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Potential of Plant Bioactive Compounds for Coronavirus Disease 2019 (Covid19) from Ylang-ylang, Ginger, and Eucalyptus Essential Oil based on Gas Chromatography - Mass Spectrometry (GC-MS) and Molecular Docking Analysis

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ABSTRACT

The needs for herbal medicine especially during the pandemic Covid19 tend to increase because of its few side effect and complications. Ylang-ylang, ginger, and eucalyptus oil have been under attention due to the progressive need for Covid19 therapy, immunity, and promote relaxation. The objective of the research was to analyze the bioactive compound of essential oil of ylang-ylang, ginger, and eucalyptus essential oil using the GC-MS method followed by molecular docking analysis of its major chemical compound potency against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The method to analyze the bioactive compound of ylang-ylang, ginger, and eucalyptus essential oil was using the GC-MS method. The molecular docking method was utilized for identifying and evaluating potential inhibitors of major chemical compounds' binding affinities. The docking process is carried out through the stages of ligand preparation, receptor preparation, docking simulation, and validation of the docking model. In addition, the suitability of these inhibitors as drugs for biological systems was predicted using Lipinski's rule. The result of GC-MS analysis showed the major components of ylang-ylang are alpha-bergemotone, ginger is Zingiberene and eucalyptus is 1,8-cineole or eucalyptol. The result showed the inhibition potency of the main protease receptor SARS-CoV-2 by the interaction of virus protein Mpro with three compounds so that it has the potential to be used in drug design against SARS-CoV-2. The molecular docking analysis showed the interaction properties of inhibitors of Mpro by three compounds so that it has the potential to be used in drug design against SARS-CoV-2.

Keywords: Ylang-ylang; Ginger; Eucalyptus; Essential Oil; GC-MS; molecular docking

1. Introduction

Currently, Coronavirus Disease 2019 (Covid19) has become a never-ending global health threat. This is compounded by the limitations of approved drugs and the high level of efficacy against the SARs-CoV-2 virus. Meanwhile, the effectiveness of the vaccine has also decreased due to various new mutations that have emerged from Covid19 [1]. Essential oils (EO) are known to have anti-inflammatory, immunomodulatory, bronchodilator, aromatherapy, and antiviral activities [2-9]. Currently, EO is being tested for its activity against the virus [10].

Cananga or ylang-ylang (*Cananga odorata* (Lam.) Hook. f. & Thomson) essential plants, ginger (*Zingiber officinale* Rosc.), and eucalyptus (*Melaleuca cajuputi*) are part of Indonesia's biodiversity that can be used as a source of essential oils for medicinal ingredients drugs. The pharmacological activities of ylang-ylang essential

oil that have been studied include antibacterial, antifungal, and cytotoxic activities [11-12]. Currently, research on ylang-ylang essential oil is being developed on its biological properties and chemical components [13]. Another EO that came from the ginger rhizome has shown the ability to help treat osteoarthritis, neurodegenerative disorders, rheumatoid arthritis, type 2 diabetes, respiratory disorders, liver disease, and primary dysmenorrhea. Pharmacological activity from ginger was anticancer, antioxidant, anti-inflammatory, antiangiogenic, antimetastatic, antimicrobial, antifungal, neuroprotective, antiemetic, antihyperlipidemic effects, antihypertensive and cardiovascular diseases [14-15]. Fresh ginger has higher antiviral activity against the human respiratory syncytial virus (HRSV) and rhinovirus [16]. Ginger contains about 76% moisture, 5.0% - 6.0% fat, 9.0% protein, 16% carbohydrates, 3.0% - 8.0% crude fiber, about 8.0% ash, 9.0% - 12.0% water, and approx. 2.0 %- 3.0% essential oil [17-18].

Eucalyptus oil has long been used as a traditional medicine in Indonesia. The oil from the leaves shows the potential of eucalyptus oil as an antimicrobial, analgesic, anti-inflammatory, and antipyretic, especially being able to relieve breathing during colds, flu, and nasal congestion [19]. The pharmacological activity of eucalyptus oil is mainly determined by the main bioactive compound contained in it, namely 1,8-cineole (cineole or eucalyptol). Other chemical compounds that are commonly contained in it are α-pinene, 1,8-cineol, and pinocarveol-trans. In addition, the antioxidant and antimicrobial activity of eucalyptus oil are related to its high flavonoid and phenolic content [20]. Eucalyptus leaf essential oil has also shown antiviral activity as an anti-herpes simplex virus, adenovirus, and mumps [21].

Information and research on the ability of essential oils to fight the Covid-19 virus are still very limited. Based on the potential of the three essential plants, this study will explore the potential of essential oils from ylang-ylang, ginger, and eucalyptus as an antiviral so that they can be used to help overcome the Covid19 pandemic. As a first step, the potential of the main bioactive compounds contained in eucalyptus, ylang-ylang, and ginger oils will be identified using GC-MS analysis. Furthermore, the potential of the main compounds of the three volatile plants will be seen for their antiviral activity against SARS-CoV-2 in silico using molecular docking. Molecular docking is a computational simulation method that is used to predict and imitate the interaction or binding between a drug/ligand and a receptor/protein in an in-vitro test [22]. Mpro is a protease that is a key enzyme of the coronavirus and plays an important role in viral replication and transcription so it has the potential to be used in drug design for SARS-CoV-2 [5-6]. This method was performed by attaching a small molecule (ligand) to the active site of the receptor, which is currently widely used in the process of discovery and development of new drugs with higher activity. The docking process is carried out through the stages of ligand preparation, receptor preparation, docking simulation, and validation of the docking model. Docking is an attempt to find a match (interaction) best between two molecules. The best interactions are interactions with the lowest energy. The interaction is strongly influenced by the orientation of the molecule which interacts. The suitability of the interaction is the main key to the function of a molecule according to its bioactivity. It is hoped that this research will become the basis for developing potential herbal medicines so that they become a more feasible and effective approach to help overcome the Covid19 virus pandemic. This research is also an attempt to study the molecular interactions of bioactive compounds from ylang-ylang, ginger, and eucalyptus oils against SARS-CoV-2 to predict the pharmacokinetic potential of volatile compounds used in this study.

2. Material and Methods

2.1. Essential Oil Material

The essential oil used in this study was eucalyptus oil from Ambon Island while ginger and ylang-ylang oil came from Central Java, Indonesia. The essential oil is obtained through traditional distillation methods. Eucalyptus oil is obtained from the leaves, ylang-ylang oil from the flowers, and ginger oil is obtained from the rhizome.

2.2. Identification of essential oil bioactive compound using GC-MS

Analysis of the bioactive compounds of eucalyptus, ginger, and ylang-ylang oil was carried out using Gas Chromatography-Mass Spectrometry (GC-MS) QP5050A (Shimadzu Co.Ltd, Kyoto, Japan) [23]. The tool uses a 15 m long capillary column TC-1701. The essential oil will be analyzed using a capillary column with a size of 30 m x 25 m and coated with 0.25 mm 5% phenylmethyl siloxane film to hold at 80°C for injection and positioned in a fire ionization detector (FID). The GC-MS program was set at minus 10°C to 150°C, minus 5°C min-1 to 250°C, and minus 10°C min-1 to 280°C for 5 minutes. The carrier in GC-MS is helium gas with a flow rate of 1 mL/min with a 2µL splitless injection. The method used in the spectrometer is the electron-impact (EI) mode, which has an electron energy of 70 eV with a

scanning range of 50-550 amu. The inlet temperature and source of ionization are 240 °C and 280 °C, respectively. Identification of essential oil composition was carried out using the Wiley database by comparing the mass spectrum pattern and the fragmentation pattern of the reference substance. Quantitative analysis was performed using the percentage of the area obtained by FID.

2.3. Protein and Ligand preparation

The crystallographic structure of SARS coronavirus main protease (COVID-19 3CLpro/Mpro) in complex with N3 was retrieved from the structure database (PDB) and its PDB ID is 6LU7 [24-25]. The structure of the SARS-CoV-2 Mpro (PDB: 6LU7) was used to generate a receptor grid for the docking simulation. The active site center of the grid is adjusted by position N3 in the structure. the Ligand structure data which was the result of the GCMS analysis was obtained from the PubChem database (http://pubchem. ncbi.nlm.nih.gov) in SDF format. The SMILES format of the compounds, namely, alpha-Bergamotone; 1,8-Cineole, and Zingiberene were retrieved. Visualization of the three compounds was carried out using Discovery Studio software which was also used to remove water molecules and native ligands on the receptor.

2.4. Lipinski Rule of Five test

The physicochemical test was carried out by entering the SMILE arrangement of the test compounds (obtained from the PubChem database http://pubchem.ncbi.nlm.nih.gov) in the 2D form on the Lipinski Rule of Five pages (http://www.scfbio-iitd.res .in/software/drug design/lipinski.jsp) [26-27].

2.5. Active Site Determination and Receptor-Ligand docking

Determination of the active site and marking of the binding map (Grid Box) on the receptor was carried out using the Autodock Tools software [28]. The molecular docking process is carried out using Autodock Vina (AV) which is operated using a command prompt [29]. AutoDock Vina will prepare the protein and ligand structure. Molecular docking was carried out with the original setting, initially without the presence of co-factor molecules and/or H2O. If a strong protein-ligand interaction is produced, the interaction in the presence of co-factor molecules and/or H2O will be stronger The result parameters observed in the docking process were the binding affinity value, the RSMD value, and the modeling of the structure of the docking result between the receptor and the ligand.

3. Results and Discussion

Trying to find a drug ingredient for SARS-CoV-2 is the hard work of all scientists. Medicinal ingredients derived from safe and natural herbs are one of the studies that have received a lot of attention because of their great potential. Despite being widely used for cosmetics and cosmeceuticals, essential oils contain various main bioactive compounds and secondary metabolites that have pharmacological effects. The main constituent hydrocarbons of essential oils are terpenes. Terpenes are unsaturated hydrocarbon compounds divided into two groups, mono, di, tri, tetra, and sesquiterpenes based on their isoprene units [30-34]. The chemical composition of the three EOs was determined by gas-chromatography–mass spectrometry (GC–MS) analysis The main bioactive compounds can be used to track its property as a Covid-19 antiviral drug potency using molecular docking [35-36].

3.1. GC-MS analysis of Essential Oil Bioactive Compounds

Ylang-ylang oil is distilled from the flowers of Cananga odorata (Lam.) Hook. f. Thomson. It is an important ingredient in fragrance products and industries such as perfumes, and detergents. The quality of the essential oil is differentiated based on its chemical constituents. The chemical composition of ylang-ylang oil based on the results of GC-MS analysis of essential oil consists of 10 compounds which are presented in **Table 1.**

Table 1. The bioactive compound of Ylang-ylang oil

Peak#	R.Time	Area	Area (%)	Name
1	12.024	16224920	1.04	1,3,6-Octatriene, 3,7-dimethyl-, (E)- (CAS)/ trans-β-Ocimene
2	21.961	17311668	1.11	.alpha.-Copaene
3	23.108	245955507	15.79	trans-Caryophyllene (β-caryophyllene)
4	23.990	70360459	4.52	.alpha.-Humulene (CAS)
5	24.650	174873488	11.23	Germacrene-D
6	25.376	851284809	**54.67**	.alpha.-Bergamotene (CAS)
7	25.712	61824025	3.97	.delta.-Cadinene (CAS)
8	28.928	47084280	3.02	t-Muurolol
9	30.267	34921631	2.24	Farnesol Isomer B
10	31.038	37425129	2.40	Benzyl benzoate
		1557265916	100.00	

The results of the GC-MS analysis of ylang-ylang EO show that 97,6% are sesquiterpenes consisting of alpha-bergamotene (54.67%), germacrene D (11.23%), -caryophyllene (15.79%), humulene (4.53%), farnesol (2.24%), alpha-Copaene (1.11%), muurolol a cadinene

sesquiterpenoid, and 1,3,6-Octatriene, 3,7-dimethyl-, new sesquiterpene alcohol from ylang-ylang essential oil.

The research results obtained were supported by the previous study which stated that the type of essential oil from ylang-ylang flowers will be different at each stage of flowering. When the flowers are in full bloom, the most essential oils will be produced, both in number and type. In ylang-ylang mature flowering, the type of essential oil produced is mostly terpenes compounds [11]. Germacrene D and Benzyl benzoate farnesol were produced at the end-flowering stage, with fully matured petals, yellow, 30 d after the bud stage. Benzyl Benzoate is found as an ester of Benzyl alcohol and Benzoic acid, where this compound is used as a preservative and a fragrance ingredient [12]. **Table 1** shows that alpha bergamotene is a chemical compound that has the highest percentage of 54.67%. Alpha-bergamotene has molecular formula C15H24 with a molecular weight of 204,35. Alpha- bergamotene is a sesquiterpene consisting of a bicyclo[3.1.1]hept-2-ene skeleton substituted at positions 2 and 6 by methyl groups and at position 6 by a 4-methylpent-3-en-1-yl group. Several characteristic compounds that are responsible for aroma were benzyl benzoate (spicy-faint balsamic odor), cadinene, and trans-caryophyllene (woody odor), also 1,3,6-Octatriene, 3,7-dimethyl-/ trans- β-Ocimene which has a floral type odor [12-13].

The composition of the chemical compounds present in the essential oil of ginger rhizome showed 10 compounds based on the results of the GC-MS analysis as shown in **Table 2**. Zingiberene was the compound with a high amount of 25.58%. Zingiberene has molecular formula C15H24 with molecular weight 204,35. It is a sesquiterpene and a cyclohexadiene. The pharmacological activity of ginger is mainly due to the content of terpene hydrocarbons and phenolic compounds [15]. The terpenes in ginger are zingiberene, bisabolene, curcumene, farnesene, sesquiphellandrene, limonene, cineole, linalool, borneol, and geranial. Ginger phenolic compounds consist of the main ingredients gingerol, paradol, shogaol, and zingerone, 1-dehydrogingerdione, 6-gingerdione and 10-gingerdione as well as gingerdiol and diarylheptanoids [37-38].

The sesquiterpene in ginger from GC-MS analysis is zingiberene, bisabolene, alpha-curcumene, beta.-Cedrene, Citral, and monoterpene are Camphene, beta.-Phellandrene, beta.-Citronellol. Hinesol is a unique sesquiterpenoid isolated from the ginger rhizome. Another study reported that Hinesol showed antitumor activity associated with apoptosis [39]. Another study found farnesene, sesquiphellandrene, limonene, cineole, linalool, borneol, and geranial. Ginger phenolic compounds consist of the main ingredients gingerol, paradol, shogaol, and zingerone, 1-dehydrogingerdione, 6-gingerdione and 10-gingerdione as well as gingerdiol and diarylheptanoids [15, 17-18]. In contrast to the high content of sesquiterpenes in Ylang-ylang and Ginger essential oils, Eucalyptus is more dominated by monoterpenes at 93%. Eucalyptol/1,8 Cineole was dominant as monoterpene and has molecular formula C10H18O with molecular weight 154,25 as exhibited in **Table 3**.

Table 2. The bioactive compound of Ginger oil

Peak#	R.Time	Area	Area (%)	Name
1	8.371	83188397	5.46	Camphene (CAS)
2	11.240	56095115	3.68	.beta.-Phellandrene
3	17.930	41295125	2.71	.beta.-Citronellol
4	18.701	46264603	3.03	Citral
5	21.919	24972541	1.64	Alpha-Copaene/ 8-Isopropyl-1,3-dimethyltricyclo [4.4.0.0~2,7~]dec-3-ene
6	24.746	476157821	31.23	Alpha-Curcumene/Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (CAS)
7	25.127	390032758	**25.58**	Zingiberene (CAS)
8	25.406	195091309	12.80	.beta.-Bisabolene (CAS)
9	25.778	187318205	12.29	.beta.-Cedrene (CAS)
10	28.304	24123170	1.58	Hinesol
		1524539044	100.00	

Table 3. The bioactive compound of Eucalyptus oil

Peak#	R.Time	Area	Area (%)	Name
1	8.039	414300485	29.30	cis-Ocimene (monoterpene)
2	9.360	31792790	2.25	2-.beta.-Pinene (monoterpene)
3	10.659	62913658	4.45	.delta.3-Carene (monoterpene)
4	11.259	637504212	**45.09**	1,8-Cineole/Eucalyptol (monoterpene)
5	12.342	37822303	2.67	gamma.-Terpinene(monoterpene)
6	13.292	27828692	1.97	.Alpha.-Terpinolene (m)
7	16.901	78285822	5.54	Apha-Terpineol/3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, (S)- (CAS) (monoterpene)
8	21.006	28387251	2.01	.alpha.-Terpinenyl acetate (m)
9	23.075	67105732	4.75	trans-Caryophyllene (sesquiterpene)
10	23.962	28057399	1.98	.alpha.-Humulene (CAS) sesquiterpene
		1413998344	100.00	

Eucalyptol is a naturally produced cyclic ether. Pharmacological activities showed its ability to control airway mucus hypersecretion and asthma via anti-inflammatory cytokine inhibition, also an effective treatment for nonpurulent rhinosinusitis [19-20]. Eucalyptol will reduce inflammation and pain when applied topically. It kills leukemia cells in vitro and shows antiviral activity [21]. 1,8-cineol is a colorless liquid with a camphor-like odor. 1,8-cineole has a spicy cooling taste and is the key compound in the eucalyptus aroma [40-41]. The antiviral activity of Melaleuca essential oil was reported by several researchers where the activity was derived from the main monoterpene and its derivatives including 1,8-cineol against herpes simplex virus type 1 (HSV-1) in vitro [42-43].

3.2. Molecular Docking

In this study, we predicted the interaction and the best bond stability of the major compounds contained in eucalyptus, ylang-ylang , and ginger oil consisting of alpha-Bergamotene, 1,8-Cineole, and Zingiberene as illustrated in **Figure 1**. These compound bonds are expected to inhibit the activity of the virus COVID-19 and the viral replication process.

The GC-MS was used to determine the main substances in the essential oils of, ylang-ylang, ginger, and eucalyptus oil. This study studied the inhibitory ability of the main active compound in eucalyptus oil in inhibiting the protein Angiotensin-Converting Enzyme 2 (ACE2) protein in the human body which is the host receptor for SARS-CoV-2 and causing SARS-CoV-2 to lose its host receptor and destroy its protein (PDB: 6LU7) using docking simulations [35]. The 6LU7 protein structure after preparation was shown in **Figure 2**. The results showed that ACE2 and 6LU7 proteins were strongly inhibited by the three main compounds. The interaction of these three main compounds showed strong inhibition of the ACE2 and 6LU7 proteins. The molecular docking results obtained indicate that Eucalyptus, ylang-ylang, and Ginger oil essential oils are potential natural sources to prevent the invasion of SARS-CoV-2 into the human body.

Figure 1. Compound structure of alpha-Bergamotene (left), 1,8-Cineole (center), and Zingiberene (right).

Figure 2. The visualization of the crystal structure of COVID-19 main protease (6LU7) in complex with an inhibitor N3 (red arrow) using Discovery Studio software.

The N3 ligand structure was prepared for molecular docking analysis. The docking value accountability evaluation has been implemented through validation. Redocking was carried out using the Mpro receptor without ligands and with ligands previously separated. The validation result is shown in **Figure 3**.

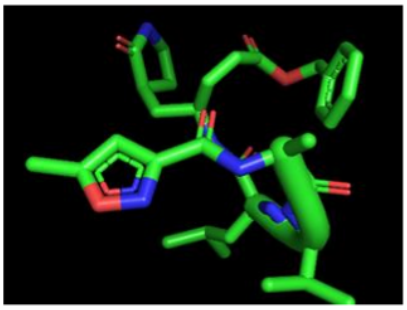

Figure 3. The result of superimposing the ligand structure is based on the redocking process of N3 with crystallography (green: crystallography result; blue: redocking result).

3.3. Lipinski Rule of Five for ADME analysis of EO Compound

The results of the Lipinski rule of five about three compound molecules in each plant was shown in **Table 4**.

Table 4. The bioactive compound of Eucalyptus oil

Bioactive Compound	Molecular Formula	Molecular weight (<500g/mol)	logP (<5)	H-bond donor (<5)	H-bond acceptor (<10)	Molar Refractivity	Fulfillment of the Lipinski Rule of Five Criteria
Alpha-Bergamotene	$C_{15}H_{24}$	204,00	4,72	0,0	0,0	66,74	Fulfill
1,8-Cineole	$C_{10}H_{18}O$	154,00	2,74	0,0	1,0	45,52	Fulfill
Zingiberene	$C_{15}H_{24}$	204,00	4,89	0,0	0,0	68,83	Fulfill

The suitability and potency of inhibitors as drugs cannot be guaranteed only based on the interaction of inhibitor antagonists with receptor proteins or enzymes so analysis of ADME inhibitors is needed for drug development. The suitability of the inhibitors of EO compound as drugs for biological systems was considered by their ADME predicted using Lipinski's rule [44]

The analysis result shows that the test of three bioactive compounds gaining molecular weight less than 500g/mol, logP <5, H-bond donor <5, H-bond acceptor <10, and molar refractivity which is meet the Lipinski rule of five criteria. All compounds obtained in the three plants in this study can be said to be able to penetrate cell membranes because they have a molecular weight of less than 500 Dalton. A compound can be said to have drug-like properties if it has a molecular mass between 180-480 Dalton [45]. Another parameter is the Log P value, this Log P value reflects the polarity of the oil. The Log P value of more than 5 causes the compound to dissociate and is difficult to dissolve in the body. The lower log P value indicates the chemical compound has higher hydrophilicity, high adsorption, and permeation. Meanwhile, the lower molecular weight will increase the adsorption rate which is a sought-after character for drug candidates. Molecules weighing less than 500 will have many advantages in biological processes because they easily cross the blood-brain and intestinal barrier.

In addition, the Lipinski rule of five tests can also predict the solubility of compounds through passive diffusion by assessing the number of hydrogen bonds in donors and acceptors (**Table 5**). This illustrates that a compound that is more difficult to disperse is a compound that has more than 5 donor hydrogen bonds and more than 10 acceptor hydrogen bonds. Natural products for the discovery of new drugs from EO are often considered incompatible with Lipinski's Rule of Five (Ro5). This is because EO as a natural product is a complex mixture of EOC which is relatively hydrophobic and volatile so it can cause interference during the filtering process. However, the results of this study indicate that the properties of EO or its natural product derivatives from Ginger, Ylang-ylang, and Eucalyptus can still follow Lipinski's rule. The results also show that Lipinski's rule of five as a rule of thumb that describes the drug ability of certain molecules can also be applied to bioactive compounds contained in Ginger, Ylang-ylang, and Eucalyptus EO, as also supported by other studies [25, 35-36]. The higher negative value indicates that the chemical compound will bind more strongly to the protein because this reflects the strength of the interaction and affinity between the ligand and the receptor.

Table 5. The binding affinity of Ylang-ylang, Ginger, and Eucalyptus oil bioactive compound

Bioactive compound	Binding Affinity Value 6LU7 (kcal/mol)	RMSD Lower bound	RMSD upper bound
Alpha-Bergamotene	-5,1	0,0	0,0
1,8-Cineole	-4,2	0,0	0,0
Zingiberene	-5,5	0,0	0,0
N3 (Native-ligand)	-6,9	0,0	0,0

This is one of the important parameters in the effort to find new drugs. Therefore, in this molecular docking study, we wanted to find a ligand that exhibits the smallest binding energy and thus has the best affinity among the molecules. From the data in Table 5, it can be seen that the binding affinity value of the three test compounds to the receptor on 6LU7 protein had close differences (range 1,4 – 2,7) compare to the binding affinity value of N3 as a native ligand. This binding affinity value determines the amount of energy required for a compound in Ylang-ylang, Ginger, and Eucalyptus oil to bind to a protein. If the value of the binding affinity of a compound is close to zero or greater, then the energy required to form bonds is also greater. In addition, the RMSD value of 2 indicates that the docking method used has been well-validated [46-47]. Based on the results of molecular docking, the Zingiberene compound has the best potential because it has the lowest binding affinity (-5.5) compared to Alpha-Bergamotene and 1.8 Cinole with an overall RMSD value of 0.

3.4. Molecular Docking Test Results in 3 Dimensions

SARS-CoV-2 consists of structural and non-structural proteins. The main structural proteins of SARS-CoV-2 include spike protein (S), membrane (M), envelope (E),

hemagglutinin-esterase (HE-protein), and nucleocapsid. (N). Non-structural proteins of SARS-CoV-2 consist of 16 proteins, one of which is nsp 5 or commonly called Mpro [11, 36]. Mpro is a key enzyme in the viral replication cycle. When viral mRNA translates polyproteins, Mpro is in charge of cleaving all 11 nonstructural proteins of the polyprotein topolypeptide required for viral replication. Thus, Mpro inhibition is expected to block virus replication. In a previous study, a Michael acceptor inhibitor—known as N3—was developed using a computer-aided drug design [11]. N3 can specifically inhibit Mpro from multiple coronaviruses, including SARS-CoV and MERS-CoV12–15 [48]. The molecular docking test result showed that all test compounds and native ligands bind to the same active site on the 6LU7. Protein which can be seen in **Figure 4**.

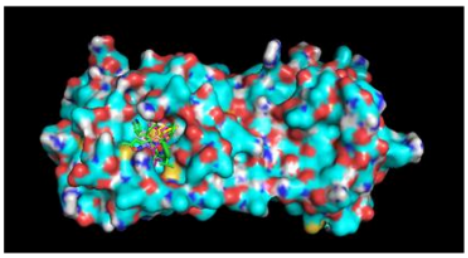

Figure 4. The 3 dimension structure of molecular docking result.

3.5. Ligand confirmation test results

The ligand confirmation test result in **Figure 5** showed that in molecular docking. The ligand molecule is anchored to the active site or the binding site of a protein that is at rest (static), whether or not the co-factor and/or H2O molecules are included [49]. The result shows that data were obtained regarding the position and orientation of the ligands in the active site or the anchorage site. It can be concluded that the functional groups of the ligands are important for their interactions so that they cannot be removed, and the functional groups can increase the strength of their interactions. This information serves as a guide for the modification of the ligand. With these interactions, the modification of the ligands and in-vitro assays of their derivatives can take place efficiently.

According to the results, a high correlation was found between the chemical composition and the antiviral assay. The results of this study have also been answered from previous research with antivirus [50]. This allowed us to deduce that the global biological activity of alpha-Bergamotene, 1,8-Cineole, and Zingiberene was mainly due to an addition or a synergism effect between the major components. It is also shown that essential oils have the potential to penetrate viral membranes due to their lyophilic nature. It means that these three compounds can affect various stages of viral replication.

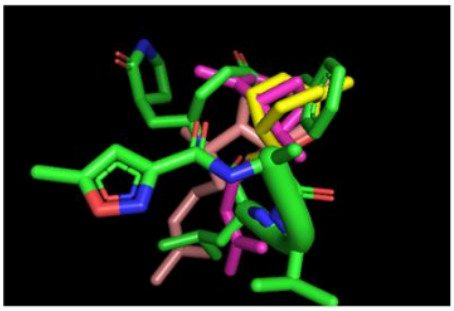

Figure 5. Ligand confirmation test result.

4. Conclusion

Characterization of the bioactive compounds of Ylang-ylang, Ginger, and Eucalyptus essential oil showed that alpha-bergemotone, Zingiberene, and 1,8-cineole or eucalyptol were the major compounds. The molecular docking analysis showed the inhibition potency of the Main protease receptor SARS-CoV-2 by the interaction of virus protein Mpro with three compounds so that it has the potential to be used in drug design against SARS-CoV-2.

5. Acknowledgments

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6. Conflict of Interest

The authors declare that there are no conflicts of interest.

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