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ANALYSIS OF THREE NON-STRUCTURAL PROTEINS, NSP1, NSP2, AND NSP10 OF SARS-COV-2 AS PIVOTAL TARGET PROTEINS FOR COMPUTATIONAL DRUG SCREENING

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ABSTRACT

Coronaviruses cause mild to severe respiratory infections. The highly contagious SARS-CoV-2 new coronavirus caused a global outbreak of atypical viral pneumonia in late 2019. Acute respiratory distress syndrome, multiple organ failure, respiratory failure, and death can result from the infection. This study aimed to evaluate the possible inhibition activity from numerous medicinal plants' bioactive compounds against three non-structural proteins, namely Nsp1, Nsp2, and Nsp10 of SARS-CoV-2, through the computational study. Molecular docking was performed on the ligands and the target protein. This study investigated multiple criteria, including binding affinity value, location, and chemical interaction. In this present study, we found the top three highest binding affinity values of bioactive compounds against Nisp1, namely cafestol, crocin, and ledene; the top three highest binding affinity values of bioactive compounds against Nisp2, including cafestol, kahweol, and theaflavin 3,3′-digallate; and top three highest binding affinity value of bioactive compounds against Nisp10 namely cafestol, kahweol, and theaflavin-3,3′-digallate. Interestingly, we also found that cafestol, crocin, and theaflavin-3,3'-digallate binds to all target proteins.

Keywords: In silico, medicinal plants, Nsp1, Nsp2, Nsp10

INTRODUCTION

It has been reported that the broad group of viruses known as coronaviruses cause minor to severe respiratory infections **(Cui et al., 2019; Hu et al., 2021).** Significantly, both animals and humans might acquire respiratory and gastrointestinal diseases caused by coronaviruses. At the end of 2019, an outbreak of atypical viral pneumonia was brought on by the SARS-CoV-2 new coronavirus, which is highly contagious and has spread quickly throughout the entire world **(Wu et al., 2020; Hu et al., 2021).** The infected people with the virus showed common symptoms such as fever, malaise, and cough **(Wang et al., 2020; Ludwig and Zarbock, 2020).** In the worst and most serious conditions, the infection might lead to developing acute respiratory distress syndrome, multiple organ failure, respiratory failure, and even death **(Huang et al., 2019; Ludwig and Zarbock, 2020).**

The SARS-CoV-2 was known to belong to the family Coronaviridae and the order Nidovirales. These viral protein structures are spherical, with a diameter of about 80 to 160 nm. The capsid is enclosed with spike (S) proteins, membrane (M) proteins, and envelope (E) proteins. Inside the capsid contain RNA viral genome and phosphorylated nucleocapsid (N) that form a spiral nucleocapsid **(Yang et al., 2020**). On the other hand, seven open reading frames (ORFs) encode extra proteins **(Hu et al., 2021).** The ORF1ab of the SARS-CoV-2 genome is processed into several non-structural proteins (Nsp). Importantly, Nsp plays an important role, especially in maintaining the life cycle and pathogenicity. For example, the virus occupied the Nsp1 to circumvent the host immune system or impair host gene expression. Moreover, the Nsp2 is expendable for viral replication, and the Nsp10–16 complex is vital in the transcription/translational process and immune evasion **(Low et al., 2022; Raj, 2020)**. During infection, the viral Nsp forms a multi-subunit RNA-synthesis complex responsible for viral genome replication and transcription **(Fung and Liu, 2019).** Because Nsp plays a vital role in viral replication, survival, and pathogenicity, they are a promising target for anti-CoV drug development.

On the other hand, COVID-19 has been tried to be treated in several ways. These include RNA-dependent RNA polymerase inhibitors (Remdesivir, Ribavirin, or Favipiravir); protease inhibitors (Lopinavir or Ritonavir); endosomal acidification inhibitors (azithromycin, chloroquine, or hydroxychloroquine); monoclonal or polyclonal antibodies; viral exocytosis inhibitors; convalescent plasma transfusion therapy; and herbal medicines **(Mirzaie et al., 2020; Jean et al., 2020).** Even though some therapeutic approaches have been developed, specific treatments for COVID-19 are still tricky. For instance, remdesivir has some positive effects in patients with severe COVID-19, but it has also been associated with several adverse side effects, including elevated liver enzymes, diarrhea, rash, hypotension, renal impairment, multiple organ dysfunction syndromes, and septic shock **(Zhou et al., 2020).**

A phytopharmaceutical preparation, known as a herbal intervention, is made from a whole plant, one of its components, or one of its exudates. It is utilized either in its natural state or as a pure pharmaceutical formulation, such as extracts, juice, dry powder, and decoction, after several procedures, such as distillation, extraction, and filtration **(Mehta et al., 2015).** Their active phytochemical ingredients include alkaloids, flavonoids, terpenoids, phenols, polyphenols, tannins, saponins, polysaccharides, proteins, lipids, and peptides, which are responsible for the pharmacological action of herbal formulations and medications. The employed herbal medications have antipyretic, anti-inflammatory, expectorant, anti-asthmatic, antitussive, and antiviral qualities, which serve several purposes against the invasion, penetration, reproduction, and expression of the virus infection **(Kumar et al., 2022).** Furthermore, natural chemicals, including phytochemicals naturally synthesized in the living organism, may offer better advantageous effects than synthetic chemicals. As a therapeutic agent, natural chemicals can lower side effects that are raised by the use of synthetic chemicals. Natural compounds often have lower molecular mass, partition coefficient, and structural diversity. Those properties increase their interaction probability with enzymes and other biological molecules. However, because of their structural complexity, many of these compounds cannot be wholly synthesized in the lab. However, natural chemicals will stay important to help the advancement of drug discovery, whereas natural chemicals will be derivates **(Mathur and Hoskins, 2017).** Thus, in this study, we aimed to evaluate the possible inhibition activity from multiple medicinal plants' bioactive compounds against three non-structural proteins, such as Nsp1, Nsp2, and Nsp10, of SARS-CoV-2 through the computational study.

MATERIAL AND METHODS

Ligands retrieval and screening

About 30 Indonesian local herbs and spices were used for bioactive compounds screening, including adas (*Foeniculum vulgare*); andaliman (*Zanthoxylum acanthopodium*); asam jawa (*Tamarindus indica*); beras hitam (*Oryza sativa*); bunga lawang (*Illicium verum*); cengkeh (*Syzygium aromaticum*); daun salam (*Syzygium polyanthum*); jahe (*Zingiber officinale*); jinten (*Plectranthus amboinicus*); kacang hijau (*Phaseolus vulgaris*); kapulaga (*Amomum compactum*); kayu manis (*Cinnamomum burmannii*); kayu secang (*Caesalpinia sappan*); kedelai (*Glycine max*); kelor (*Moringa oleifera*); kemiri (*Aleurites moluccanus*); kemukus (*Piper cubeba*); kencur (*Kaempferia galanga*); ketumbar (*Coriandrum sativum*); kluwek (*Pangium edule*); lada (*Piper ningrum*); lengkuas (*Alpinia galanga*); pala (*Myristica fragrans*); saffron (*Crocus sativus*); serai (*Cymbopogon citratus*); talas (*Colocasia esculenta*); teh hitam (*Camelia sinensis*); ubi (*Ipomoea batatas*); vanili (*Vanilia planifolia*); and wijen (*Sesamum indicum*). The main bioactive compound structure of those herbs and spices was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The drug-like screening of these compounds was performed according to the Lipinski Rule of Five parameters through the Supercomputing Facility for Bioinformatics and Computational Biology, IT Delhi (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) server **(Jayaram et al., 2012).**

Proteins retrieval and preparation

Three target proteins used in this study were Nsp1, Nsp2, and Nsp10. The 3D structure was built up through SWISS-MODEL (https://swissmodel.expasy.org/). The structure with the highest similarity with the protein sequences was chosen for further procedures. Proteins template code used were 6zmi.81.A (Nsp1), 7msw.1.A (Nsp2), and 7jyy.1B (Nsp10).

Molecular docking and analysis

The PyRx software (https://pyrx.sourceforge.io/) is utilized for the docking procedure **(Trott & Olson, 2009).** All elements, including proteins and ligands structures, have been converted to AutoDock format (pdbqt). The docking coordinates are determined after selecting the target proteins and chemicals. The coverage area (Å) of Nsp1 is X: 28.3102, Y: 25.9080, Z: 22.6631, and the center coordinate is X: 297.960, Y: 186.474, Z: 245.8685. In addition, the coverage area and the center coordinates of Nsp2 are X: 61.6453, Y: 67.7670, Z: 133.3540 and X: 114.584, Y: 115.232, Z: 133.3540, respectively. Furthermore, the coverage area of Nsp10 is X: 33.7077, Y: 54.1150, Z: 38.5372, while the center coordinate is X: 64.1639, Y: 15.8248, Z: -17.3762. Through visualization, the primary docking outcomes are determined according to the binding affinity, the binding site position, and the protein-ligand interaction known through the visualization process.

RESULTS AND DISCUSSION

According to the findings of the computational prediction, ten different compounds were shown to have a higher binding affinity potential than the control against the Nsp1 protein. These compounds include cafestol, crocin, ledene, cubebin, sesaminol, β-selinene, γ-selinene, γ-elemene, β-elemene, and theaflavin-3.3′-digallate respectively (Figure 1). In this study, we also showed the 3D and 2D binding pattern of the protein-ligands complex (Figures 2 and 3). Further, the interaction of the ligands against the target protein was evaluated, consisting of two main interactions, hydrophobic and hydrogen bonds (Table 1).

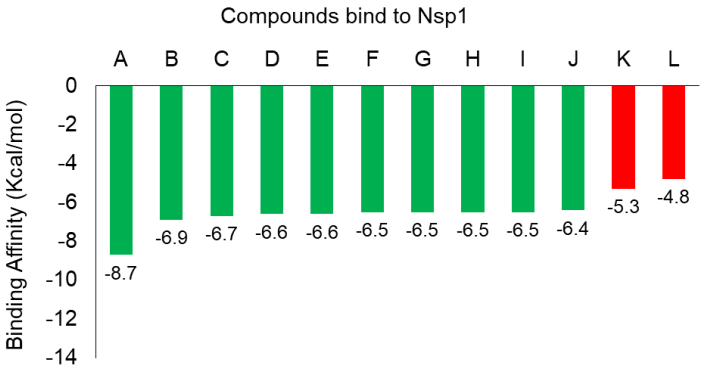

Figure 1 The binding affinity result of *M. oleifera* bioactive compounds and control drugs toward the Nsp1 protein. (A). Cafestol; (B). Crocin; (C). Ledene; (D). Cubebin; (E). Sesaminol; (F). β-selinene; (G). γ-Selinene; (H). γ-Elemene; (I). β-elemene; (J). Theaflavin-3.3′-digallate; (K). Arbidol; and (L). Chloroquine.

Furthermore, approximately ten different compounds were shown to have a higher binding affinity potential than the control against the Nsp2 protein. These compounds include cafestol, kahweol, theaflavin-3.3′-digallate, crocin, tannic acid, sesaminol, thearubigin, brazilein, sesamin, and eugeniin, respectively (Figure 4). In this study, we also showed the 3D and 2D binding pattern of the protein-ligands complex (Figures 5 and 6). Further, the interaction of the ligands against the target protein was evaluated (Table 2).

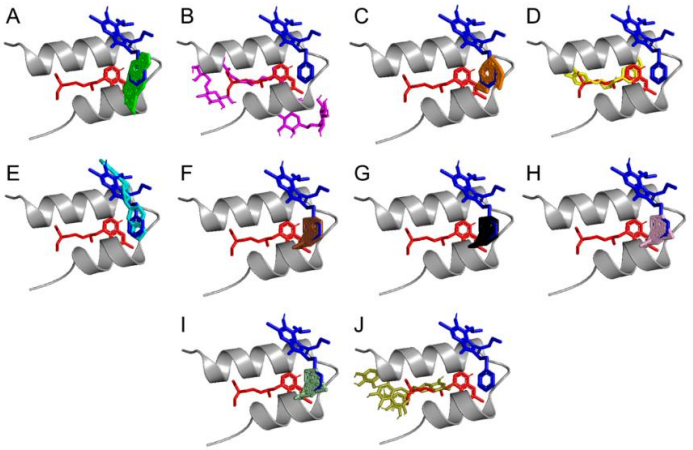

Figure 2 The 3D structure visualization of *M. oleifera* bioactive compounds and control drugs toward the Nsp1 protein. (A). Cafestol and control drugs; (B). Crocin and control drugs; (C). Ledene and control drugs; (D). Cubebin and control drugs; (E). Sesaminol and control drugs; (F). β-selinene and control drugs; (G). γ-Selinene and control drugs; (H). γ-Elemene and control drugs; (I). β-elemene and control drugs; and (J). Theaflavin-3.3′-digallate and control drugs.

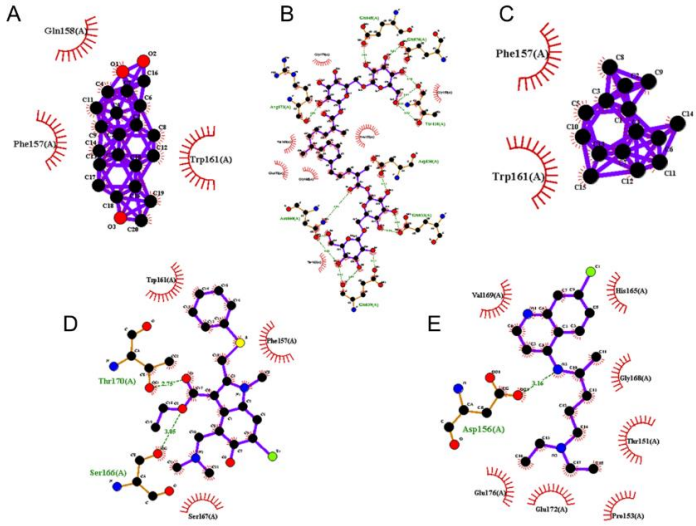

Figure 3 The 2D structure visualization of ligands (top three) and control drugs against the Nsp1 protein. (A). Cafestol; (B). Crocin; (C). Ledene; (D). Arbidol; and (E). Chloroquine.

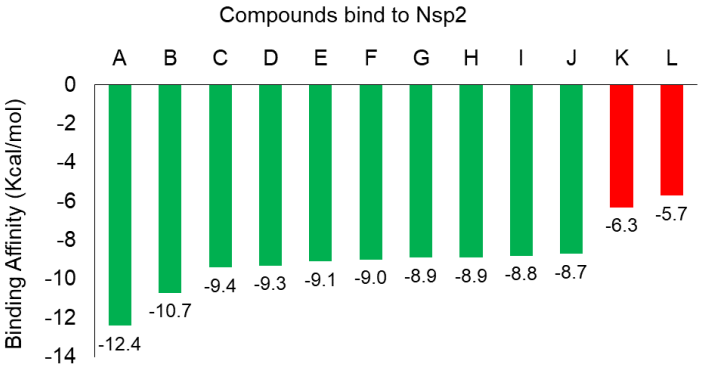

Figure 4 The binding affinity result of *M. oleifera* bioactive compounds and control drugs toward the Nsp2 protein. (A). Cafestol; (B). Kahweol; (C). Theaflavin-3.3′-digallate; (D). Crocin; (E). Tannic acid; (F). Sesaminol; (G). Thearubigin; (H). Brazilein; (I). Sesamin; (J). Eugeniin; (K). Arbidol; and (L). Chloroquine.

Table 1 List of amino acids residue and chemical interaction of top three bioactive compounds and control drug against Nsp1 protein.

No.	Compound	Source	Amino Acids Residue	Interaction
1.	Cafestol CID. 108052	Common bean (*Phaseolus vulgaris*)	Gln158(A); Phe157(A); Trp161(A)	Hydrophobic interaction
2.	Crocin CID. 5281233	Saffron (*Crocus sativus*)	Arg175(A); Glu148(A); Glu176(A); Thr151(A); Glu155(A); Glu159(A); ASN160(A)	Hydrogen bond
			Gly179(A); Gly180(A); Gly150(A); Glu172(A); Asp152(A); Gly168(A); Thr163(A)	Hydrophobic interaction
3.	Ledene CID. 10910653	Star anise (*Illicium verum*)	Phe157(A); Trp161(A)	Hydrophobic interaction
4.	Arbidol CID. 131411	Antiviral drug (control)	Thr170(A); Ser166(A)	Hydrophobic interaction
			Trp161(A); Phe157(A); Ser167(A)	Hydrophobic interaction
5.	Chloroquine CID. 2719	Antiviral drug (control)	Asp156(A)	Hydrophobic interaction
			Val169(A); His165(A); Gly168(A); Thr151(A); Pro153(A); Glu172(A); Glu176(A)	Hydrophobic interaction

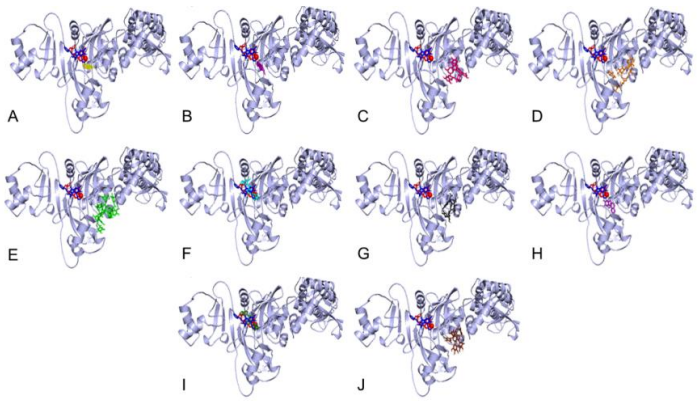

Figure 5 The 3D structure visualization of *M. oleifera* bioactive compounds and control drugs toward the Nsp2 protein. (A). Cafestol and control drugs; (B). Kahweol and control drugs; (C). Theaflavin-3.3′-digallate and control drugs; (D). Crocin and control drugs; (E). Tannic acid and control drugs; (F). Sesaminol and control drugs; (G). Thearubigin and control drugs; (H). Brazilein and control drugs; (I). Sesamin and control drugs; and (J). Eugeniin and control drugs.

On the other hand, about ten different compounds were shown to have a higher binding affinity potential than the control against the Nsp10 protein. These compounds include cafestol, kahweol, theaflavin-3.3′-digallate, tannic acid, crocin, eugeniin, ledene, β-selinene, γ-selinene, and tocotrienol respectively (Figure 7). In this study, we also showed the 3D and 2D binding pattern of the protein-ligands complex (Figures 8 and 9). Further, the interaction of the ligands against the target protein was evaluated (Table 3).

As an above explanation, we demonstrated that multiple bioactive compounds have the possibility as inhibitor candidates against the non-structural protein, Nsp1, Nsp2, and Nsp10 (Figure 10). Interestingly, we found evidence that some active compounds such as cafestol, crocin, and theaflavin-3,3'-digallate binds to all target proteins (Figure 11). Accumulating evidence showed that cafestol could be found in common bean/ *Phaseolus vulgaris* **(Lima et al., 2017; Oliveira et al., 2019)**; crocin could be found in saffron/ *Crocus sativus* **(Rahaiee et al., 2015; De Monte et al., 2015)**; and theaflavin-3,3'-digallate could be extracted from black tea/ *Camelia sinensis* **(Mhatre et al., 2020; Sen et al., 2020; Prasansuklab et al., 2021)**.

Table 2 List of amino acids residue and chemical interaction of top three bioactive compounds and control drug against Nsp2 protein.

No.	Compound	Source	Amino Acids Residue	Interaction
1.	Cafestol CID. 108052	Common bean (*Phaseolus vulgaris*)	Ser358(A)	Hydrogen bond
			Ser299(A); Phe356(A); Cys253(A); Phe329(A); Asn328(A); Asn254(A); Leu180(A); Tyr179(A); Pro181(A); Ala357(A)	Hydrophobic interaction
2.	Kahweol CID. 114778	Common bean (*Phaseolus vulgaris*)	Ala357(A); Pro181(A); Ser299(A); Asn328(A); Cys326(A); Phe356(A); Phe329(A); Tyr179(A); Ser358(A)	Hydrophobic interaction
3.	Theaflavin-3,3'-digallate CID. 136277567	Black tea (*Camelia sinensis*)	Arg362(A); Ile393(A); Asn133(A); Ala357(A); Thr304(A); Ser394(A); Thr160(A)	Hydrogen bond
			Glu162(A); Ser366(A); Gly392(A); Val363(A); Gln134(A); Glu359(A); Gly154(A); Thr153(A)	Hydrophobic interaction
4.	Arbidol CID. 131411	Antiviral drug (control)	Ala225(A); Thr170(A)	Hydrogen bond
			Ile216(A); Ile224(A); Lys214(A); Phe156(A); Thr175(A); Phe226(A); Pro13(A); Asp14(A); Val94(A)	Hydrophobic interaction
5.	Chloroquine CID. 2719	Antiviral drug (control)	Phe156(A); Ala225(A)	Hydrogen bond
			Ile224(A); Lys214(A); Gly260(A); Val94(A); Ile216(A); Val259(A); Thr176(A); Phe226(A); Thr170(A); Thr139(A)	Hydrophobic interaction

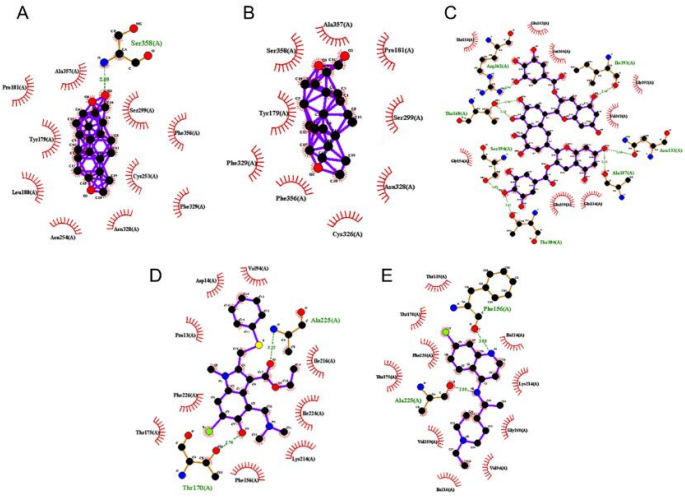

Figure 6 The 2D structure visualization of ligands (top three) and control drugs against the Nsp2 protein. (A). Cafestol; (B). Kahweol; (C). Theaflavin-3.3′-digallate; (D). Arbidol; and (E). Chloroquine.

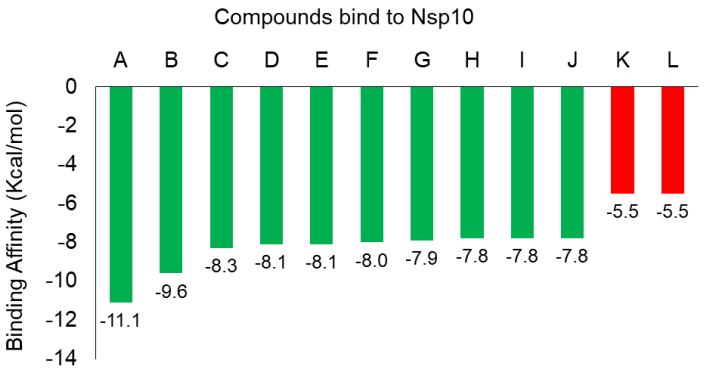

Figure 7 The binding affinity result of *M. oleifera* bioactive compounds and control drugs toward the Nsp10 protein. (A). Cafestol; (B). Kahweol; (C). Theaflavin-3.3′-digallate; (D). Tannic acid; (E). Crocin; (F). Eugeniin; (G). Ledene; (H). β-selinene; (I). γ-Selinene; (J). Tocotrienol; (K). Arbidol; and (L). Chloroquine.

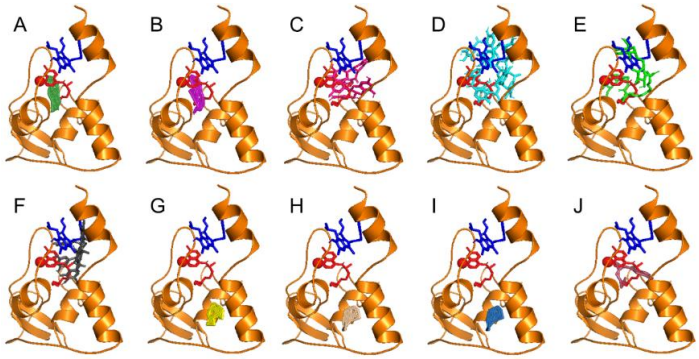

Figure 8 The 3D structure visualization of *M. oleifera* bioactive compounds and control drugs toward the Nsp10 protein. (A). Cafestol and control drugs; (B). Kahweol and control drugs; (C). Theaflavin-3.3′-digallate and control drugs; (D). Tannic acid and control drugs; (E). Crocin and control drugs; (F). Eugeniin and control drugs; (G). Ledene and control drugs; (H). β-selinene and control drugs; (I). γ-Selinene and control drugs; and (J). Tocotrienol and control drugs.

Cafestol is a naturally occurring diterpene that may also be extracted from coffee beans **(Ren et al., 2019).** In the early stages, researchers found that cafestol effectively increased human plasma triacylglycerol and low-density lipoprotein, which suggests that it may be a factor in the development of several cardiovascular disorders **(Godos et al., 2014; De Roos et al., 2000).** However, when viewed from a broader angle, cafestol reveals a unique effect that can be interpreted in two different ways. Extensive research has shown that cafestol possesses various pharmacological activities, such as anti-inflammatory, anti-angiogenic, and anti-tumorigenic. In addition, it also negatively impacts serum lipid levels and the activity of liver enzymes in some individuals.

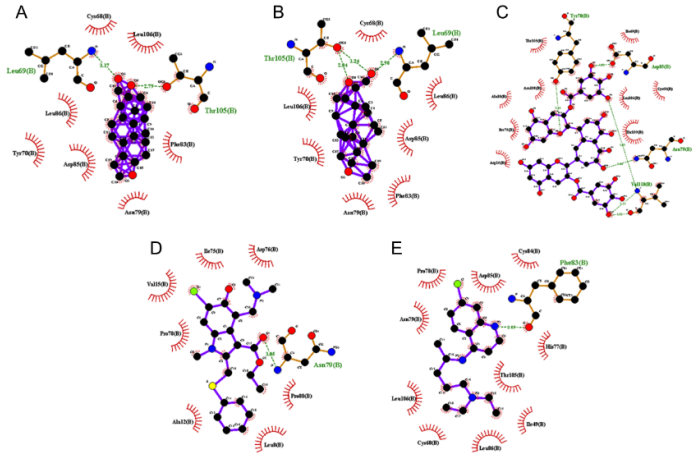

Figure 9 The 2D structure visualization of ligands (top three) and control drugs against the Nsp10 protein. (A). Cafestol; (B). Kahweol; (C). Theaflavin-3.3′-digallate; (D). Arbidol; and (E). Chloroquine.

Table 3 List of amino acids residue and chemical interaction of top three bioactive compounds and control drug against Nsp10 protein.

No.	Compound	Source	Amino Acids Residue	Interaction
1.	Cafestol CID. 108052	Common bean (*Phaseolus vulgaris*)	Leu69(B); Thr105(B)	Hydrogen bond
			Cys68(B); Ley106(B); Phe83(B); Asn79(B); Asp85(B); Tyr70(B); Leu86(B)	Hydrophobic interaction
2.	Kahweol CID. 114778	Common bean (*Phaseolus vulgaris*)	Thr105(B); Leu69(B)	Hydrogen bond
			Cys68(B); Leu86(B); Asp85(B); Phe83(B); Asn79(B); Tyr70(B); Leu106(B)	Hydrophobic interaction
3.	Theaflavin-3,3'-digallate CID. 136277567	Black tea (*Camelia sinensis*)	Asp85(B); Asn79(B); Val110(B); Tyr70(B)	Hydrogen bond
			Ile49(B); Cys68(B); Leu106(B); Thr109(B); Asp16(B); Pro78(B); Ala18(B); Asn180(B); Thr105(B)	Hydrophobic interaction
4.	Arbidol CID. 131411	Antiviral drug (control)	Asn79(B)	Hydrogen bond
			Pro80(B); Leu8(B); Ala12(B); Pro78(B); Val15(B); Ile75(B); Asp76(B)	Hydrophobic interaction
5.	Chloroquine CID. 2719	Antiviral drug (control)	Phe83(B)	Hydrogen bond
			His77(B); Thr105(B); Ile49(B); Leu86(B); Cys68(B); Leu106(B); Asn79(B); Pro78(B); Asp85(B); Cys84(B)	Hydrophobic interaction

The major carotenoid in saffron, crocin, has the potential to lower the severity and development of the SARS-CoV2 infection for several reasons, including its strong antioxidant characteristics, controlled cytokine production that causes acute lung injury, upregulate PPAR and downregulate NF-κB expression, and diminish the viral infection **(Ghasemnejad-Berenji, 2021; Gholami et al., 2021).** Theaflavins are a polyphenol most prevalent in black tea **(Jain et al., 2022).** Through computational analysis, it has been shown that theaflavin-3,3′-digallate may block the Zika virus protease (Cui et al., 2020) and several stages of the SARS-CoV-2 life cycle **(Jain et al., 2022).** Further research is necessary to accomplish the immediate future in structure-based drug designs (SBDD), such as X-ray crystallography, NMR, and homology modeling, to obtain structural information. This step will improve the limitations of docking algorithms because, in reality, the prediction conformation between ligands and receptors might not show strong correlations **(Pinzi et al., 2019).**

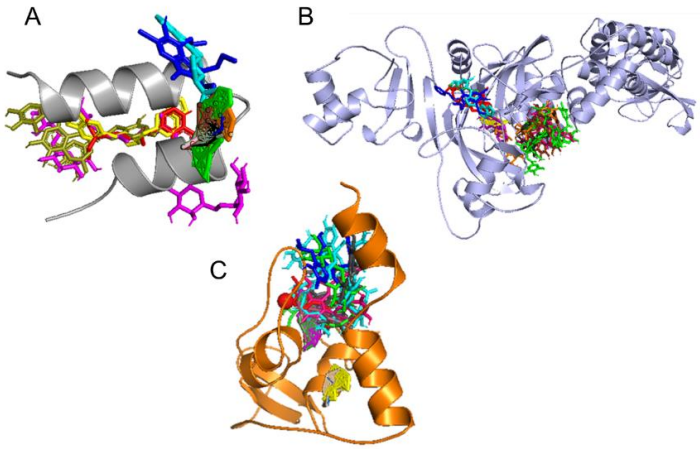

Figure 10 The 3D structure visualization of three SARS-Cov19 non-structural protein-ligand complex, (A). Nsp1; (B). Nsp2; and (C). Nsp10.

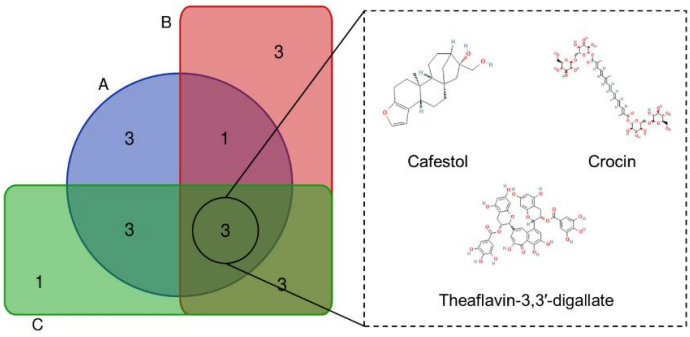

Figure 11 A Venn diagram showed three compounds of *M. oleifera*, such as cafestol, crocin, and theaflavin-3,3'-digallate bind to all target proteins, Nsp1, Nsp2, and Nsp10.

CONCLUSION

In this present study, we found the top three highest binding affinity values of bioactive compounds against Nisp1, namely cafestol, crocin, and ledene; the top three highest binding affinity values of bioactive compounds against Nisp2, including cafestol, kahweol, and theaflavin-3.3′-digallate; and the top three highest binding affinity value of bioactive compounds against Nisp10, such as cafestol, kahweol, and theaflavin-3.3′-digallate. Interestingly, we also found that cafestol, crocin, and theaflavin-3,3'-digallate binds to all target proteins.

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