Targeting the Coronavirus SARS-CoV-2's Envelope, Nucleocapsid, and Spike/ Spike RBD Protein: Computational Insight from Multiple Bioactive Compounds as Potential Anti-Viral Drug Candidates

Wira Eka Putra[1*], Muhammad Fikri Heikal[2], Sustiprijatno[3]
Arief Hidayatullah[4] and Diana Widiastuti [5]
[1]Department of Applied Sciences, [2]Department of Biology
Universitas Negeri Malang, East Java, Indonesia
[*]*wira.putra.fmipa@um.ac.id*

[3]Research Center for Applied Botany, National Research and Innovation Agency
West Java, Indonesia

[4]Health Governance Initiative, United Nations Development Programme Indonesia
Jakarta, Indonesia

[5]Department of Chemistry, Universitas Pakuan
West Java, Indonesia

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Abstract

Global health, social, and economic systems have been seriously threatened by the coronavirus disease (COVID-19) pandemic. In addition to the increasing number of deaths, thousands of COVID-19 survivors continue to experience life-altering illness. This study aimed to evaluate multiple bioactive compounds from various indigenous medicinal plants against the structural proteins of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), including the envelope, nucleocapsid, and spike/spike receptor-binding domain (RBD) proteins, in search of potential antiviral drug candidates. Computational analysis was used to screen for binding affinities and assess chemical interactions between ligands and target proteins. The findings revealed the top three potential compounds to bind to the envelope protein (cafestol, kahweol, and ledene), nucleocapsid protein (cafestol, kahweol, and thearubigin), spike protein (tannic acid, eugeniin, and kahweol), and spike RBD protein (kahweol, cafestol, and tannic acid). Moreover, the study identified four types of plants that contain potential bioactive compounds against SARS-CoV-2 structural proteins, including black tea (Camellia sinensis), clove (Syzygium aromaticum), common bean (Phaseolus vulgaris), and star anise (Illicium verum). Interestingly, kahweol exhibited possible binding activity against all four target proteins. This result suggests that bioactive compounds from the listed medicinal plants could potentially be developed into antiviral drugs against COVID-19.

***Keywords**:* anti-viral drug, bioactive compounds, *in silico*, SARS-CoV-2, structural protein

1. Introduction

The COVID-19 pandemic, which was declared in March 2020, is an enormous threat to the world's health, social, and economic systems (McKee and Stuckler, 2020; Bueno-Notivol *et al.*, 2021). More than 45 million cases of infection have been confirmed in more than 180 countries, with one million deaths (Chu, 2021). Not only did COVID-19 cause a terrible number of excess deaths, but thousands of people who survived it have permanent problems (Clark and Turner, 2021). This viral illness is marked by a high fever, a prolonged cough, bone pain, breathing difficulties, and can ultimately lead to pneumonia (Haleem *et al.*, 2020; Subramanian *et al.*, 2022). After making a full recovery from acute COVID-19, a significant number of individuals continue to exhibit symptoms of a physical, psychological, or cognitive nature (Ballering *et al.*, 2022).

The SARS-CoV-2 genomic sequence, its links with other SARS-CoV viruses, and the structure of its spike protein have been explored to uncover novel targets and effective new therapeutic techniques to reduce COVID-19 transmission and death (Malik *et al.*, 2020). To treat SARS-CoV-2, pharmaceutical companies are also creating drugs that target various phases of the viral life cycle. These stages involve viral adherence and entry into the host cell, endocytosis, replication, viral protease activity, suppression of the cytokine storm, and reduction of the freely circulating viral load (Al-Tawfiq, 2020). Importantly, clinical trials are being conducted to test a variety of medications, convalescent plasma therapy, monoclonal antibodies, immunoglobulin therapy, and cell therapy to ensure the best strategy to tackle COVID-19 (Niknam *et al.*, 2022).

Century-old folk healers have advocated the use of herbal medicines and dietary herbs to treat a range of diseases (Shahrajabian *et al.*, 2020; Demeke *et al.*, 2021). The extensive use of plant-based pharmaceuticals with proven therapeutic efficacy has led to the discovery of several effective remedies from plant sources (Putra and Rifa'i, 2019; Cragg and Newman, 2021). Numerous findings suggest that natural treatments could mitigate the effects of COVID-19 and prevent infection (Chan *et al.*, 2020; Vellingiri *et al.*, 2020; Putra *et al.*, 2023). In China and India, herbal medicine is used alongside modern medications to strengthen patients' immune systems (Ni *et al.*, 2020; Shankar *et al.*, 2020). Herbal treatments have also helped lessen the symptoms of viral illnesses like SARS-CoV-2. Natural therapies may help reduce and manage the risk of COVID-19. The use of herbal medicine to treat COVID-19 has

been advocated in various approaches in addition to conventional medication (Ang *et al.*, 2020). Herbal medicine significantly contributes to the management of COVID-19 across cultures. Herbal treatments have the potential to directly or indirectly impact the immune system, host receptors, viral-host interactions, signaling cascades, molecular targets, and microenvironments (Hidayatullah *et al.*, 2021, 2022; Liana and Phanumartwiwath, 2022). With this, the present study utilized a computational approach to predict potential bioactive compounds from various indigenous medicinal plants against the structural proteins of SARS-CoV-2, including the envelope, nucleocapsid, and spike/spike RBD protein.

2. Methodology

2.1 Ligands Preparation and Screening

To screen bioactive components, 30 commonly and daily used indigenous Indonesian herbs and spices were evaluated (Table 1). The overall flowchart of this study is shown in Figure 1. Through PubChem database, the study was able to determine the structure of the primary bioactive chemicals that are present in the mentioned herbs and spices. The Indian Institute of Technology Delhi's Supercomputing Facility for Bioinformatics and Computational Biology server was used to conduct the drug-like screening of these compounds (Jayaram *et al.*, 2012; Putra, 2018; Hidayatullah *et al.*, 2023).

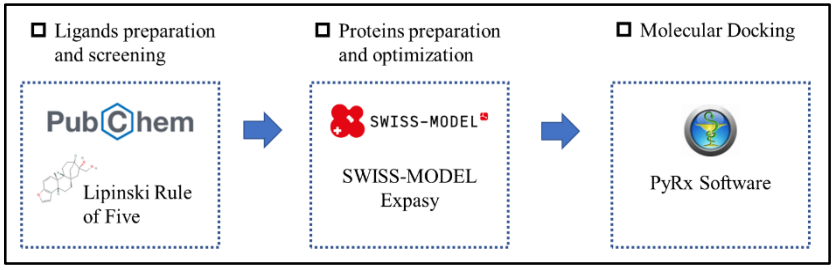


Figure 1. Flow chart of overall in silico study

2.2 Proteins Preparation and Optimization

This current investigation focused on different target proteins: the SARS-CoV-2's envelope, the nucleocapsid, and the spike/spike RBD protein. The SWISS-MODEL server was utilized in order to construct the three-

dimensional (3D) model. The study moved forward with the processes based on the structure that had the greatest amount of overlap with the protein sequences. The template codes for the proteins that were employed were 2mm4.1.A for the envelope protein, 7n0r.1 for the nucleocapsid protein, 6bfu.1.A for the spike protein, and 7b17.1 for the spike RBD.

Table 1. The list of common indigenous Indonesian herbs and spices

No.	Local name	Scientific name
1	Wigen	*Sesamum indicum*
2	Vanili	*Vanillia planifolia*
3	Ubi	*Ipomoea batatas*
4	Te hitam	*Camellia sinensis*
5	Talas	*Colocasia esculenta*
6	Serai	*Cymbopogon citratus*
7	Saffron	*Crocus sativus*
8	Pala	*Myristica fragrans*
9	Lengkuas	*Alpinia galanga*
10	Lada	*Piper nigrum*
11	Kluwek	*Pangium edule*
12	Ketumbar	*Coriandrum sativum*
13	Kencur	*Kaempferia galanga*
14	Kemukus	*Piper cubeba*
15	Kemiri	*Aleurites moluccanus*
16	Kelor	*Moringa oleifera*
17	Kedelai	*Glycine max*
18	Kayu secang	*Caesalpinia sappan*
19	Kayu manis	*Cinnamomum burmannii*
20	Kapulaga	*Amomum compactum*
21	Kacang hijau	*Phaseolus vulgaris*
22	Jinten	*Plectranthus amboinicus*
23	Jahe	*Zingiber officinale*
24	Daun salam	*Syzygium polyanthum*
25	Cengkeh	*Syzgium aromaticum*
26	Bunga luwang	*Illicium verum*
27	Beras hitam	*Oryza sativa*
28	Asam jawa	*Tamarindus indica*
29	Andaliman	*Zanthoxylum acanthopodium*
30	Adas	*Foeniculum vulgare*

2.3 Docking, Visualization, and Analysis

During the docking process, the PyRx software was utilized (Dallakyan and Olson, 2015; Putra *et al.*, 2017; Widiastuti *et al.*, 2023). All of the elements, including the structures of the proteins and ligands, have been converted to the AutoDock format (pdbqt). After deciding which proteins and compounds would serve as targets, the docking coordinates and coverage area (Å) were calculated (Table 2). The primary docking outcomes were determined based on the binding affinity, the binding site position, and the protein-ligand interaction that were discovered through the visualization process according to the authors' previous study (Putra *et al.*, 2020, 2021).

Table 2. Coordinates and coverage area (Å) setting during molecular docking

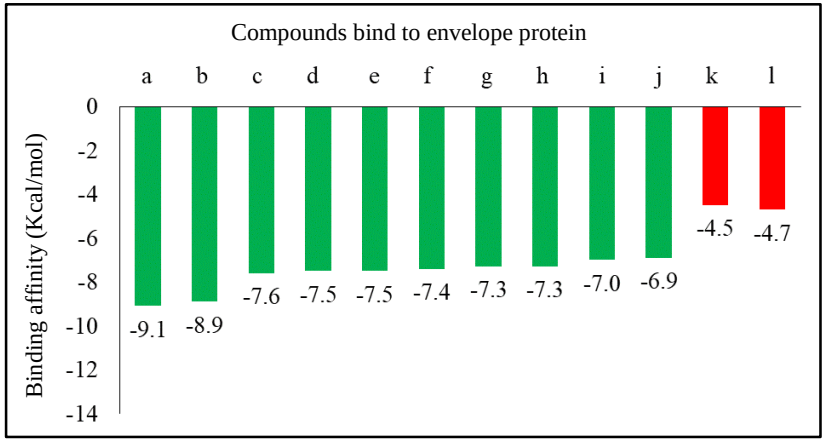
Center	X	Y	Z
Envelope protein	41.7375	19.7655	-21.4917
Nucleocapsid protein	0.0000	0.0000	0.3159
Spike protein	212.813	212.813	196.1010
Spike RBD	305.926	306.389	306.3797
Dimensions	X	Y	Z
Envelope protein	16.3004	47.6566	20.5333
Nucleocapsid protein	48.9776	51.3146	42.3157
Spike protein	107.9392	112.7436	142.0711
Spike RBD	66.9458	52.9292	87.0893

3. Results and Discussion

Computational analysis revealed that several bioactive compounds such as cafestol, kahweol, ledene, β-elemene, γ-elemene, tocotrienol, β-selinene, γ-selinene, γ-oryzanols, and β-sitosterol had great potency to bind with SARS-CoV-2 envelope protein compare with control drugs, Arbidol and Chloroquine (Figure 2). The 3D and 2D structure visualization of the complexes also showed the similar pattern of binding area to each other (Figures 3 and 4).

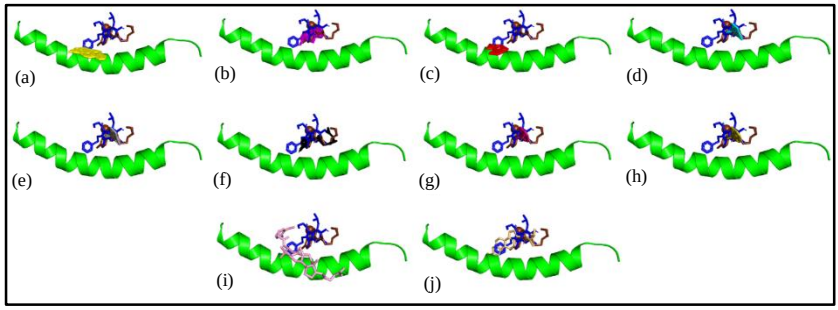
These findings suggested that the bioactive compounds might have competitiveness in inhibitory activity against the target protein compared with the control drugs. A study demonstrated that ligands with similar binding modes tend to have similar structures and closely related biological functions.

However, dissimilar ligand structures may still exhibit similar binding modes, although their biological functions might vary from each other (Xu and Zou, 2021).



Cafestol (a); kahweol (b); ledene (c); β-elemene (d); γ-elemene (e); tocotrienol; (g). β-selinene (g); γ-selinene (h); γ-oryzanols (i); β-sitosterol (j); arbidol (k); chloroquine (l)

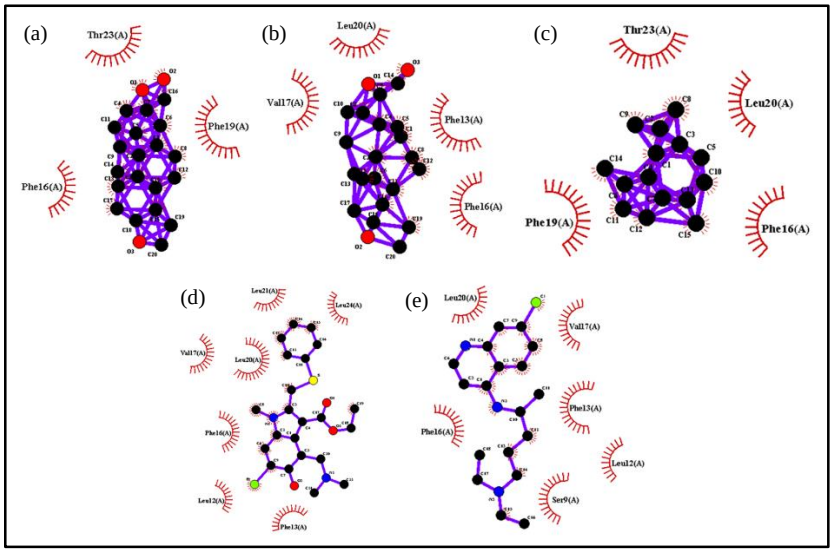
Figure 2. The binding's affinity score of envelope protein – ligand complex



Cafestol (a); kahweol (b); ledene (c); β-elemene (d); γ-elemene (e); tocotrienol (f); β-selinene (g); γ-selinene (h); γ-oryzanols (i); β-sitosterol (j)

Figure 3. The 3D structure visualization of envelope protein – ligand complex

Together, the computational prediction showed the presence of hydrophobic interactions in cafestrol, kahweol, and ledene, which were the top three compounds with the greatest binding affinity after molecular docking using PyRx software (Table 3).



Cafestol (a); kahweol (b); ledene (c); arbidol (d); chloroquine (e)

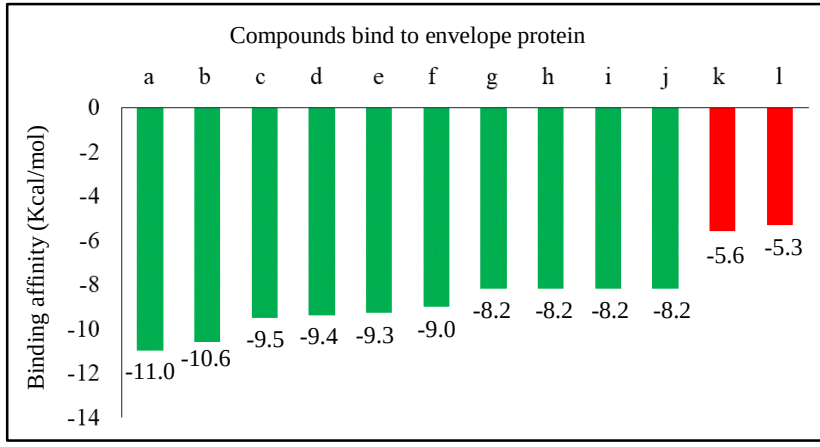
Figure 4. The 2D structure visualization of envelope protein – ligand complex

Table 3. List of amino acids residue and chemical interaction of top three bioactive compounds and control drugs against envelope protein

No.	Compound	Source	Amino Acids Residue	Interaction
1	Cafestol CID. 108052	Common bean (*P. vulgaris*)	Thr23(A); Phe19(A); Phe16(A)	Hydrophobic interaction
2	Kahweol CID. 114778	Common bean (*P. vulgaris*)	Leu20(A); Phe13(A); Phe16(A); Val17(A)	Hydrophobic interaction
3	Ledene CID. 10910653	Star anise (*I. verum*)	Thr23(A); Leu20(A); Phe16(A); Phe19(A)	Hydrophobic interaction
4	Arbidol CID. 131411	Antiviral drug (control)	Leu24(A); Phe13(A); Leu12(A); Phe16(A); Leu20(A); Val17(A); Leu21(A)	Hydrophobic interaction
5	Chloroquine CID. 2719	Antiviral drug (control)	Val17(A); Phe13(A); Leu12(A); Ser9(A); Phe16(A); Leu20(A)	Hydrophobic interaction

As an additional step in the drug discovery process, binding affinity is evaluated to assist in the development of drugs that interact with their targets precisely and selectively (Putra and Rifa'i, 2020; Thafar *et al.*, 2022; Maslikah

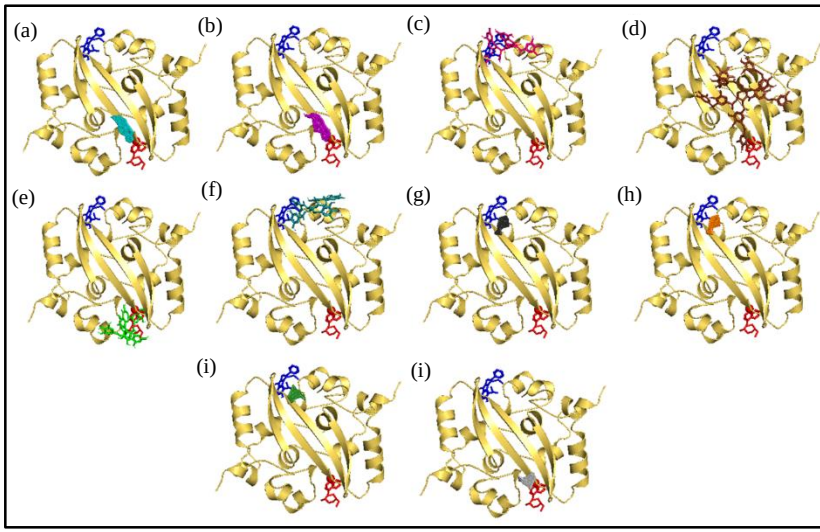
and Putra, 2024). Importantly, more negative values of binding affinity suggest a stronger interaction between ligands/drugs and receptor proteins (Uzzaman *et al.*, 2019). In the present study, several bioactive compounds, including cafestol, kahweol, thearubigin, tannic acid, theaflavin-3,3'-digallate, eugeniin, β-selinene, γ-selinene, γ-elemene, and β-elemene, showed potency to bind with the SARS-CoV-2 nucleocapsid protein compared with the control drugs, Arbidol and Chloroquine (Figure 5). The 3D and 2D visualizations, which provide the ligand's position, are shown in Figures 6 and 7, respectively. Interestingly, the top three compounds showed hydrogen bonds and hydrophobic interactions in complexes with the nucleocapsid protein (Table 4). Hydrogen bonding plays a crucial role in drug discovery as it contributes to the stabilization of the 3D structures of therapeutic targets, such as proteins and RNA (Putra *et al.*, 2019; Costales *et al.*, 2020).



Cafestol (a); kahweol (b); thearubigin (c); tannic acid (d); theaflavin-3,3'-digallate (e); eugeniin (f); β-selinene (g); γ-selinene (h); γ-elemene (i); β-elemene (j); arbidol (k); chloroquine (l)

Figure 5. The binding's affinity score of nucleocapsid protein – ligand complex

Consistent with previous findings on the envelope and nucleocapsid proteins, the present study also identified several potential bioactive compounds with proper binding affinity, such as tannic acid, eugeniin, kahweol, thearubigin, theaflavin-3,3'-digallate, crocin, sesamolin, cubebin, fisetin, and ledene, against the SARS-CoV-2 spike protein (Figure 8). The 3D and 2D visualizations, providing the ligand's position, are shown in Figures 9 and 10, respectively. Similar to the nucleocapsid interaction, the top three compounds exhibited hydrogen bonding and hydrophobic interactions in complexes with the spike protein (Table 5).



Cafestol (a); kahweol (b); thearubigin (c); tannic acid (d); theaflavin-3,3′-digallate (e); eugeniin (f); β-selinene (g); γ-selinene (h); γ-elemene (i); β-elemene (j)

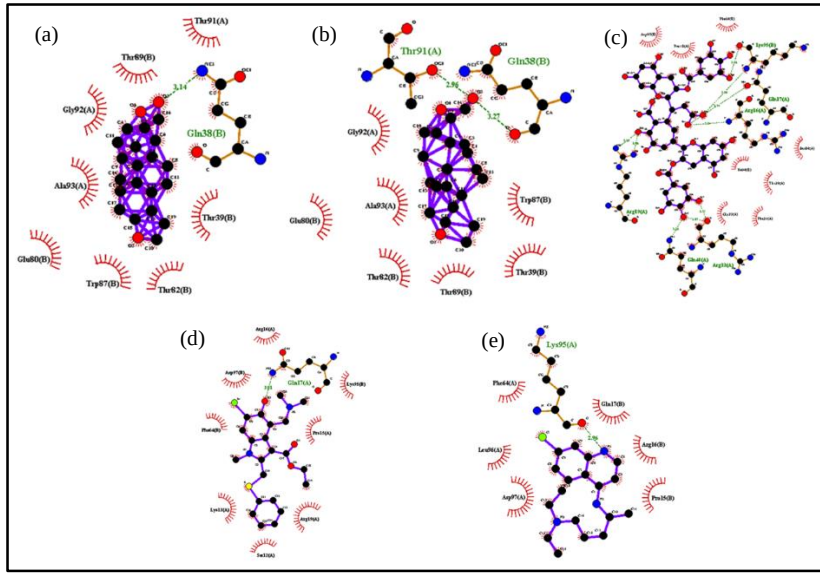
Figure 6. The 3D structure visualization of nucleocapsid protein – ligand complex

Table 4. List of amino acids residue and chemical interaction of top three bioactive compounds and control drug against nucleocapsid protein

No.	Compound	Source	Amino acids residue	Interaction
1	Cafestol CID. 108052	Common bean (*P. vulgaris*)	Gln38(B)	Hydrogen bond
			Thr39(B); Thr82(B); Trp82(B); Glu80(B); Ala93(B); Gly92(B); Thr89(B); Thr91(B)	Hydrophobic interaction
2	Kahweol CID. 114778	Common bean (*P. vulgaris*)	Gln38(B); Thr91(B)	Hydrogen bond
			Trp87(B); Thr39(B); Thr89(B); Thr82(B); Ala93(B); Glu80(B); Gly92(B)	Hydrophobic interaction
3	Thearubigin CID. 100945367	Black tea (*C. sinensis*)	Gly41(A); Arg33(A); Gln40(A); Lys95(B); Asp97(B)	Hydrogen bond
			Lys99(B); Ser84(A); Thr39(A); Trp87(A);	Hydrophobic interaction

Table 4 continued.

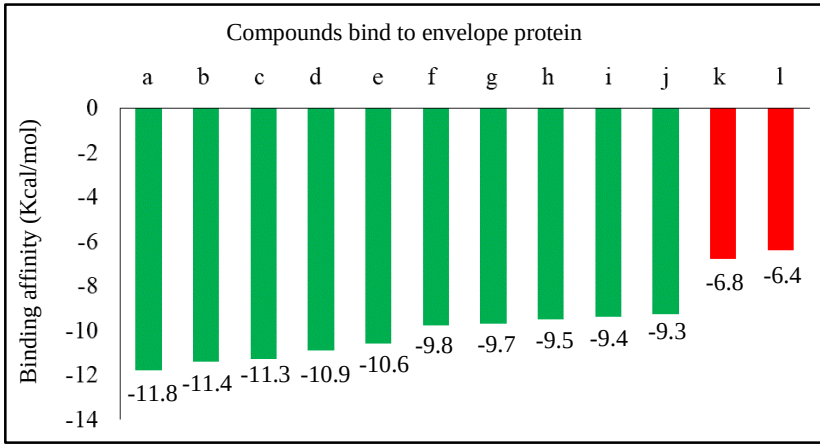
			Phe31(A); Arg34(A); Gly32(A); Arg19(A); Ile94(B); Leu96(B); Gln17(A); Phe64(B); Gln63(B); Asp100(B); Gln106(B)	
4	Arbidol CID. 131411	Antiviral drug (control)	Gln17(A)	Hydrogen bond
			Lys95(B); Pro15(A); Arg19(A); Ser12(A); Lys13(A); Phe64(B); Asp97(B); Arg16(A)	Hydrophobic interaction
5	Chloroquine CID. 2719	Antiviral drug (control)	Lys95(A)	Hydrogen bond
			Gln17(B); Arg16(B); Pro15(B); Asp97(A); Leu96(A); Phe64(A)	Hydrophobic interaction



Cafestol (a); kahweol (b); thearubigin (c); arbidol (d); chloroquine (e)

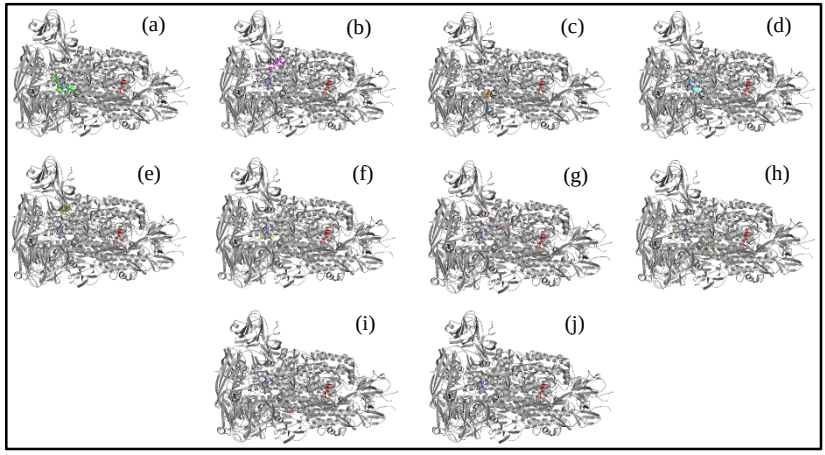
Figure 7. The 2D structure visualization of nucleocapsid protein – ligand complex

Furthermore, as the final target protein in this study, several bioactive compounds were demonstrated against the spike RBD protein, namely kahweol, cafestol, tannic acid, ledene, sesaminol, morin, γ-selinene, thearubigin, β-selinene, and genistein, which displayed greater binding affinity compared with the control drugs (Figure 11). The 3D and 2D visualizations, providing the ligand's position, are shown in Figures 12 and 13, respectively, illustrating the chemical interaction among the complexes of ligands and target proteins (Table 6).



Tannic acid (a); eugeniin (b); kahweol (c); thearubigin (d); theaflavin-3,3'-digallate (e); crocin (f); sesamolin (g); cubebin (h); fisetin (i); ledene (j); arbidol (k); chloroquine (l)

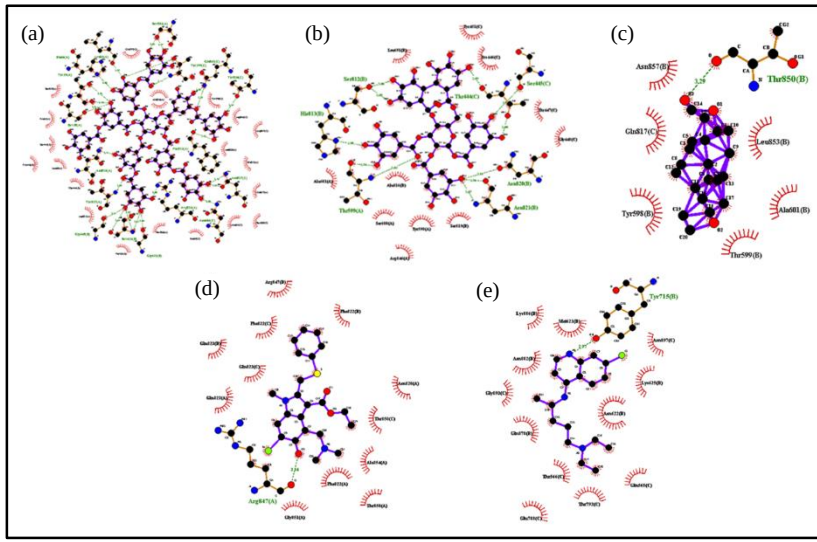
Figure 8. The binding's affinity result of spike protein – ligand complex



Tannic acid (a); eugeniin (b); kahweol (c); thearubigin (d); theaflavin-3,3'-digallate (e); crocin (f); sesamolin (g); cubebin (h); fisetin (i); ledene (j)

Figure 9. The 3D structure visualization of spike protein – ligand complex

According to the above explanation regarding the present findings, the study discovered an intriguing aspect: a bioactive compound isolated from common beans, called kahweol, appeared in all target proteins, including envelope, nucleocapsid, and spike/spike RBD proteins, exhibiting the greatest binding affinity among other ligands (Figure 14). Furthermore, among 30 herbs and spices analyzed, four types of plants were found to contain potential bioactive compounds against SARS-CoV-2 structural proteins: black tea, clove, common beans, and star anise (Tables 3 to 6).



Tannic acid (a); eugeniin (b); kahweol (c); arbidol (d); chloroquine (e)

Figure 10. The 2D structure visualization of spike protein – ligand complex

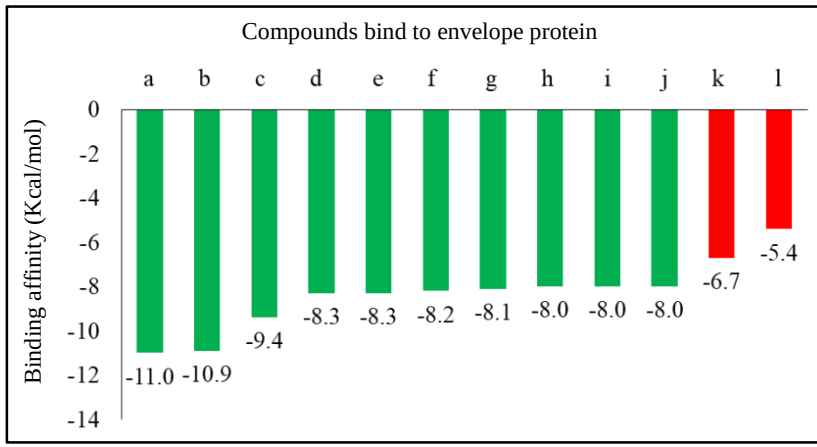
Table 5. List of amino acids residue and chemical interaction of top three bioactive compounds and control drug against spike protein

No.	Compound	Source	Amino acids residue	Interaction
1	Tannic acid CID. 16129778	Black tea (*Camelia sinensis*)	Phe822(A); Thr850(C); Asn857(C); Asn604(C); Thr599(C); Ser812(A); Ser58(A); Leu65(A); Thr59(A); His66(A); Gly448(B); Ser425(B); Gly423(B); Arg835(A); Asn821(A); Thr827(A); Asn821(A); Gln843(C)	Hydrogen bond

Table 5 continued.

			Asp846(C); Arg847(C); Asn820(A); His813(A); Ile605(C); Gln817(A); Ser600(C); Ala601(C); Tyr598(C); Ala816(A); Gln597(C); Pro61(A); Ser445(B); Pro446(B); Leu60(A); Thr444(B); Asp831(A); Val424(B); Ser826(A)	Hydrophobic interaction
2	Eugeniin CID. 442679	Clove (*S. aromaticum*)	Ser812(B); Thr444(C); Ser445(C); Asn820(B); Asn821(B); Thr599(A); His813(B)	Hydrogen bond
			Tyr451(C); Pro446(C); Thr447(C); Gly448(C); Ser819(B); Tyr598(B); Ser600(A); Ala816(B); Ala601(A); Leu691(B)	Hydrophobic interaction
3	Kahweol CID. 114778	Common bean (*P. vulgaris*)	Thr850(B)	Hydrogen bond
			Leu852(B); Ala601(A); Thr599(B); Tyr598(B); Gln817(C); Asn857(B)	Hydrophobic interaction
4	Arbidol CID. 131411	Antiviral drug (control)	Arg847(A)	Hydrogen bond
			Asn820(A); Thr850(C); Ala854(A); Thr850(A); Phe822(A); Gly851(A); Gln823(A); Gln823(C); Gln823(B); Phe822(C); Arg847(B); Phe822(B)	Hydrophobic interaction
5	Chloroquine CID. 2719	Antiviral drug (control)	Tyr715(B)	Hydrogen bond
			Asn897(A); Lys625(B); Asn622(B); Gln565(C); Thr792(C); Glu788(C); Thr566(C); Gln878(B); Gly893(C); Asn882(B); Lys886(B); Met623(B)	Hydrophobic interaction

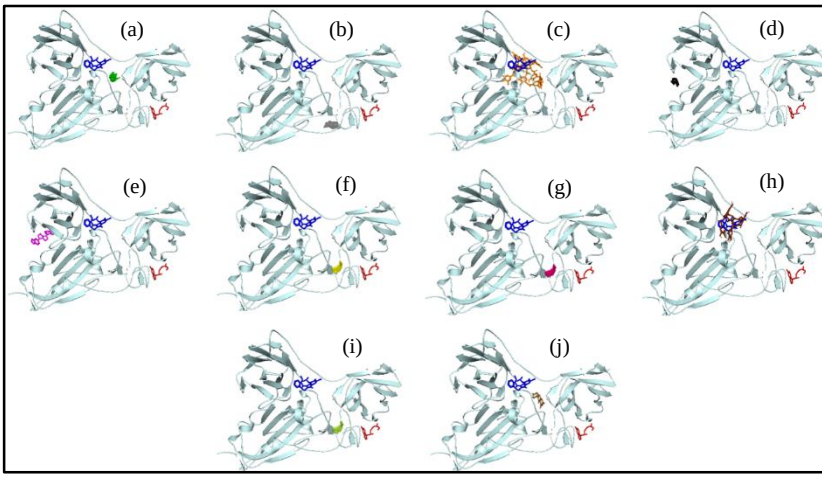
Based on these findings, kahweol showed promising potential as a candidate for developing multi-target drugs. Multi-target drugs can regulate several target proteins in specific diseases, offering benefits in improving efficacy and avoiding side effects (Talevi, 2015; Viana *et al.*, 2018). In cancer-related research, kahweol is known for its impact on promoting several important molecular targets, including AP-1, NF-kB, CREB, STAT3, and STAT1 (Chen *et al.*, 2022). Conversely, kahweol has demonstrated therapeutic potential in promoting inflammatory mediators such as IL-8, MCP1, and ICAM1 (Hao *et al.*, 2019). Furthermore, kahweol is recognized for its function in stimulating the expression of Nrf2 and reducing oxidative stress during liver injury (Seo *et al.*, 2020). From the perspective of viral infections, Nrf2 exerts a protective effect against infection (Wang *et al.*, 2023).



Kahweol (a); cafestol (b); tannic acid (c); ledene (d); sesaminol (e); morin (f); γ-selinene (g); thearubigin (h); β-selinene (i); genistein (j); arbidol (k); chloroquine (l)

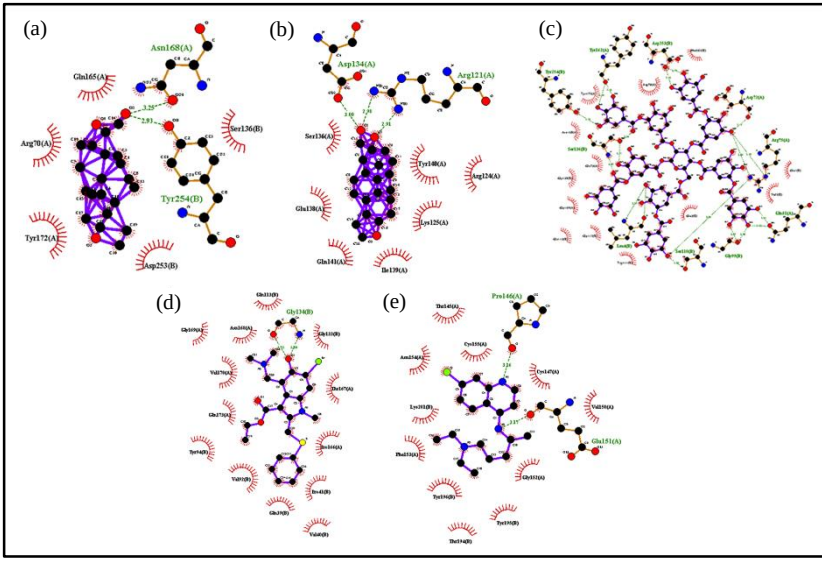
Figure 11. The binding's affinity result of spike RBD – ligand complex

In recent years, there has been intensive research on a wide range of natural compounds to better understand their antiviral properties. One such class of chemicals known for its potent antiviral properties is polyphenols (Kitazato *et al.*, 2007). Components of black tea extract, such as theaflavins, have demonstrated potent antiviral activity against calicivirus, herpes simplex virus 1, human immunodeficiency virus 1, and influenza A (Chowdhury *et al.*, 2018; Mhatre *et al.*, 2021). Clove, one of the most precious spices, has been used for many years both as a food preservative and as a component in various medicinal preparations. A research group noted that clove exhibits antiviral activity against herpes simplex virus type 1 (Cortés-Rojas *et al.*, 2014).



Kahweol (a); cafestol (b); tannic acid (c); ledene (d); sesaminol (e); morin (f); γ-selinene (g); thearubigin (h); β-selinene (i); genistein (j)

Figure 12. The 3D structure visualization spike RBD – ligand complex



Kahweol (a); cafestol (b); tannic acid (c); arbidol (d); chloroquine (e)

Figure 13. The 2D structure visualization of spike RBD – ligand complex

Table 6. List of amino acids residue and chemical interaction of top three bioactive compounds and control drug against spike RBD protein

No.	Compound	Source	Amino acids residue	Interaction
1	Kahweol CID. 114778	Common bean (*P. vulgaris*)	Asn168(A); Tyr254(B)	Hydrogen bond
			Ser136(B); Asp253(B); Tyr172(A); Arg70(A); Gln165(A)	Hydrophobic interaction
2	Cafestol CID. 108052	Common bean (*P. vulgaris*)	Asp134(A); Arg121(A)	Hydrogen bond
			Tyr140(A); Arg124(A); Gln141(A); Lys124(A); Ile139(A); Glu138(A); Ser136(A)	Hydrophobic interaction
3	Tannic acid CID. 16129778	Black tea (*C. sinensis*)	Tyr254(B); Ser136(B); Tyr162(A); Leu4(B); Ser110(B); Gly99(B); Gln81(A); Arg75(A); Asp72(A); Asp253(B)	Hydrogen bond
			Asn168(A); Gly169(A); Gln113(B); Gly112(B); Trp111(B); Gln3(B); Gln1(B); Val2(B); Glu73(A); Met252(B); Arg70(A); Tyr172(A); Gly135(B)	Hydrophobic interaction
4	Arbidol CID. 131411	Antiviral drug (control)	Gly134(B)	Hydrogen bond
			Pro166(A); Pro41(B); Val40(B); Gln39(B); Val92(B); Try94(B); Gln173(A); Val170(A); Gly169(A); Asn168(A); Gln113(B); Gly133(B); Thr167(A)	Hydrophobic interaction
5	Chloroquine CID. 2719	Antiviral drug (control)	Glu151(A); Pro146(A)	Hydrogen bond
			Cys147(A); Val150(A); Gly152(A); Tyr195(B); Thr194(B); Tyr196(B); Phe153(A); Lys201(B); Asn154(A); Cys155(A); Thr145(A)	Hydrophobic interaction

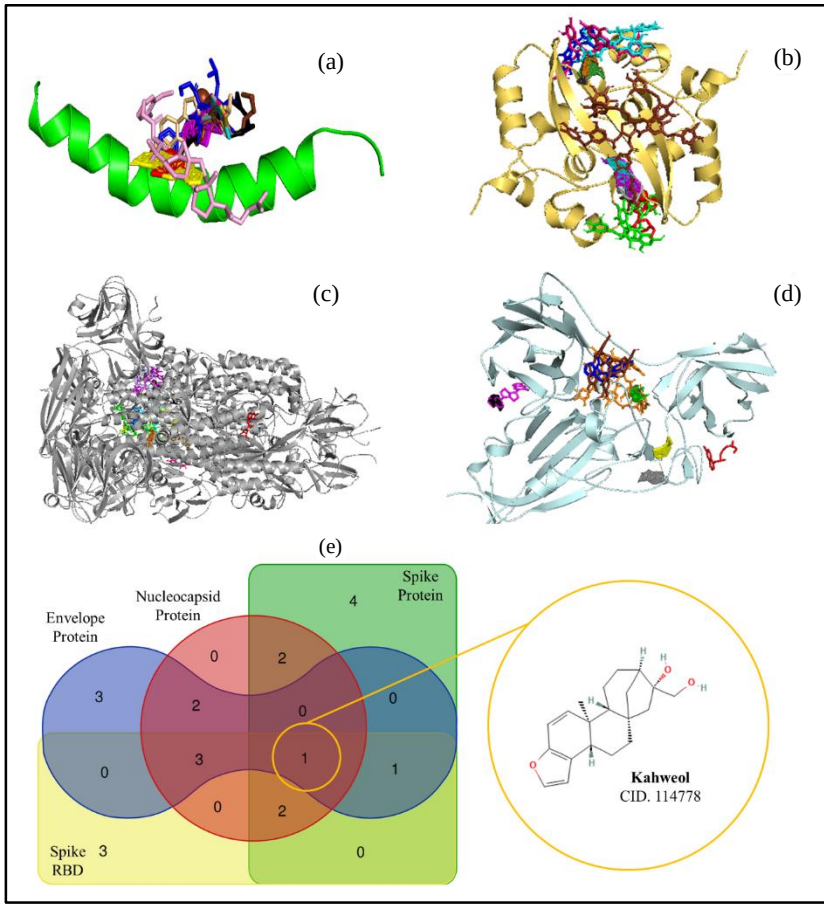


Figure 14. The 3D structure visualization of bioactive compounds (top ten) and control drugs toward the envelope protein (a), nucleocapsid protein (b), spike protein (c), and spike RBD protein (d); the Venn diagram showed kahweol is present in four target proteins (e)

Interestingly, clove has been explored as an anti-COVID-19 remedy through decoction preparation, involving boiling clove in water for 15 min (Vicidomini *et al.*, 2021). Similarly, common bean has demonstrated antiviral activity, showing reverse transcriptase activity against human immunodeficiency virus (Patel *et al*., 2021). In addition to its culinary use as a seasoning, star anise is a key component of Chinese medicinal herbs and is renowned for its antiviral effects. It is well-documented that star anise possesses antiviral properties, particularly against influenza A, influenza B (Patra *et al*., 2020), and grouper iridovirus infection (Liu *et al*., 2020). Given the evidence presented for their antiviral properties, these medicinal plants and

their components hold significant potential for development into anti-COVID-19 medications.

4. Conclusion and Recommendation

Approximately four out of thirty types of plants evaluated in this study exhibit potential bioactive compounds against SARS-CoV-2 structural proteins, including black tea (*C. sinensis*), clove (*S. aromaticum*), common bean (*P. vulgaris*), and star anise (*I. verum*). Interestingly, our findings demonstrate that kahweol, widely found in all of these medicinal plants, is the only compound that exhibits multi-target properties against SARS-CoV-2's envelope protein, nucleocapsid protein, spike protein, and spike RBD protein. These preliminary findings suggest great potential for developing an anti-SARS-CoV-2 drug candidate. Further research is needed to explore the biological activity of kahweol through in vitro or in vivo studies to evaluate its pharmacokinetics, toxicity, and efficacy as a multi-target drug candidate against SARS-CoV-2.

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6. References

Al-Tawfiq, J.A. (2020). Asymptomatic coronavirus infection: MERS-CoV and SARS-CoV-2 (COVID-19). Travel Medicine and Infectious Disease, 35, 1-2. https://doi.org/10.1016/j.tmaid.2020.101608

Ang, L., Song, E., Lee, H.W., & Lee, M.S. (2020). Herbal medicine for the treatment of coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis of randomized controlled trials. Journal of Clinical Medicine, 9(5), 1-20. https://doi.org/10.3390/jcm9051583

Ballering, A.V., van Zon, S.K.R., Hartman, T.C., Rosmalen, J.G.M., & Lifelines Corona Research Initiative (2022). Persistence of somatic symptoms after COVID-19

in the Netherlands: An observational cohort study. The Lancet, 400(10350), 452-461. https://doi.org/10.1016/S0140-6736(22)01214-4

Bueno-Notivol, J., Gracia-García, P., Olaya, B., Lasheras, I., López-Antón, R., & Santabárbara, J. (2021). Prevalence of depression during the COVID-19 outbreak: A meta-analysis of community-based studies. International Journal of Clinical and Health Psychology, 21(1), 1-11. https://doi.org/10.1016/j.ijchp.2020.07.007

Chan, K.W., Wong, V.T., & Tang, S.C.W. (2020). COVID-19: An update on the epidemiological, clinical, preventive and therapeutic evidence and guidelines of integrative Chinese-Western medicine for the management of 2019 novel coronavirus disease. American Journal of Chinese Medicine, 48(3), 737-762. https://doi.org/10.1142/S0192415X20500378

Chen, J.Y., Xiao-Yun, T., Wei, S.S., Yang, Y.J., Deng, S., Jiao, C.J., Wang, C.J., Chu, K.D., Ma, X.Q., & Xu, W. (2022). Perspectives of herbs and their natural compounds, and herb formulas on treating diverse diseases through regulating complicated JAK/STAT signaling. Frontiers in Pharmacology, 13, 1-22. https://doi.org/10.3389/fphar.2022.993862

Chowdhury, P., Sahuc, M.E., Rouillé, Y., Rivière, C., Bonneau, N., Vandeputte, A., Brodin, P., Goswami, M., Bandyopadhyay, T., Dubuisson, J., & Séron, K. (2018). Theaflavins, polyphenols of black tea, inhibit entry of hepatitis C virus in cell culture. PLoS One, 13(11), 1-16. https://doi.org/10.1371/journal.pone.0198226

Chu, J. (2021). A statistical analysis of the novel coronavirus (COVID-19) in Italy and Spain. PLoS One, 16(3), 1-36. https://doi.org/10.1371/journal.pone.0249037

Clark, S.J., & Turner, A.N. (2021). Monitoring epidemics: Lessons from measuring population prevalence of the coronavirus. Proceedings of the National Academy of Sciences of the United States of America, 118(9), 1-3. https://doi.org/10.1073/pnas.2026412118

Cortés-Rojas, D.F., de Souza, C.R., & Oliveira, W.P. (2014). Clove *(Syzygium aromaticum*): A precious spice. Asian Pacific Journal of Tropical Biomedicine, 4(2), 90-6.

Costales, M.G., Childs-Disney, J.L., Haniff, H.S., & Disney, M.D. (2020). How we think about targeting RNA with small molecules. Journal of Medicinal Chemistry, 63, 8880-8900. https://doi.org/10.1021/acs.jmedchem.9b01927

Cragg, G.M., & Newman, D.J. (2001). Natural product drug discovery in the next millennium. Pharmaceutical Biology, 39(1), 8-17. https://doi.org/10.1076/phbi.39.s1.8.0009

Dallakyan, S., & Olson, A.J. (2015). Small-molecule library screening by docking with PyRx. Methods in Molecular Biology, 1263, 243-50.

Demeke, C.A., Woldeyohanins, A.E., & Kifle, Z.D. (2021). Herbal medicine use for the management of COVID-19: A review article. Metabolism Open, 12, 1-6.

Haleem, A., Javaid, M., & Vaishya, R. (2020). Effects of COVID-19 pandemic in daily life. Current Medicine Research and Practice, 10(2), 78-79.

Hao, W.R., Sung, L.C., Chen, C.C., Chen, P.Y., Cheng, T.H., Chao, H.H., Liu, J.C., & Chen, J.J. (2019). Cafestol inhibits cyclic-strain-induced interleukin-8, intercellular adhesion molecule-1, and monocyte chemoattractant protein-1 production in vascular endothelial cells. Oxidative Medicine and Cellular Longevity, 2019, 1-10. https://doi.org/10.1155/2018/7861518

Hidayatullah, A., Putra, W.E., Rifa'i, M., Sustiprijatno, Widiastuti, D., Heikal, M.F., Susanto, H., & Salma, W.O. (2022). Molecular docking and dynamics simulation studies to predict multiple medicinal plants' bioactive compounds interaction and its behavior on the surface of DENV-2 E protein. Karbala International Journal of Modern Science, 8(3), 531-542.

Hidayatullah, A., Putra, W.E., Salma, W.O., Muchtaromah, B., Permatasari, G.W., Susanto, H., Widiastuti, D., & Kismurtono, M. (2021). Discovery of drug candidate from various natural products as potential novel dengue virus nonstructural protein 5 (NS5) inhibitor. Natural and Life Sciences Communications, 20(1), 1-17.

Hidayatullah, A., Putra, W.E., Sustiprijatno, Widiastuti, D., Salma, W.O., & Heikal, M.F. (2023). Molecular docking and molecular dynamics simulation-based identification of natural inhibitors against druggable human papilloma virus type 16 target. Trends in Sciences, 20(4), 1-12. https://doi.org/10.48048/tis.2023.4891

Jayaram, B., Singh, T., Mukherjee, G., Mathur, A., Shekhar, S., & Shekhar, V. (2012). Sanjeevini: A freely accessible web-server for target directed lead molecule discovery. BMC Bioinformatics, 13, 1-13. https://doi.org/10.1186/1471-2105-13-S17-S7

Kitazato, K., Wang, Y., & Kobayashi, N. (2007). Viral infectious disease and natural products with antiviral activity. Drug Discoveries and Therapeutics, 1(1), 14-22.

Liana, D., & Phanumartwiwath, A. (2022). Leveraging knowledge of Asian herbal medicine and its active compounds as COVID-19 treatment and prevention. Journal of Natural Medicines, 76(1), 20-37. https://doi.org/10.1007/s11418-021-01575-1

Liu, M., Yu, Q., Xiao, H., Yi, Y., Cheng, H., Putra, D.F., Huang, Y., Zhang, Q., & Li, P. (2020). Antiviral activity of *Illicium verum* Hook. f. extracts against grouper iridovirus infection. Journal of Fish Diseases, 43(5), 531-540. https://doi.org/10.1111/jfd.13146

Malik, S., Gupta, A., Zhong, X., Rasmussen, T.P., Manautou, J.E., & Bahal, R. (2020). Emerging therapeutic modalities against COVID-19. Pharmaceuticals (Basel), 13(8), 1-32.

Maslikah, S.I., & Putra, W.E. (2024). Molecular dynamics simulation of various bioactive compounds of red betel (*Piper crocatum*) as anti-inflammatory drug-like candidates in rheumatoid arthritis treatment. Trends in Sciences, 21(3), 1-8. https://doi.org/10.48048/tis.2024.7334

McKee, M., & Stuckler, D. (2020). If the world fails to protect the economy, COVID-19 will damage health not just now but also in the future. Nature Medicine, 26(5), 640-642. https://doi.org/10.1038/s41591-020-0863-y

Mhatre, S., Srivastava, T., Naik, S., & Patravale, V. (2021). Antiviral activity of green tea and black tea polyphenols in prophylaxis and treatment of COVID-19: A review. Phytomedicine, 85, 1-8.

Ni, L., Zhou, L., Zhou, M., Zhao, J., & Wang, D.W. (2020). Combination of western medicine and Chinese traditional patent medicine in treating a family case of COVID-19. Frontiers in Medicine, 14(2), 210-214. https://doi.org/10.1007/s11684-020-0757-x

Niknam, Z., Jafari, A., Golchin, A., Danesh Pouya, F., Nemati, M., Rezaei-Tavirani, M., & Rasmi, Y. (2022). Potential therapeutic options for COVID-19: An update on current evidence. European Journal of Medical Research, 27(1), 1-15. https://doi.org/10.1186/s40001-021-00626-3

Patel, B., Sharma, S., Nair, N., Majeed, J., Goyal, R.K., & Dhobi, M. (2021). Therapeutic opportunities of edible antiviral plants for COVID-19. Molecular and Cellular Biochemistry, 476(6), 2345-2364.

Patra, J.K., Das, G., Bose, S., Banerjee, S., Vishnuprasad, C.N., Del Pilar Rodriguez-Torres, M., & Shin, H.S. (2020). Star anise (*Illicium verum*): Chemical compounds, antiviral properties, and clinical relevance. Phytotherapy Research, 34(6), 1248-1267. https://doi.org/10.1002/ptr.6614

Putra, W.E., & Rifa'i, M. (2019). Immunomodulatory activities of *Sambucus javanica* extracts in DMBA exposed BALB/c mouse. Advanced Pharmaceutical Bulletin, 9(4), 619-623.

Putra, W.E., & Rifa'i, M. (2020). Evaluating the molecular interaction of Sambucus plant bioactive compounds toward TNF-R1 and TRAIL-R1/R2 as possible anti-cancer therapy based on traditional medicine: The bioinformatics study. Scientific Study & Research - Chemistry & Chemical Engineering, Biotechnology, Food Industry, 21(3), 357-365.

Putra, W.E., Salma, W.O., & Rifa'i, M. (2019). Anti-inflammatory activity of Sambucus plant bioactive compounds against TNF-α and TRAIL as solution to overcome inflammation associated diseases: The insight from bioinformatics study. Natural Product Sciences, 25(3), 215-221.

Putra, W.E., Salma, W.O., Widiastuti, D., & Kismurtono, M. (2020). In silico screening of peroxisome proliferator activated receptor gamma (PPARG)-agonist from Eugenia jambolana bioactive compounds as potential anti-diabetic agent. Malaysian Journal of Biochemistry and Molecular Biology, 23(2), 142-146.

Putra, W.E., Susanto, H., Lestari, S.R., Gofur, A., Salma, W.O., Widiastuti, D., Ali, H.F., Assalimi, Q.N., Kusumadewi, N.I., Artamevia, D.A., Pratiwi, A.A., Muhsin, F.A.H.A.N., Christina, Y.I., & Wahyudi, D. (2021). In silico screening of bioactive compounds from various medicinal plants for α-2 neuronal nicotinic acetylcholine receptor inhibitor candidate. AIP Conference Proceedings, 2353, 1-5. https://doi.org/10.1063/5.0052775

Putra, W.E., Sustiprijatno, Hidayatullah, A., Heikal, M.F., Widiastuti, D., & Isnanto, H. (2023). Analysis of three non-structural proteins, Nsp1, Nsp2, and Nsp10 of Sars-Cov-2 as pivotal target proteins for computational drug screening. Journal of Microbiology, Biotechnology and Food Sciences, 12(5), 1-6. https://doi.org/10.55251/jmbfs.9586

Putra, W.E., Waffareta, E., Ardiana, O., Januarisasi, I.D., Soewondo, A., & Rifa'i, M. (2017). Dexamethasone-administrated BALB/c mouse promotes proinflammatory cytokine expression and reduces CD4+CD25+ regulatory T cells population. Bioscience Research, 14(2), 201-213.

Putra, W.E. (2018). In silico study demonstrates multiple bioactive compounds of Sambucus plant promote death cell signaling pathway via Fas receptor. Federal University Wukari Trends in Science Journal, 3(2), 682-685.

Seo, H.Y., Lee, S.H., Lee, J.H., Hwang, J.S., Kim, M.K., & Jang, B.K. (2020). Kahweol activates the Nrf2/HO-1 pathway by decreasing Keap1 expression independently of p62 and autophagy pathways. PLoS One, 15(10), 1-13.

Shahrajabian, M.H., Sun, W., & Cheng, Q. (2020). Traditional herbal medicine for the prevention and treatment of cold and flu in the autumn of 2020, overlapped with COVID-19. Natural Product Communications, 15(8), 1-10. https://doi.org/10.1177/1934578X20951431

Shankar, A., Dubey, A., Saini, D., & Prasad, C.P. (2020). Role of complementary and alternative medicine in prevention and treatment of COVID-19: An overhyped hope. Chinese Journal of Integrative Medicine, 26(8), 565-567.

Subramanian, A., Nirantharakumar, K., Hughes, S., Myles, P., Williams, T., Gokhale, K.M., … Haroon, S. (2022). Symptoms and risk factors for long COVID in non-hospitalized adults. Nature Medicine, 28, 1706-1714. https://doi.org/10.1038/s41591-022-01909-w

Talevi, A. (2015). Multi-target pharmacology: Possibilities and limitations of the "skeleton key approach" from a medicinal chemist perspective. Frontiers in Pharmacology, 6, 1-7. https://doi.org/10.3389/fphar.2015.00205

Thafar, M.A., Alshahrani, M., Albaradei, S., Gojobori, T., Essack, M., & Gao, X. (2022). Affinity2Vec: Drug-target binding affinity prediction through representation learning, graph mining, and machine learning. Scientific Reports, 12(1), 4751.

Uzzaman, M., Chowdhury, M.K., & Hossen, M.B. (2019). Thermochemical, molecular docking and ADMET studies of Aspirin metabolites. Frontiers in Drug, Chemistry and Clinical Research, 2, 1-5.

Vellingiri, B., Jayaramayya, K., Iyer, M., Narayanasamy, A., Govindasamy, V., Giridharan, B., Ganesan, S., Venugopal, A., Venkatesan, D., Ganesan, H., Rajagopalan, K., Rahman, P.K.S.M., Cho, S.G., Kumar, N.S., & Subramaniam, M.D. (2020). COVID-19: A promising cure for the global panic. Science of the Total Environment, 725, 1-19. https://doi.org/10.1016/j.scitotenv.2020.138277

Viana, J.O., Félix, M.B., Maia, M.S., Serafim, V.L., Scotti, L., & Scotti, M.T. (2018). Drug discovery and computational strategies in the multitarget drugs era. Brazilian

Journal of Pharmaceutical Sciences, 54, 1-25. https://doi.org/10.1590/s2175-97902018000001010

Vicidomini, C., Roviello, V., & Roviello, G.N. (2021). Molecular basis of the therapeutical potential of clove (*Syzygium aromaticum* L.) and clues to its anti-COVID-19 utility. Molecules, 26(7), 1-12. https://doi.org/10.3390/molecules26071880

Wang, Y., Ma, J., & Jiang, Y. (2023). Transcription factor Nrf2 as a potential therapeutic target for COVID-19. Cell Stress Chaperones, 28(1), 11-20.

Widiastuti, D., Warnasih, S., Sinaga, S.E., Pujiyawati, E., Supriatno, & Putra, W.E. (2023). Identification of active compounds from *Averrhoa bilimbi* L. (belimbing wuluh) flower using LC-MS and antidiabetic activity test using in vitro and in silico approaches. Trends in Sciences, 20(8), 1-9. https://doi.org/10.48048/tis.2023.6761

Xu, X., & Zou, X. (2021). Dissimilar ligands bind in a similar fashion: A guide to ligand binding-mode prediction with application to CELPP studies. International Journal of Molecular Sciences, 22(22), 1-11. https://doi.org/10.3390/ijms222212320