YPL). Amino acid colours: Red—charged negative; deep blue—charged positive; pale blue—polar; yellow-green—hydrophobic. Bonds: Purple arrow—H-bond; blue-red line—salt bridge; red line—π-cation; green line—π-π stacking.

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The catalytic activities of NDM-1 and MBLs generally are influenced by the coordination of Zn ions to some active site residues such as His 120, 122, 189, and 250. Other amino acid residues including Gln 123, Asp 124, Glu 152, Cys 208, Lys 211, Asn 220, and 223 inclusively constitute the binding pocket of NDM-1 and mediate the Zn-dependent catalytic functions (Salari-jazi et al. 2021; Kar et al. 2021; Wang et al. 2020). Hypothetically, the binding of inhibitors to these critical residues could limit their flexibility for antibiotic inactivation and distort the catalytic integrity of the enzymes to promote antibiotic effects (Moreira et al. 2021). From Table 4.5 and Figure 4.7, the selected phenolic compounds bind to many of the relevant residues, including Zn(II) ions, in similar patterns to the reference inhibitor imipenem. This indicates plausibility for covalent bonding and Zn-targeting inhibitory interactions in some cases, whereas shreds of experimental pieces of evidence have shown that Zn-sensitive ligands could be ideal selective inhibitors of MBLs (Bush 2018; A. Y. Chen 2020). Overall, the application of a bioinformatic tool, molecular docking, has revealed the inhibitory potentials of phenolic compounds in NS on MBLs. The results promote them as promising candidates amenable to combating the global AR challenges upon experimental validation and other translational studies.

4.7 ANTIVIRAL ACTIVITIES USING BIOINFORMATICS APPROACHES

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4.7.1 Andrographis paniculata

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About 650 flavonoids from some medicinal plants of Pakistan and India were evaluated for inhibitory potentials against the non-structural protein (nsp) 1–4 of chikungunya virus (CHIKV) using bioinformatics techniques. The crystal structures for nsp 2 and nsp 3, PDB entry 3TRK, and PDB entry 3GPG were retrieved from the RCSB PDB (Burley et al. 2021), while the homology protein structures for nsp 1 and nsp 4 were modelled using I-TASSER (Zhang 2008) and validated by Ramachandran plots using the RAMPAGE server (Lovell et al. 2003). In particular, some phenolic compounds from AP, 3',4',5,7-tetrahydroxyflavone 4',5,7-trihydroxyflavone, tamarixetine (AP4), rhamnetin (AP5), luteolin (AP6), and medioresinol (AP7) showed excellent binding affinities to nsp 1 and nsp 3, especially the first two compounds. For instance, the first interacted with some key amino acid residues at the conserved binding pocket of nsp 1 including Ala 19, Ala 21, Arg 22, Asp 27, Arg 28, and Lys 29 with an overall binding energy of −9.4 kcal/mol. Both highlighted compounds bonded strongly to nsp 3 with the respective binding energy of −9.0 and −8.9 kcal/mol, engaging Ala 22, Val 33, Leu 108, Thr 111, Gly 112, and Tyr 114 via various bonding interactions. Further analyses through the density functional theory (DFT) calculations and *in silico* predictions of properties for absorption, distribution, metabolism, excretion, and toxicity (ADMET) support the two compounds as potent inhibitors of CHIKV replication acting through interactions with the nsps for experimental validation (Hussain, Amir, and Rasool 2020).

Similarly, 5-hydroxy-7,6,2',3'-tetramethoxy flavone (AP8), 5-hydroxy-7,8,2',5'-tetramethoxy flavone (AP9), and 5-hydroxy-7,8-dimethoxy flavone (AP10) from AP also displayed strong binding energies ranging from −7.20 to −9.0 kcal/mol, while

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remdesivir, a nucleotide inhibitor of SARS-CoV-2, in clinical application scored between 7.60 and 9.50 kcal/mol against the targets. The predicted ADMET profiles of the phenolic compounds also support their use as drugs, suggesting them worthy of further evaluation as promising candidates for the ravaging SARS-CoV-2 (Hiremath et al. 2021). Similarly, AutoDock Vina and Chimera bioinformatics software tools were also employed to investigate the inhibitory potentials of phytochemicals from AP against the 3CLpro of SARS-CoV-2 (PDB entry 6LU7). Among the phytochemicals, two flavonoids, 5,4'-dihydroxy-7-O-β-D-pyran-glycuronate butyl ester (AP11) and 7,8-dimethoxy-2'-hydroxy-5-O-β-d-glucopyranosyloxyflavone (AP12), displayed strong binding affinities to the protease. This was indicated by the docking scores of −8.37 and −8.20 kcal/mol, respectively, whereas the positive control inhibitor, remdesivir and indinavir each were recorded with −8.23 kcal/mol. The predicted toxicological profiles favour the two compounds relative to others and, as such, portray them as putative candidates for further study as inhibitors of SARS-CoV-2 Mpro (Sukardiman et al. 2020). In another study, Glide molecular docking, in silico ADMET predictions, and binding free energy calculation by molecular mechanics-generalized Born surface area (MMGB/SA) support dihydroxy-dimethoxy flavone from AP as a more potent inhibitor of SARS-CoV-2 Mpro (PDB entry 5R82) than hydroxychloroquine and nelfinavir, and comparably to remdesivir (Kalirajan et al., 2020).

To further express the multi-target therapeutic potentials of phytoconstituents of AP, some phenolic contents, 2,4-dihydroxycinnamic acid (AP13), 3-O-caffeoyl-D-quinic acid (AP14), 5-hydroxy-7,8,2',3'-tetramethoxyflavone (AP15), and 5-hydroxy-7,8,2'-trimethoxyflavone (AP16) were reported with inhibitory interactions for mitigating several activities in terms of SARS-CoV-2 infection, the life cycle, and virulent factors. The activities targeted for suppression include viral entry, replication/synthesis, transcription, RNA binding, promotion of cytokines, formation of ion channels for viral particle release, and blocking of the host's RNA translation and dominant immunogens. The interactive implications for such effects were expressed *in silico* using molecular docking, targeting some relevant viral accessory, structural, and non-structural proteins, including the envelope (E) protein (PDB entry 5X29), membrane (M) protein (PDB entry 3I6G), S protein (PDB entry 6CRV), Mpro (PDB entry 6LU7), nsp 3 (PDB entry 6VXS), nsp 9 (PDB entry 6WXD), nsp 15 (PDB entry 6VWW), Open Reading Frame (ORF) 1a (PDB entry 2G9T) and ORF3a polyprotein (PDB entry 6XDC), and RNA binding domain (RBD) (PDB entry 6M0J). The complexes of the compounds with the proteins' structures were established for stability upon molecular dynamics (MD) simulations, while the predicted ADMET profiles support their candidacy as promising multi-target–directed inhibitors of SARS-CoV-2 amenable for further translational assessments (Swaminathan et al. 2021).

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Eighty-two phytochemicals detected in extracts of AP and *Tinospora cordifolia* were investigated for larvicidal activities via *in silico* and experimental models. The compounds were observed for interactions with the sterol-carrying protein-2 (SCP-2) of *Aedes aegypti*, a mosquito vector of the dengue virus (DENV). Among the

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phytochemicals, panicolin (AP17), a phenolic compound in AP, displayed a strong binding affinity for SCP-2 (PDB entry 1PZ4) with an energy score of −9.86 kcal/mol and interacted with essential amino acid residues for disrupting the viral life cycle upon molecular docking analysis. The *in silico* results further support the experimental outcomes, suggesting the compound as a potential larvicidal agent for controlling the spread of DENV through *A. aegypti* (Paul et al. 2021).

Again, phenolic phytoconstituents of some medicinal plants were investigated for inhibitory potentials against some relevant crucial structures for replication and transcription of DENV using molecular docking. The selected targets include the DENV NS3 protease-helicase (PDB entry 2VBC), DENV 1 NS2/NS3 protease (PDB entry 3L6P), DENV NS2B/NS3 protease (PDB entry 2FOM), DENV 3 protease (PDB entry 3U1J), the second conformation of DENV NS3 protease-helicase (PDB entry 2WHX), DENV 1 E protein (PDB entry 3UZQ), DENV 2 E protein (PDB entry 1TG8), DENV 3 E protein (PDB entry 1UZG), DENV 4 E protein (PDB entry 3UYP), and DENV NS5 RdRp (PDB entry 2J7W). In particular, AP8/NS7 and caffeic acid (AP18/NS16) from AP were among the mostly strongly bound ligands to the targets indicated by high binding energies. The two compounds also interacted with key amino acid residues essential for anti-dengue pharmacology, comparable to the standard inhibitors, thus representing promising multi-target–directed candidates for preventing and treating DENV infections upon further study (Pawar and Patravale 2020).

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4.7.2 Phyllanthus amarus

Thirty-five phytochemicals from PA and AP were investigated for inhibitory potentials against some key life cycle– and virulence- determining proteins of SARS-CoV-2, spike glycoprotein opened (PDB entry 6VYB) and closed form (PDB entry 6VXX), 3-main protease (Mpro)/3-chymotrypsin-like protease (3CLpro; PDB entry 6LU7), papain-like protease (PDB entry 4OVZ), and RNA-dependent RNA polymerase (RdRp; PDB entry 6NUS) using the AutoDock Vina molecular docking tool. Notably, five phenolic compounds, quercetin (PA1/NS7), quercitrin (PA2), quercetin-3-*O*-glucoside (PA3), astragalin (PA4/NS22), and kaempferol (PA5/NS14) from PA, interestingly bound potently to the active pockets of the crystal structures quantitatively with binding affinities between −7.50 and −10.30 kcal/mol. Some phenolic phytochemicals from PA of Nigerian extraction were investigated for inhibitory interactions against the nsp 16 of SARS-CoV-2 (PDB entry 6YZ1) using the AutoDock Vina bioinformatic approach, followed by *in silico* ADMET prediction. The relevant phytochemicals include corilagin (PA6), isocorilagin (PA7), ellagic acid (PA8), gallic acid (PA9/NS1), gallocatechin (PA10), and 4-O-galloylquinic acid (PA11). Although the compounds showed weaker binding energy than the six hits selected by the authors, including andrographolide from AP, whose docking score was recorded as −7.9 kcal/mol. The latter displayed strong H-bond interactions with relevant active pocket residues such as Asn 43, Asp 99, and Asp 130 and was predicted with ideal ADMET properties for drug likeness (Saliu et al. 2021).

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4.7.3 Nigella sativa

Historically, NS is renowned for its anti-inflammatory, antibacterial, and immunomodulatory effects among its vast medicinal properties (Shawky, Nada, and Ibrahim 2020; Pandey et al. 2021). Employing bioinformatics techniques, including molecular docking, with relevant targets CTN and GO enrichment analyses, a recent study revealed the influence of interesting network pharmacological pathways and other potential therapeutic approaches for combating SARS-CoV-2, aided by NS phytochemicals (Shawky, Nada, and Ibrahim 2020). The infusion of SARS-CoV-2 into host cells understandably occurs through the interactions between the viral S protein and the human angiotensin-converting enzyme 2 (ACE2). The process is primarily mediated by the activation of the enzyme ACE2 by the transmembrane protease serine 2 (TMPRSS2). As such, the protease is recognized as a therapeutic target for preventing viral entry and replication, and its renowned inhibitor, camostat, has been reported as active against SARS-CoV-2 (Y.O. Ayipo et al. 2021). The extract of NS reportedly enhanced the helper T-cell as well as suppressor T-cell ratio. Hence, it has increased natural killer cell activity in humans. The extract has also inhibited murine cytomegalovirus and the human immunodeficiency virus (HIV) (Muhammad Torequl Islam, Khan, and Kumar 2019). In a related study, the extract of NS decreased the replication of the virus when the Hela-epithelial carcinoembryonic antigen-related cell adhesion molecule cell was infected with the mouse hepatitis virus (Muhammad T. Islam et al. 2020). In the treatment of SARS-COV-2, the extract of NS was listed among the famous natural immune boosters (Boozari and Hosseinzadeh 2020).

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From bioinformatic perspectives, in an *in silico* evaluation of some spice-derived phytochemicals as prospects for COVID-19, NS18 was shown to interact more potently with the active pocket of the homology model of TMPRSS2, with a docking score of −3.90 kcal/mol, than camostat, which scored −3.19. The compound reportedly bound tightly to the oxyanion hole and catalytic triad of the enzyme, engaging the catalytic domain residues His 296, Asp 345, and Ser 441 in bonding interactions. The MD simulation and ADMET prediction also support the drug potential of the phenolic compound as a promising candidate for prophylaxis and treatment of SARS-CoV-2, deserving further scientific attention (Yadav, Jaiswal, and Singh 2021). Similarly, in an exploration of NS for identifying potent chemotherapeutics for COVID-19 using bioinformatics approaches of molecular docking, MD, and ADMET predictions, NS18, NS20, and NS21 displayed strong inhibitory interactions with the SARS-CoV-2 RBD-ACE2 interface (PDB entry 6VW1). Their affinities were quantified by the docking scores of −7.0, −6.0, and −6.1 kcal/mol, respectively, consistently supported by post-MD molecular mechanics Poisson-Boltzmann surface area (MMPB/SA) scores. In particular, NS18 scored closely to chloroquine, a reference control which was recorded at −7.2 kcal/mol. The MD simulations favour their stability with the protein structures, and the predicted ADMET properties support their drug likeness and safety, making them worthy of further consideration (Ahmad et al. 2021). Although NS7, NS20, and NS22 were not among the selected 10 candidates when 25 phytochemicals of NS were investigated against the nucleocapsid (N) protein (PDB entry 6M3M), Mpro (PDB entry 6M03), and PLpro (PDB 6W9C) for SARS-CoV-2 replication using bioinformatic techniques. However, the compounds also displayed

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fair binding interactions indicated by respective docking scores of −7.15, −4.04, and −7.22 kcal/mol with Mpro and −5.16, −4.95, and −5.31 kcal/mol with PLpro and ideal ADMET profiles (Siddiqui et al. 2022). Similarly, remdesivir and α-hederin, non-phenolic phytoconstituents of NS, showed higher binding affinities to the RdRp of SARS-CoV-2 (PDB entry 6M71) with docking scores of −7.6 and −8.6 kcal/mol, respectively, compared to the phenolic NS18, NS19, NS20, and NS21, which scored −4.5, −6.0, −4.3, and −4.6 kcal/mol, respectively, upon molecular docking analysis (Mir et al. 2022). However, their interactions with RdRp deserve further scientific attention through experimental validation in the quest for RdRp inhibitors for treating COVID-19. Interestingly, the four compounds again expressed higher binding affinities than remdesivir and hydroxychloroquine (HCQ) when investigated against some key targets for suppressing viral infusion, replication, and transcription. These include the 3CLpro (PDB entry 6LU7), RBD-ACE2 interface (PDB entry 6VW1), prefusion S protein (PDB entry 6VSB), and S protein (PDB entry 6VXX). The four phenolic phytochemicals were recorded with docking scores ranging from −6.01 to −4.30 kcal/mol against the receptors, whereas remdesivir and HCQ showed binding energies in the range of −4.09 to 16.82 kcal/mol and −4.83 to −3.61 kcal/mol against the same target. Further, NS18, NS20, and NS21 were selected phenolic phytochemicals among the 13 major phytoconstituents of NS screened for inhibitory potentials against the 3CLpro and nsp 15 of SARS-CoV-2 using bioinformatics approaches involving molecular docking, MD simulation, and ADMET prediction. The compounds were recorded with docking scores of −5.20, −5.19 and −5.35, respectively, against the 3CLpro (PDB entry 6LU7), although the values were lower than −7.95 kcal/mol for lopinavir, a control inhibitor of the target. However, the compounds with the respective binding affinities of −5.50, −5.27, and −5.90 kcal/mol, also bound potently to nsp 15 (PDB entry 6VWW) comparably to the selected control, benzopurpurin B, which scored −5.87 kcal/mol. The predicted physicochemical and ADMET properties, as well as stability with the proteins upon MD simulation, suggest the compounds worthy of further study as prospective candidates for mitigating SARS-CoV-2 replication and transcription (Rizvi et al. 2021). Moreover, an investigation of 58 phytochemicals reportedly extractable from NS using bioinformatics approaches including molecular docking and ADMET predictions favoured NS17, NS19, and NS22 as promising multi-target–directed inhibitors of SARS-CoV-2 among other phenolic contents. The compounds were recorded with respective binding affinities in the ranges of −6.9 to −9.8 kcal/mol, −6.3 to −8.8 kcal/mol, and −6.2 to −8.7 kcal/mol, respectively, when virtually screened against some relevant viral proteins for replication and transcription. These include the crystal structures for Mpro free enzyme (PDB entry 6Y2E), PLpro (nsp 3; PDB entry 6W9C), RdRp (nsp 12; PDB entry 6M71), RBD (PDB entry 6M17), endoribonuclease (nsp 15; PDB entry 6VWW), RNA-binding protein (nsp 9; PDB entry 6W4B), RBD of N protein (PDB entry 6VYO), and nsp 13 helicase (PDB entry 6ZSL). Meanwhile, chloroquine, a reference drug, scored significantly lower with binding affinities ranging from −4.7 to −6.3 kcal/mol against the enlisted targets. Interestingly, the selected compounds were predicted with ideal profiles for drug potential and safety, making them promising therapeutic prospects for COVID-19 with network pharmacological effects amenable for further investigation (Baig and Srinivasan 2022).

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Another study was conducted using a bioinformatics approach to promote therapeutic effects of the "miraculous herb" NS for all diseases including emerging ones such as COVID-19 according to prophetic and biblical assertions. In the study, the results from the molecular docking, MD simulations, and predictions of physicochemical properties projected NS19 and NS23 as promising inhibitors of SARS-CoV-2 Mro (PDB entry 6LU7). For instance, the compounds showed respective higher docking scores of −7.8 kcal/mol and −8.2 kcal/mol than a renowned Mpro ligand, leupeptin, with −7.6 kcal/mol, although N3, another Mpro ligand, scored the highest with −9.1 kcal/mol (Hardianto et al. 2021). The voluminous interesting reports from the *in silico* and bioinformatics evaluation of NS further support the worthiness of the medicinal plant as a promising candidate for COVID-19.

To further express the inhibitory potential of NS, its major phytochemicals were screened for interaction with ACE2 using molecular docking analysis. Two phenolic compounds, NS18 and NS21, displayed strong binding affinities to the active pocket of ACE2 (PDB entry 1R4L), especially the latter, whose docking score (−5.466 kcal/mol) was very close to that of the top-ranked non-phenolic ligand, α-hederin (−6.263 kcal/mol). Meanwhile, bioinformatic tools for network interaction analysis have been explored to further validate the molecular correlations between the ACE2 and some relevant biomolecules for hypoxia- and inflammatory-related mechanisms associated with COVID-19. The interaction of the ligands with ACE2 further suggests the propensity of NS phytochemicals for therapeutic designs against the virulent checkpoint in the COVID-19 episode (Jakhmola Mani et al. 2022). The summary of antiviral activities of some phenolic phytochemicals from the three selected Ayurvedic botanicals as investigated using bioinformatics is presented in Table 4.6.

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4.8 CONCLUSION AND FUTURE PROSPECTS

Bacterial and viral diseases continuously pose serious threats to human wellness, and they have been responsible for most of the pandemics the world has witnessed. Incessant resistance development by the relevant pathogens, low efficacy, accessibility, and unbearable effects of the currently available medications inclusively constitute therapeutic challenges. This makes continuous scientific efforts towards the discovery of improved alternatives essential. Although the entire process of introducing a new drug for clinical applications is cumbersome, demanding exorbitant costs and other resources, lengthy time frames, and environmental risks, the incorporation of CADD, specifically the bioinformatics approach, ameliorates the challenges and facilitates the process. In this chapter, investigations of antibacterial and antiviral performances of major phenolic phytoconstituents of three Ayurvedic botanicals, *A. paniculata, P. amarus,* and *N. sativa,* were showcased through bioinformatics techniques. Several active phenolic compounds, notably, rutin, astragalin, kaempferol-3-glucoside, and chlorogenic acid, have demonstrated interesting broad-spectrum inhibitory interactions against relevant therapeutic targets of major pathogenic organisms including the multi-drug-resistant metallo-β-lactamase–producing Enterobacteriaceae and the ravaging SARS-CoV-2. The extracts bearing the

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TABLE 4.6
Summary of Antiviral Investigations of Representative Polyphenols and Some Other Metabolites from Selected Ayurvedic Botanicals Using a Bioinformatics Approach

Ayurvedic Botanica	Phenolic Phytochemicals	Target Organism	Mechanisms of Antiviral Action	Bioinformatics Tool	Interactive Structure	PDB Entry	Reference
AP	5,7,3',4'-Tertahydroxyflavone, 5,7,4'-trihydroxyflavone, tamarixetin, rhamnetin, luteolin and medioresinol	CHIKV	Inhibition of viral replication	Homology protein structure modelling; Molecular docking; ADMET prediction	nsp 1 nsp 2 nsp 3 nsp 4	HM 3TRK 3GPG HM	(Hussain, Amir, and Rasool 2020)
AP	Panicolin	DENV	Larvicidal effect of *A. aegypti*	Molecular docking	SCP-2	1PZ4	(Paul et al. 2021)
AP	Quercetin and caffeic acid	DENV	Inhibition of viral replication and transcription	Molecular docking	NS3 protease-helicase NS2/NS3 protease NS2B/NS3 protease DENV 3 protease 2nd conformation NS3 protease-helicase DENV 1 E protein DENV 2 E protein DENV 3 E protein DENV 4 E protein NS5 RdRp	2VBC 3L6P 2FOM 3U1J 2WHX 3UZQ 1TG8 1UZG 3UYP 2J7W	(Pawar and Patravale 2020)

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TABLE 4.6 (*Continued*)
Summary of Antiviral Investigations of Representative Polyphenols and Some Other Metabolites from Selected Ayurvedic Botanicals Using a Bioinformatics Approach

Ayurvedic Botanica	Phenolic Phytochemicals	Target Organism	Mechanisms of Antiviral Action	Bioinformatics Tool	Interactive Structure	PDB Entry	Reference
AP	2,4-dihydroxycinnamic acid, 3-O-caffeoyl-D-quinic acid, 3,7,8-trimethoxy-2-(2-methoxyphenyl)-4 H, 5-hydroxy-7,8,2',3'-tetramethoxyflavone and 5-hydroxy-7,8,2'-trimethoxyflavone	SARS-CoV-2	Suppression of viral entry, replication/synthesis, transcription, RNA-binding, promotion of cytokines, formation of ion channels for viral particles release, blocking of host's RNA translation and dominant immunogens	Molecular docking; Molecular dynamics; ADMET prediction	Mpro nsp 3 nsp 9 nsp 15 E protein M protein S protein ORF1a ORF3a RBD	6LU7 6VXS 6WXD 6VWW 5X29 3I6G 6CRV 2G9T 6XDC 6M0J	(Swaminathan et al. 2021)
AP	5,4'-Dihydroxy-7-O-β-D-pyran-glycuronate butyl ester and 7,8-dimethoxy-2'hydroxy-5-O-β-d-glucopyranosyloxyflavone	SARS-CoV-2	Inhibition of viral replication and transcription.	Molecular docking; Toxicity prediction	3CLpro	6LU7	(Sukardiman et al. 2020)
AP	Dihydroxy-dimethoxy flavone	SARS-CoV-2	Inhibition of viral replication and transcription.	Molecular docking; ADMET prediction; MMGB/SA	Mpro	5R82	(Kalirajan and Varakumar 2020)
AP	5-Hydroxy-7,6,2',3'-tetramethoxy flavone, 5-hydroxy-7,8,2',5'-tetramethoxy flavone and 5-hydroxy-7,8-dimethoxy flavone	SARS-CoV-2	Inhibition of viral replication and transcription.	Molecular docking; ADMET prediction	S-protein (opened) S-protein (closed) 3CLpro PLpro RdRp	6VYB 6VXX 6LU7 4OVZ 6NUS	(Hiremath et al. 2021)

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NS	Carvacrol	SARS-CoV-2	Inhibition of viral entry	Molecular docking; ADMET prediction	TMPRSS2	HM	(Yadav, Jaiswal, and Singh 2021)
NS	Carvacrol, nigellidine, thymol, thymohydroquinone	SARS-CoV-2	Disruption of viral-host entry interface; inhibition of viral replication	Molecular docking; ADMET prediction	RBD-ACE2 3CLpro Prefusion S protein S protein (opened)	6VW1 6LU7 6VSB 6VXX	(Ahmad et al. 2021; Pandey et al. 2021)
NS	Carvacrol and thymohydroquinone	SARS-CoV2	Inhibitory interactions with hypoxia- and inflammatory-related checkpoint for COVID-19 virulence	Network interaction analysis; Molecular docking	ACE2	1R4L	(Jakhmola Mani et al. 2022)
NS	Astragalin, quercetin, and thymol	SARS-CoV-2	Inhibition of viral replication and transcription	Molecular docking; MD; ADMET prediction	N protein Mpro PLpro	6M3M 6M03 6W9C	(Siddiqui et al. 2022)
NS	Rutin, nigellidine, and astragalin	SARS-CoV-2	Inhibition of viral entry, replication/synthesis, transcription, RNA binding, and RNA translation	Molecular docking; ADMET prediction	Mpro PLpro RdRp RBD nsp 15 nsp 9 RBD of N protein nsp 13 helicase	6Y2E 6W9C 6M71 6M17 6VWW 6W4B 6VYO 6ZSL	(Baig and Srinivasan 2022)
NS	Carvacrol, nigellidine, thymol, and thymohydroquinone	SARS-CoV-2	Inhibition of viral replication and synthesis	Molecular docking	RdRp	6M71	(Mir et al. 2022)
NS	Carvacrol, nigellidine, nigellidine-4-O-sulfite, thymol, and thymohydroquinone	SARS-CoV-2	Inhibition of viral replication and transcription	Molecular docking; MD; ADMET prediction	3CLpro nsp 15	6LU7 6VWW	(Rizvi et al. 2021; Hardianto et al. 2021)

Abbreviations: AP = *Andrographis paniculata;* HM = homology model.

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representative phytochemicals have also been reported with attractive activities implicating the synergistic effects of the phytoconstituents. The activities of the extracts further validate the results from bioinformatics investigation of individual phenolic compounds, posing them as promising future antibiotic and antiviral alternatives. As such, they represent promising candidates for future antibiotic and antiviral designs.

Considering the unwavering pool of applications in the literature, the bioinformatics approach has become almost inevitable in modern drug discovery. Consistently in this context, it has promoted the therapeutic potentials of phenolic phytochemicals in selected Ayurvedic botanicals for further study as worthy candidates for antibacterial and antiviral drug alternatives. This chapter represents a model for knowledge-based therapeutic science for combating bacterial and viral diseases through the incorporation of bioinformatics techniques.

Despite its enormous advantages, the application of bioinformatics essentially requires capital investments, especially in sophisticated computing and data processing resources, and expertise and skills for protocol configuration. Moreover, an extensive understanding of the diseases' biochemistry is crucial for selecting appropriate bioinformatics tools to minimize failure. In addition, being theoretical, its reliability for accuracy and precision necessarily require experimental validation. Therefore, the enlisted phenolic phytochemicals from the selected Ayurvedic botanicals are hereby presented for experimental scientists for further validation as bona fide antibacterial and antiviral prospects through more robust *in vitro* and *in vivo* investigations.

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4.9 ACKNOWLEDGEMENT

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