REVIEW: NATURAL BIOACTIVE COMPOUNDS POTENTIAL ON INHIBITION OF TRANSMEMBRANE SERINE PROTEASE 2 WITH STRUCTURE-BASED VIRTUAL SCREENING METHOD

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ABSTRACT

The COVID-19 pandemic has led to a global health emergency. Suitable medications are required to prevent severe SARS-CoV-2 infection. Human transmembrane serine protease 2, which is required for viral entry into the host cells, was identified as the target protein. The present study was designed to synthesize, through a systematic review, evidence of phytochemicals found in plants that can inhibit transmembrane serine protease 2 in silico. The databases ScienceDirect, PubMed, Scopus, Nature, and SpringerLink were used for systematic exploration. Among the 113 studies retrieved, 11 were selected for the entire read and 7 studies were deemed appropriate for the qualitative synthesis. Flavonoids, including the bioactive substances luteolin, vicenin 2, naringin, 8-geranylapigenin, phenylethyl-D-rutinoside morusin, sanggenol L, and kaempferol, are the most widely studied classes of secondary metabolites. Other classes that were also evaluated were lactones, terpenoids, and saponins with withanoside-V, 11-hydroxy-2-(3,4-dihydroxybenzoyloxy)abieta-5,7,9(11),13-tetraene-12- one, and licorice as active substances, respectively. This review indicates the most bioactive components of each group of metabolites that demonstrated the greatest binding affinity for the transmembrane serine protease 2 receptor, as an initial point for selecting substances and exploring additional laboratory research and clinical studies to identify novel medication candidates for COVID-19 treatment.

Keywords: *Secondary metabolite, SARS-CoV-2, structure-based virtual screening, transmembrane serine protease 2, Plants*

INTRODUCE

The explosion in cases of Coronavirus Disease (COVID-19) that has occurred so far is caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (Cascella et al., 2022). As of April 12, 2023, the WHO has reported more than 760 million confirmed cases of COVID-19, with more than 6,8 million cases of deaths (WHO, 2023).

Based on the 4th edition of the Guidelines for the Management of COVID-19, the antivirals of choice for patients with confirmed COVID-19 in Indonesia are favipiravir, molnupiravir, paxlovid (a combination of nirmatrelvir and ritonavir), and remdesivir (Burhan et al., 2022). However, there are still limitations to the currently available antivirals for COVID-19, such as the occurrence of side effects and the potential for significant drug interactions based on preclinical trials, case reports, and clinical trials (Brunetti et al., 2021; Hanai et al., 2022; Koseki et al., 2022; Touafchia et al., 2021; U.S. Food and Drug Administration, 2022a, 2022b). Therefore, the development of a new anti-SARS-CoV-2 agent as a better therapeutic option is urgently needed.

Studies on the life cycle and infectious mechanisms of SARS-CoV-2 have also paved the way for developing effective antivirals (Gao et al., 2021). Inhibition of human receptors

or enzymes involved in the coronavirus entry process has been highlighted as a potential new treatment strategy (Joshi et al., 2020; Kim et al., 2020). The virus adheres to the cell surface and disrupts the stability of the trimer before fusing with the spike protein when the SARS-CoV-2 spike protein binds to human ACE2. The transmembrane serine protease 2 (TMPRSS2) in the S2 subunit is generally the next to be cleaved after the furin enzyme in the S1/S2 subunit. This is the way spike proteins are often cleaved (Ma et al., 2021). SARS-CoV-2 uses TMPRSS2 to modify and activate the spike protein (Hoffmann et al., 2020). TMPRSS2 plays a crucial role in the proteolytic activation of SARS-CoV-2 through cleavage of the spike protein and initiation of fusion of viral membranes with human cellular membranes (Hu et al., 2021). The important role of TMPRSS2 in limiting viral entry into human cells is a potential target for the discovery of new drugs against COVID-19.

Drug discovery using experimental methods in the laboratory is time-consuming and relatively expensive (Horizny, 2019). Therefore, a computational approach is an important resource for shortening drug discovery time. Computational methods design, search, compare, model, predict binding energies, predict pharmacokinetics, and optimize processes (Tiwari and Singh, 2022). Computational studies on TMPRSS2 inhibition by drug compounds or their derivatives include mozenavir (Mamidala et al., 2022), camostat mesylate derivatives (Sharma et al., 2022), gabexate (Hu et al., 2021), capreomycin, aspoxicillin, and fosamprenavir (Hatmal et al., 2021).

The discovery of lead compounds is an important step in the research and developing new drugs. Medicinal plants have been chosen as alternatives for therapy and disease prevention because they can minimize the dangers and side effects of synthetic drugs (Suharyani et al., 2021). Natural products have been widely used to treat viral infections and enhance immune responses (Ma et al., 2021); therefore, herbal medicines have been extensively explored for the potential therapy and prevention of COVID-19. Therefore, it is necessary to carry out further studies on the potential of natural bioactive compounds to inhibit the TMPRSS2 receptor using a structure-based virtual screening method that can be developed into candidates for potential antiviral compounds against SARS-CoV-2.

RESEARCH METHODS

1. Search Strategy

This systematic review is a literature review using secondary data sources compiled based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria. The article search strategy was a data source reviewed for the last five years, from 2019 to 2023. Articles were obtained from ScienceDirect, PubMed, Scopus, Nature, and SpringerLink searches using the keywords “natural bioactive inhibitor TMPRSS2 in silico.”

2. Inclusion and Exclusion Criteria

The inclusion criteria set were full-text research articles with the results of studies identifying bioactive compounds in plants that can inhibit transmembrane serine protease 2 receptors in silico and written in English. Articles in the form of reviews, opinions, irrelevant studies, and duplicate articles were excluded. Articles were selected based on the title, abstract, and inclusion and exclusion criteria, and the information obtained from the articles was recorded in an Excel spreadsheet.

3. Data Extraction and Quality Assessment

Articles that met the requirements for in-depth analysis were carried out with a full-text assessment, and the data were extracted as follows:1) author's name and year of publication, 2) country, 3) TMPRSS2 protein PDB ID, 4) total of test ligands, 5) name of the plants, 6) classification of bioactive compounds, 7) main results, and 8) the program used. The authors agree to discuss and consult senior reviewers if there are conflicts of disagreement in the literature search and results obtained.

RESULTS AND DISCUSSION

1. Results of Literature Study

Overall, 113 articles were obtained from keyword searches, including 47 articles from the initial literature search in the ScienceDirect database and an additional 66 articles from searches in the PubMed database (1 article), Scopus (2 articles), Nature (6 articles), and SpingerLink (57 articles). Of these 113 articles, 11 were shortlisted for the full-text evaluation. Seven publications that fulfilled the inclusion criteria were selected for review after careful assessment (Aini et al., 2022; Dhanjal et al., 2021; Gyebi et al., 2021; Jindal and Rani, 2022; Kumar et al., 2022; Puttaswamy et al., 2020; Shakya et al., 2022). The reason for issuing the other 4 articles is that the results of the study did not use natural ligands from plants but instead synthesized derivative compounds (Lawal et al., 2023; Waidha et al., 2021) or repurposed existing chemical drugs (Esam et al., 2023; Singh et al., 2020).

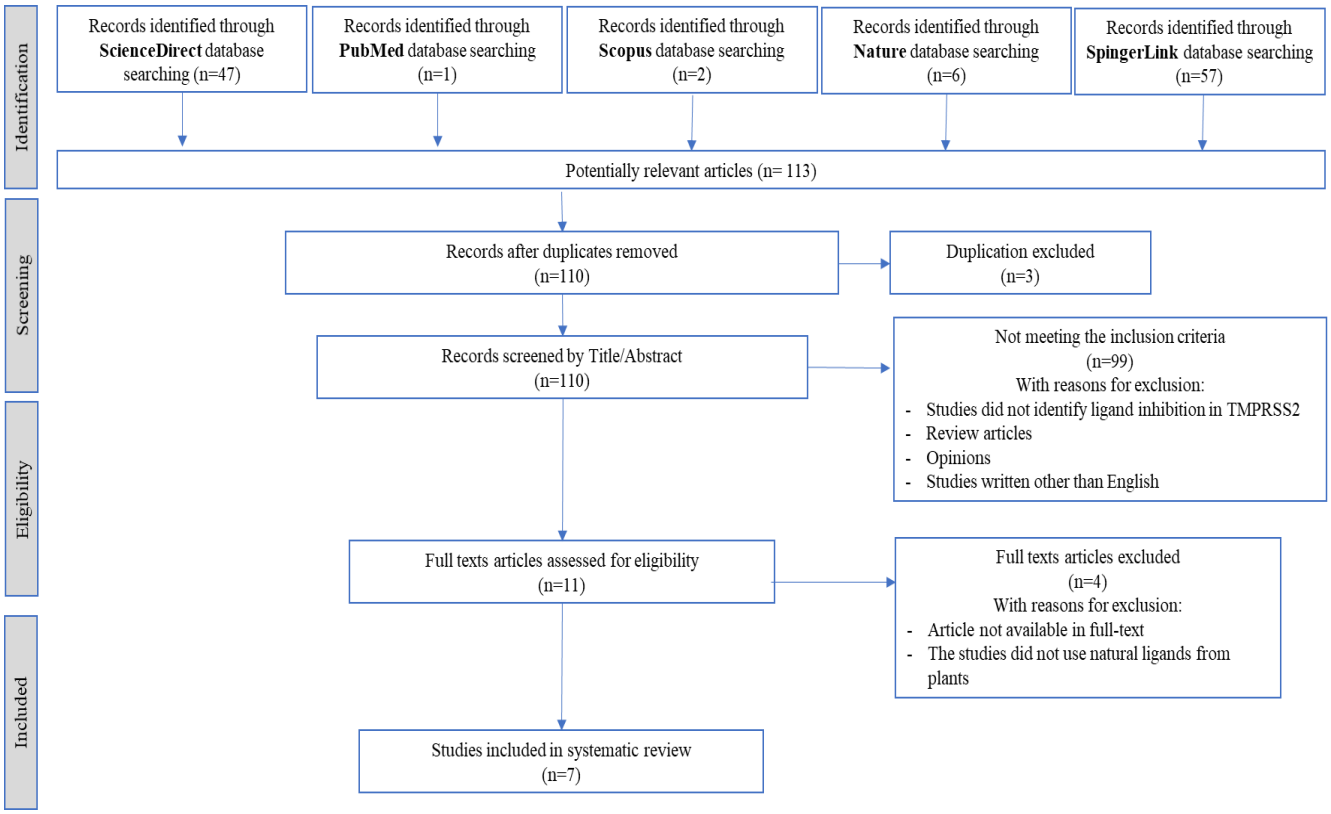

Figure 1 presents an overview of the study selection process.

Figure 1. Process flow for selecting and identifying articles

2. Characteristics of the Study

A total of 5.115 test ligands were identified in the 7 eligible articles. As shown in Table I, the study with the highest number of test ligands was 4.704 compounds (Puttaswamy et al., 2020), while the smallest number of test ligands was 6 (Aini et al.,

2022). The included studies were published from 2020 to 2023 and conducted in Indonesia (Aini et al., 2022), India (Dhanjal et al., 2021; Jindal & Rani, 2022; Kumar et al., 2022; Puttaswamy et al., 2020; Shakya et al., 2022), and Nigeria (Gyebi et al., 2021). All studies used test ligands from plant bioactive compounds with different classifications of metabolites, such as flavonoids (Aini et al., 2022; Jindal & Rani, 2022; Kumar et al., 2022; Shakya et al., 2022), steroid lactones (Dhanjal et al., 2021), terpenoids (Gyebi et al., 2021), and saponins (Puttaswamy et al., 2020). The 3D structure of TMPRSS2 protein was obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) website (https://www.rcsb.org/) with PDB ID 6VWL (Gyebi et al., 2021), 7MEQ (Jindal & Rani, 2022), and 2OQ5 (Kumar et al., 2022). Several studies have not used the PDB ID but have built 3D structures by homology modeling using the TMPRSS2 sequence (Dhanjal et al., 2021; Puttaswamy et al., 2020; Shakya et al., 2022).

Table I. Study Characteristics of TMPRSS2 Group of Bioactive Compounds

No	Study (Authors name, Year)	Country	PDB ID TMPRSS2	Total of Test Ligands	Plants	Classification of Bioactive Compounds
1	Aini, et al., 2022	Indonesia	No information	6 compounds	Purslane (*Portulaca oleracea* L.)	Flavonoids
2	Dhanjal, et al., 2021	India	None (using Swiss model repository ID: O15393)	9 compounds	Ashwagandha (*Withania somnifera*)	Lakton steroid
3	Gyebi, et al., 2021	Nigeria	6VWL	106 compounds	*Plectranthus ecklonii* dan *Plectranthus lucidus*	Terpenoids
4	Jindal & Rani, 2023	India	7MEQ	100 compounds	*Piper longum* dan *Ocimum sanctum*	Flavonoid glycosides, polyphenols
5	Kumar, et al., 2022	India	2OQ5	46 compounds	*Podocarpus fasciculus, Citrus latipes*	Flavonoids
6	Puttaswamy, et al., 2020	India	None (using TMPRSS2 sequence from NCBI protein database (NP_001128571.1)	4.704 compounds	*Glycyrrhiza glabra* L.	Triterpenoid saponins
7	Shakya, et al., 2022	India	None (using Swiss-Model TMPRSS2 sequence from UniProtKB database: Accession number O15393)	144 compounds	*Morus alba*	Flavonoids

3. Structure-Based Virtual Screening Method

A computational method named "structure-based virtual screening" is used in the initial stages of drug discovery to explore new compounds against specific drug targets or receptors. This method uses a three-dimensional (3D) receptor structure obtained from NMR, X-ray, or computational modeling such as homology modeling. These 3D structures of biological targets were used to screen the ligands for receptor binding sites and select potential ligand compounds according to the estimated binding scores for further biological investigation. The structure-based virtual screening method consists of

three pillars: (1) molecular docking, (2) 3D receptor structure, and (3) a database of active compounds (Li & Shah, 2017).

Molecular docking is an increasingly applied method in the initial phases of new medication development to identify new ligands at the desired target. Using the predicted binding affinities, this method generates appropriate ligand-binding poses, grading, and ranking of compounds. Then, thoroughly verified and assessed highly rated hits are released (Hui Zhu et al., 2022). Over the last two decades, more than 60 molecular docking programs have been developed by both academia and commercial parties, including GOLD, AutoDock, MOE-Dock, FlexX, LigandFit, Surflex, ICM, Glide, UCSF Dock, Cdocker, MCDock, DOCK, FRED, MOE-Dock, LeDock, AutoDock Vina, rDock (Allen et al. 2015), and many other programs (Pagadala et al., 2017). Further analysis was performed using molecular dynamics simulations to predict the stability of the complex formed between the protein and ligand.

Molecular dynamics simulation is an in silico method that provides information on the dynamic behavior and conformational changes of protein-ligand complexes by predicting stability and intermolecular interactions over time (Bharadwaj et al., 2021). These simulations have been improved from simulating a few hundred atoms to simulating large biological systems, such as whole proteins in solution, including explicit solvent modeling, proteins found in membranes, or large macromolecular structures such as nucleosomes or ribosomes (Hospital et al., 2015). There are 34 molecular dynamics simulation software packages, including AMBER, NAMD, CHARMm, GROMACS, Moldy, and others (University of Illinois, 2022).

Knowing a compound's pharmacokinetic profile in drug discovery, such as absorption, distribution, metabolism, and excretion (ADME), is important. The in silico method for improving ADME prediction contributes to the optimization of drug development (Durán-Iturbide et al., 2020). Web-based programs for predicting ADME parameters include ADMETlab, admetSAR, BioTransformer3.0, CYPRules, DrugLogit, FAF-Drugs4, Lazar, MetaTox, NERDD, OCHEM, OSCADD, pKCSM, preADMET, SmartCyp, SwissADME, vNN-ADMET, Way2Drug, and XenoSite (Dulsat et al., 2023).

The program for in silico studies using the structure-based virtual screening method and the main results of these studies are presented in Table II.

Table II. Programs Used and Main Results of Virtual Screening

Plants	Main Results	Programs	Studies
Purslane (*Portulaca oleracea* L.)	Luteolin is the best ligand that inhibits TMPRSS2 (docking score: -7.3 kcal/mol) compared to the comparison compound Nirmatrelvir (docking score: -6.4 kcal/mol)	PASS Online web server, PyRx software, PyMol, Biovia Discovery Studio	Aini, et al., 2022
Ashwagandha (*Withania somnifera*)	* Withanoside-V (docking score: -7.96 kcal/mol; MMGBSA score: -36.19 + 7.83 kcal/mol) and Withanoside-IV (docking score: -6.93 kcal/mol; MMGBSA score: -42.80 + 7.46 kcal/mol) is the best ligand in inhibiting TMPRSS2 * Withanoside-V has the highest quantity of hydrogen bonds with TMPRSS2 during the molecular dynamic's simulation	Maestro Schrodinger suite, OPL3e force field, Desmond tool	Dhanjal, et al., 2021

Plectranthus ecklonii dan *Plectranthus lucidus*	Abietane diterpenes (T3 = 11-hydroxy-2-(3,4-dihydroxybenzoyloxy) abieta-5,7,9(11), 13-tetraene-12-one) interacts strongly with TMPRSS2 * Docking score: -10.0 kcal/mol, * RoG value (16.75 Å) does not differ from that of the camostat (16.77 Å) * RMSD average value: 2.14 Å * RMSF average value: 0.73 Å * Bond affinity with ARG41 residue is stronger than camostat comparator * MMGBSA value: -16.00 + 4.08 kcal/mol	MGL-AutoDockTools (ADT, v1.5.6), Open babel, Chemdraw versi 19, AutoDock Vina, Discovery Studio Visualizer versi 16, NAMD V 2.13, VMD 1.9.3, TTClust V 4.9.0, Amber Tools 20, ADMET web server	Gyebi, et al., 2021
Piper longum dan *Ocimum sanctum*	* The binding affinity of TMPRSS2-vicenin 2 (-7.913 kcal/mol) and TMPRSS2-rosmarinic acid (-7.137 kcal/mol) is better than the comparison compound TMPRSS2-nafamostat (-2.188 kcal/mol) *Rosmarinic acid has good absorption and bioavailability based on ADME analysis	Schrodinger Suite, Maestro software, SwissADME, KEGG, STRING, Molinspiration	Jindal & Rani, 2023
Podocarpus fasciculus, *Citrus latipes*	Naringin has good binding affinity and stability with TMPRSS2 * Naringin exhibits good water solubility, does not penetrate the brain barrier, and is a substrate of P glycoprotein * Docking score: -8.7 kcal/mol * MMGBSA value: -65.83854 kcal/mol * RMSD deviation < 2 Å	MTI-OpenScreen web server, UCSF Chimera-1.14, SwissADME, ProTox-II, Chimera-Autodock Vina, Maestro v12.9, Desmond v5.6	Kumar, et al., 2022
Glycyrrhiza glabra L.	* Liquorice docking score: -9.7 kcal/mol and Glycyrrhizic acid: -9.7 kcal/mol) gives the best results * However, based on structural similarity analysis and bond energy correlation analysis, more than 32% are flavonoid glycoside compounds that interact with the TMPRSS2 target site	Marvin Sketch, SWISS-MODEL online server, UCSF Chimera Tool, Biovia Discovery Studio v.3, PyRx, Autodock Vina, PyMOL, Data Warrior v5.2.1, SwissADME	Puttaswamy, et al., 2020
Morus alba	Five potential TMPRSS2 inhibitors, namely morusin, sanggenol L, phenylethyl-D-rutinoside, 8-geranylapigenin, and kaempferol with a Glide dock score of -7,888; -7.426; -9.574; -7,980; and -7,807 kcal/mol. MMGBSA bond energy respectively -70.472; -56,060; -50,510; -46,680; and 60,830 kcal/mol. Based on the trajectory analysis of molecular dynamics simulation, the TMPRSS2-ligand complex is stable.	Glide Schrodinger Suite, SiteMap tool, MOE tool, Autodock Tools (ADT), Amber18	Shakya, et al., 2022

4. TMPRSS2's Role in the Invasive Process of SARS-CoV-2

Critical components of the SARS-CoV-2 (RNA virus) infection process involve the spike protein (S), nucleocapsid (N), membrane protein (M), and envelope protein (E) (Mohan et al., 2020). The spike protein consists of the S1 and S2 subunits responsible for viral attachment to host cells after TMPRSS2-mediated activation (Hashemian et al., 2022).

Transmembrane serine protease 2 (TMPRSS2) is a serine protease expressed on the surface of the host cell membrane (Joshi et al., 2020). The amino acid residues His296, Asp345, and Ser441 comprise a triad of the catalytic domain of the protease. TMPRSS2 is expressed in the lungs, prostate, colon, liver, kidney, and pancreas (Shen et al., 2017). TMPRSS2 is a key step in activating the SARS-CoV-2 spike protein (S) membrane. Inhibition of the proteolytic activity of TMPRSS2 blocks the membrane fusion of SARS-CoV-2 with host cells (Evans & Liu, 2021; Simmons et al., 2013; Zhu et al., 2021). Drug

targets that inhibit TMPRSS2 do not cause problems developing drug resistance because the receptor is a human protease, so TMPRSS2 is one of the most promising targets for anti-SARS-CoV-2 (Haixia Zhu et al., 2021)

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5. Potentially Inhibitory Natural Bioactive Compounds for TMPRSS2

Natural products are major sources of bioactive compounds and drug candidates. The number of databases on natural products that are open to the public has increased significantly in recent years. Natural products offer various therapeutic alternatives because of their capacity to produce a variety of bioactive compounds, compared to the difficult synthesis of medicinal compounds (Durán-Iturbide et al., 2020).

Within the scope of this systematic review, the outcomes of in silico experiments with bioactive substances from plants that can interact with the TMPRSS2 receptor were assessed. Based on the results of a systematic review that has been carried out (Table II), bioactive compounds that have inhibition of TMPRSS2 include luteolin from the Purslane plant (*Portulaca oleracea* L.) (Aini et al., 2022), withanoside-V and withanoside-IV from Ashwagandha plant (*Withania somnifera*) (Dhanjal et al., 2021), 11-hydroxy-2-(3,4-dihydroxybenzoyloxy)abieta-5,7,9(11),13-tetraene-12-one from *Plectranthus ecklonii* and *Plectranthus lucidus* (Gyebi et al., 2021), vicenin 2 and rosmarinic acid from *Piper longum* and *Ocimum sanctum* (Jindal & Rani, 2022), naringin from *Podocarpus fasciculus* and *Citrus latipes* (Kumar et al., 2022), liquorice from *Glycyrrhiza glabra* L. (Puttaswamy et al., 2020), and morusin, sanggenol L, phenylethyl-D-rutinoside, 8-geranylapigenin, and kaempferol from *Morus alba* (Shakya et al., 2022).

The studies included in this review demonstrated that the most studied classes of metabolites were flavonoids, such as luteolin, vicenin 2, naringin, phenylethyl-D-rutinoside, 8-geranylapigenin, morusin, kaempferol, and sanggenol L, with lower binding energies by docking scores of-7.3, -7,913, -8.7, -9.574, -7,980, -7,888, -7,807, and -7.426 kcal/mol, respectively (Table II). Based on trajectory analysis of molecular dynamics simulations, TMPRSS2-naringin, TMPRSS2-phenylethyl-D-rutinoside, TMPRSS2-8-geranylapigenin, TMPRSS2-morusin, TMPRSS2-kaempferol, and TMPRSS2-sanggenol L complexes were stable and showed great potential in this class in TMPRSS2 inhibition.

Other classes of compounds in the study that also have TMPRSS2 inhibitory activity are lactones, terpenoids, and saponins. Withanoside-V (lactone), 11-hydroxy-2-(3,4-dihydroxybenzoyloxy) abieta-5,7,9(11),13-tetraene-12-one (terpenoid), and licorice (saponins) provide binding affinity -7.96; -10.0; and -9.7 kcal/mol, respectively. The TMPRSS2-withanoside-V and TMPRSS2-11-hydroxy-2-(3,4-dihydroxybenzoyloxy) abieta-5,7,9(11),13-tetraene-12-one complexes showed stable results based on molecular dynamics analysis. This shows that lactones, terpenoids, and saponins can also act as drug candidates for the treatment of COVID-19.

This review has numerous limitations, including the fact that not all studies employ comparison ligands and that no quantitative analysis is feasible, given the variability of the data from various study methodologies. These results are exploratory only, but the individual bias of the primary research is diminished by the systematic and critical evaluation approach utilized in this review. The synthesized data could contribute to the development of additional studies in this field.

CONCLUSION

This systematic review collected and extracted accessible evidence from in silico experiments using a structure-based virtual screening method to determine the potential of natural bioactive compounds that can inhibit TMPRSS2. The most evaluated secondary metabolite classes were flavonoids luteolin, vicenin 2, naringin, 8-geranylapigenin, phenylethyl-D-rutinoside morusin, sanggenol L, and kaempferol. In addition, there are lactones with withanoside-V, terpenoids with 11-hydroxy-2-(3,4-dihydroxybenzoyloxy) abieta-5,7,9(11),13-tetraene-12-one, and saponins with licorice as active substances.

Therefore, this review can be used as an initial point for selecting substances and guiding additional in vitro, in vivo, and clinical trial research to develop new drug candidates for COVID-19 therapies.

DECLARATION OF COMPETING INTERESTS

The authors declare that they have no known financial or interpersonal conflicts that would have affected the research presented in this study.

REFERENCES

Aini, N. S., Kharisma, V. D., Widyananda, M. H., Murtadlo, A. A. A., Probojati, R. T., Turista, D. D. R., Tamam, M. B., Jakhmola, V., Novaliendry, D., Mandeli, R. S., Oktavia, B., Albari, M. T., Al Aziz, S., Ghifari, M. R., Suryani, O., Azhari, P., Ghifari, M. A., Purnamasari, D., Samala, A. D., … Zainul, R. (2022). Bioactive Compounds from Purslane (Portulaca oleracea L.) and Star Anise (Illicium verum Hook) as SARS-CoV-2 Antiviral Agent via Dual Inhibitor Mechanism: In Silico Approach. *Pharmacognosy Journal*, *14*(4), 352–357. https://doi.org/10.5530/pj.2022.14.106

Bharadwaj, S., Rao, A. K., Dwivedi, V. D., Mishra, S. K., & Yadava, U. (2021). Structure-based screening and validation of bioactive compounds as Zika virus methyltransferase (MTase) inhibitors through first-principle density functional theory, classical molecular simulation and QM/MM affinity estimation. *Journal of Biomolecular Structure and Dynamics*, *39*(7), 2338–2351. https://doi.org/10.1080/07391102.2020.1747545

Brunetti, N. D., Poliseno, M., Bottalico, I. F., Centola, A., Montemurro, L., Sica, S., Santantonio, T., & Lo Caputo, S. (2021). Safety and heart rate changes in Covid-19 patients treated with Remdesivir. *International Journal of Infectious Diseases*, *112*, 254–257. https://doi.org/10.1016/j.ijid.2021.09.036

Burhan, E., Susanto, A. D., Isbaniah, F., Nasution, S. A., Ginanjar, E., Pitoyo, C. W., Susilo, A., Firdaus, I., Santoso, A., Juzar, D. A., Arif, S. K., LolongWulung, N. G. ., Muchtar, F., Pulungan, A. B., Yanuarso, P. B., Sjakti, H. A., Prawira, Y., & Putri, N. D. (Eds.). (2022). *Pedoman Tatalaksana COVID-19* (Edisi 4). Perhimpunan Dokter Paru Indonesia (PDPI), Perhimpunan Dokter Spesialis Kardiovaskular Indonesia (PERKI), Perhimpunan Dokter Spesialis Dalam Indonesia (PAPDI), Perhimpunan Dokter Anestesiologi dan Terapi Intensif Indonesia (PERDATIN), Ikatan Dokter Anak In.

Cascella, M., Rajnik, M., Aleem, A., Dulebohn, S. C., & Napoli, R. Di. (2022). *Features, Evaluation, and Treatment of Coronavirus (COVID-19) [Updated 2022 Feb 5]*. Treasure Island (FL): StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK554776/

Dhanjal, J. K., Kumar, V., Garg, S., Subramani, C., Agarwal, S., Wang, J., Zhang, H., Kaul, A., Kalra, R. S., Kaul, S. C., Vrati, S., Sundar, D., & Wadhwa, R. (2021). Molecular mechanism of anti-SARS-CoV2 activity of Ashwagandha-derived withanolides. *International Journal of Biological Macromolecules*, *184*(December 2020), 297–312. https://doi.org/10.1016/j.ijbiomac.2021.06.015

Dulsat, J., López-Nieto, B., Estrada-Tejedor, R., & Borrell, J. I. (2023). Evaluation of Free Online ADMET Tools for Academic or Small Biotech Environments. *Molecules*, *28*(2). https://doi.org/10.3390/molecules28020776

Durán-Iturbide, N. A., Díaz-Eufracio, B. I., & Medina-Franco, J. L. (2020). In Silico ADME/Tox Profiling of Natural Products: A Focus on BIOFACQUIM. *ACS Omega*, *5*(26), 16076–16084. https://doi.org/10.1021/acsomega.0c01581

Esam, Z., Akhavan, M., Lotfi, M., Pourmand, S., & Bekhradnia, A. (2023). In silico investigation of the therapeutic and prophylactic potential of medicinal substances bearing guanidine moieties against COVID-19. *Chemical Papers*, *77*(2), 1129–1148. https://doi.org/10.1007/s11696-022-02528-y

Evans, J. P., & Liu, S. L. (2021). Role of host factors in SARS-CoV-2 entry. *Journal of Biological Chemistry*, *297*(1), 100847. https://doi.org/10.1016/j.jbc.2021.100847

Gao, S., Huang, T., Song, L., Xu, S., Cheng, Y., Cherukupalli, S., Kang, D., Zhao, T., Sun, L., Zhang, J., Zhan, P., & Liu, X. (2021). Medicinal chemistry strategies towards the development of effective SARS-CoV-2 inhibitors. *Acta Pharmaceutica Sinica B*, *12*(2), 581–599. https://doi.org/10.1016/j.apsb.2021.08.027

Gyebi, G. A., Ogunyemi, O. M., Ibrahim, I. M., Ogunro, O. B., Adegunloye, A. P., & Afolabi, S. O. (2021). SARS-CoV-2 host cell entry: an in silico investigation of potential inhibitory roles of terpenoids. *Journal of Genetic Engineering and Biotechnology*, *19*(1). https://doi.org/10.1186/s43141-021-00209-z

Hanai, Y., Yoshizawa, S., Matsuo, K., Uekusa, S., Miyazaki, T., Nishimura, K., Mabuchi, T., Ohashi, H., Ishii, Y., Tateda, K., Yoshio, T., & Nishizawa, K. (2022). Evaluation of risk factors for uric acid elevation in COVID-19 patients treated with favipiravir. *Diagnostic Microbiology and Infectious Disease*, *000*, 115640. https://doi.org/10.1016/j.diagmicrobio.2022.115640

Hashemian, S. M. R., Pourhanifeh, M. H., Hamblin, M. R., Shahrzad, M. K., & Mirzaei, H. (2022). RdRp inhibitors and COVID-19: Is molnupiravir a good option? *Biomedicine and Pharmacotherapy*, *146*(October 2021), 112517. https://doi.org/10.1016/j.biopha.2021.112517

Hatmal, M. M., Abuyaman, O., & Taha, M. (2021). Docking-generated multiple ligand poses for bootstrapping bioactivity classifying Machine Learning: Repurposing covalent inhibitors for COVID-19-related TMPRSS2 as case study. *Computational and Structural Biotechnology Journal*, *19*, 4790–4824. https://doi.org/10.1016/j.csbj.2021.08.023

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N. H., Nitsche, A., Müller, M. A., Drosten, C., & Pöhlmann, S. (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*, *181*(2), 271-280.e8. https://doi.org/10.1016/j.cell.2020.02.052

Horizny, C. (2019). *The Drug Discovery Process*. Taconic Models for Life. https://www.taconic.com/taconic-insights/quality/drug-development-process.html

Hospital, A., Goni, J. R., Orozco, M., & Gelpi, J. L. (2015). Molecular Dynamics Simulations: Advances and Applications. *Advances and Applications in Bioinformatics and Chemistry*, *8*, 37–47. https://doi.org/10.3390/molecules27072105

Hu, X., Shrimp, J. H., Guo, H., Xu, M., Chen, C. Z., Zhu, W., Zakharov, A. V., Jain, S., Shinn, P., Simeonov, A., Hall, M. D., & Shen, M. (2021). Discovery of TMPRSS2 Inhibitors from Virtual Screening as a Potential Treatment of COVID-19. *ACS Pharmacology and Translational Science*, *4*(3), 1124–1135. https://doi.org/10.1021/acsptsci.0c00221

Jindal, D., & Rani, V. (2022). In Silico Studies of Phytoconstituents from Piper longum and Ocimum sanctum as ACE2 and TMRSS2 Inhibitors: Strategies to Combat COVID-19. *Applied Biochemistry and Biotechnology*, 2618–2635. https://doi.org/10.1007/s12010-022-03827-6

Joshi, S., Joshi, M., & Degani, M. S. (2020). Tackling SARS-CoV-2: Proposed targets and repurposed drugs. *Future Medicinal Chemistry*, *12*(17), 1579–1601. https://doi.org/10.4155/fmc-2020-0147

Kim, P. S., Read, S. W., & Fauci, A. S. (2020). Therapy for Early COVID-19. *JAMA*, *324*(21), 2149. https://doi.org/10.1001/jama.2020.22813

Koseki, T., Nakajima, K., Iwasaki, H., Yamada, S., Takahashi, K., Doi, Y., & Mizuno, T. (2022). Baseline uric acid levels and steady-state favipiravir concentrations are associated with occurrence of hyperuricemia among COVID-19 patients. *International Journal of Infectious Diseases*, *115*, 218–223. https://doi.org/10.1016/j.ijid.2021.12.324

Kumar, S., Paul, P., Yadav, P., Kaul, R., Maitra, S. S., Jha, S. K., & Chaari, A. (2022). A multi-targeted approach to identify potential flavonoids against three targets in the SARS-CoV-2 life cycle. *Computers in Biology and Medicine*, *142*(November 2021),

105231. https://doi.org/10.1016/j.compbiomed.2022.105231

Lawal, B., Tsai, S. K., Wu, A. T. H., & Huang, H. S. (2023). In silico study of novel niclosamide derivatives, SARS-CoV-2 nonstructural proteins catalytic residue-targeting small molecules drug candidates. *Arabian Journal of Chemistry*, *16*(5), 104654. https://doi.org/10.1016/j.arabjc.2023.104654

Li, Q., & Shah, S. (2017). *Structure-Based Virtual Screening*. *1558*, 111–124. https://doi.org/10.1007/978-1-4939-6783-4

Ma, L. le, Liu, H. min, Liu, X. mei, Yuan, X. yu, Xu, C., Wang, F., Lin, J. zhi, Xu, R. chun, & Zhang, D. kun. (2021). Screening S protein – ACE2 blockers from natural products: Strategies and advances in the discovery of potential inhibitors of COVID-19. *European Journal of Medicinal Chemistry*, *226*. https://doi.org/10.1016/j.ejmech.2021.113857

Mamidala, E., Davella, R., Praveen Kumar, M., Swamy, S., Abhiav, M., Ali Kaimkhani, Z., Al-Ghanim, K. A., & Mahboob, S. (2022). In silico prediction of mozenavir as a potential drug for SARS-CoV-2 infection via binding multiple drug targets. *Saudi Journal of Biological Sciences*, *29*(2), 840–847. https://doi.org/10.1016/j.sjbs.2021.10.023

Mohan, C., Chauhan, S., Shekhar, S., Kaushik, P., Madhu, M., Sharma, M., & Kumar, V. (2020). A Brief Study on Rate of Morphological and Genomic Similarities between SARS and COVID-19. *International Journal of Pharma Research and Health Sciences*, *8*(3), 3167–3171. https://doi.org/10.21276/ijprhs.2020.03.03

Pagadala, N. S., Syed, K., & Tuszynski, J. (2017). Software for molecular docking: a review. *Biophysical Reviews*, *9*(2), 91–102. https://doi.org/10.1007/s12551-016-0247-1

Puttaswamy, H., Gowtham, H. G., Ojha, M. D., Yadav, A., Choudhir, G., Raguraman, V., Kongkham, B., Selvaraju, K., Shareef, S., Gehlot, P., Ahamed, F., & Chauhan, L. (2020). In silico studies evidenced the role of structurally diverse plant secondary metabolites in reducing SARS-CoV-2 pathogenesis. *Scientific Reports*, *10*(1), 1–24. https://doi.org/10.1038/s41598-020-77602-0

Shakya, A., Chikhale, R. V., Bhat, H. R., Alasmary, F. A., Almutairi, T. M., Ghosh, S. K., Alhajri, H. M., Alissa, S. A., Nagar, S., & Islam, M. A. (2022). Pharmacoinformatics-based identification of transmembrane protease serine-2 inhibitors from Morus Alba as SARS-CoV-2 cell entry inhibitors. *Molecular Diversity*, *26*, 265–278. https://doi.org/https://doi.org/10.1007/s11030-021-10209-3

Sharma, T., Baig, M. H., Khan, M. I., Alotaibi, S. S., Alorabi, M., & Dong, J.-J. (2022). Computational screening of camostat and related compounds against human TMPRSS2: A potential treatment of COVID-19. *Saudi Pharmaceutical Journal*, *xxxx*, 0–7. https://doi.org/10.1016/j.jsps.2022.01.005

Shen, L. W., Mao, H. J., Wu, Y. L., Tanaka, Y., & Zhang, W. (2017). TMPRSS2: A potential target for treatment of influenza virus and coronavirus infections. *Biochimie*, *142*, 1–10. https://doi.org/10.1016/j.biochi.2017.07.016

Simmons, G., Zmora, P., Gierer, S., Heurich, A., & Pöhlmann, S. (2013). Proteolytic activation of the SARS-coronavirus spike protein: Cutting enzymes at the cutting edge of antiviral research. *Antiviral Research*, *100*(3), 605–614. https://doi.org/10.1016/j.antiviral.2013.09.028

Singh, N., Decroly, E., Khatib, A. M., & Villoutreix, B. O. (2020). Structure-based drug repositioning over the human TMPRSS2 protease domain: search for chemical probes able to repress SARS-CoV-2 Spike protein cleavages. *European Journal of Pharmaceutical Sciences*, *153*(June), 105495. https://doi.org/10.1016/j.ejps.2020.105495

Suharyani, I., Falya, Y., Hakim, A., Fajira, D., Sadira, N. A., & Astuti, S. (2021). Review Artikel: Potensi Senyawa Aktif Pada Tanaman Obat Untuk Penanganan Covid-19 Dengan Metode Molecular Docking. *Medical Sains : Jurnal Ilmiah Kefarmasian*, *6*(2), 115–122. https://doi.org/10.37874/ms.v6i2.270

Tiwari, A., & Singh, S. (2022). Computational approaches in drug designing. In

Bioinformatics (pp. 207–217). Elsevier. https://doi.org/10.1016/B978-0-323-89775-4.00010-9

Touafchia, A., Bagheri, H., Carrié, D., Durrieu, G., Sommet, A., Chouchana, L., & Montastruc, F. (2021). Serious bradycardia and remdesivir for coronavirus 2019 (COVID-19): a new safety concerns. *Clinical Microbiology and Infection*, *27*(5), 791.e5-791.e8. https://doi.org/10.1016/j.cmi.2021.02.013

U.S. Food and Drug Administration. (2022a). Fact Sheet for Healthcare providers: Emergency Use Authorization for Molnupiravir. In *US Food & Drug Administration (FDA)*. https://www.fda.gov/media/143823/download

U.S. Food and Drug Administration. (2022b). Fact Sheet for Healthcare Providers: Emergency Use Authorization for Paxlovid. In *US Food & Drug Administration (FDA)*. https://www.fda.gov/media/143823/download

University of Illinois. (2022). *Structure Biology Software Database Application Index*. Theoretical and Computational Biophysics Group (TCBG). https://www.ks.uiuc.edu/Development/biosoftdb/biosoft.cgi?&category=2

Waidha, K., Saxena, A., Kumar, P., Sharma, S., Ray, D., & Saha, B. (2021). Design and identification of novel annomontine analogues against SARS-CoV-2: An in-silico approach. *Heliyon*, *7*(4), e06657. https://doi.org/10.1016/j.heliyon.2021.e06657

WHO. (2023). *WHO Coronavirus (COVID-19) Dashboard*. https://covid19.who.int/

Zhu, Haixia, Du, W., Song, M., Liu, Q., Herrmann, A., & Huang, Q. (2021). Spontaneous binding of potential COVID-19 drugs (Camostat and Nafamostat) to human serine protease TMPRSS2. *Computational and Structural Biotechnology Journal*, *19*, 467–476. https://doi.org/10.1016/j.csbj.2020.12.035

Zhu, Hui, Zhang, Y., Li, W., & Huang, N. (2022). A Comprehensive Survey of Prospective Structure-Based Virtual Screening for Early Drug Discovery in the Past Fifteen Years. *International Journal of Molecular Sciences*, *23*(24). https://doi.org/10.3390/ijms232415961