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Characterization *in silico* of bioactive compound in tea plant as a potentials inhibitor of SARS-CoV-2 Mpro

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ABSTRACT

One of the screenings of the chemical structure that has the potential as an active major protease (Mpro) inhibitor in SARS-CoV-2 is bioactive compounds, such as oolonghomobisflavan-A, theaflavin-3-O-gallate, and theaflavin (TF). These bioactive compounds are main components of catechin oxidation, which contribute color, taste, and aroma to black tea. Enzymatic oxidation events in black tea processing have started at the beginning of the mill. *In silico* studies of active site Mpro as an inhibitor of SARS-CoV-2 were conducted using Protein Data Bank from a web platform. This analysis was carried out using the AutoDock Vina software integrated with PyRx 0.8. The molecular docking results were visualized in 3D and 2D with the BIOVIA Discovery Studio software with the result that amino acid residues and chemical bonds formed were visible, indicating the binding site of a target protein. The production of bioactive compounds through the tea fermentation process accelerated the oxidation rate of catechins into the contained bioactive compounds, which was analyzed using a spectrophotometer with a wavelength of 380 nm. Bioactive compound analysis used the response surface methodology. The results of docking the oolonghomobisflavan compound with Mpro indicated the highest binding affinity, namely −8.0 kcal/mol; however, the oolonghomobisflavan compound with Mpro did not show the same interaction as the control. On the contrary, for the docking of theaflavin-3'-O-gallate with Mpro, the binding affinity was −6.3 kcal/mol and showed the same interaction with the control, namely, LysA:137, where the compound formed hydrogen bonds, and analysis of the selected compound was carried out on the theaflavin-3'-O-gallate compound. The optimal operating conditions for the extraction process were at a flow rate of 17.65 l/minute with a fermentation time of 50 minutes, which produced a maximum theaflavin level of 0.938%.

INTRODUCTION

Recently, the world was shaken by an acute respiratory disease caused by the coronavirus or SARS-CoV-2. Coronavirus, which belongs to the Coronaviridae family, can infect the respiratory system in humans and animals. The SARS-CoV-2 genome is 96.2% identical to the bat CoV RaTG13, while 79.5% is identical to SARS-CoV (Khanal *et al*., 2021; Kumar *et al*., 2020; Peretto *et al*., 2020). Based on the viral genome sequencing and evolutionary analysis results, bats are the original host of the virus, and SARS-CoV-2 from bats infects humans (Chen *et al*., 2020). Tang *et al*. (2020) analyzed the COVID-19 genotype in different patients from several provinces and found that SARS-CoV-2 had mutations in different patients in China. They carried out a population genetic analysis of 103 SARS-CoV-2 genomes and classified two types of SARS-

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CoV-2 progression: L type (≈70%) and S type (≈30%). Strains in type L, derived from type S, are evolutionary, more aggressive, and infectious. However, the level of difference in SARS-CoV-2 is smaller than the H7N9 avian influenza mutation (Wu *et al*., 2015). The SARS-CoV cell receptor is found in the respiratory tract, a pathogenic replica of the coronavirus Angiotensin Converting Enzyme (ACE2), and regulates species transmission and human-to-human transmission. The S-glycoprotein on the surface of the coronavirus can attach to the receptor, ACE2, on the surface of human cells. The S-glycoprotein includes two subunits: S1 and S2. S1 determines the virus-host range and cellular tropism with the main functional domain, Reseptor Binding Domain (RBD), whereas S2 mediates cell-virus membrane fusion with two main gates, heptad repeats 1 (Chen *et al*., 2009, 2020; Lung *et al*., 2020).

Currently, considerable research on potential antiviral and drug candidates is being conducted. One of the screenings of the chemical structure that has the potential as an active RNA-dependent RNA polymerase (RdRp) inhibitor in SARS-CoV-2 includes theaflavin compounds (ZINC3978446) tea leaves. Lung *et al*. (2020), regarding a molecular docking study on the RdRp of SARS-CoV-2, SARS-CoV, and mer-CoV, showed that theaflavins had a relatively lower iDock score than the RdRp of SARS-CoV-2, namely −9.11 kcal/mol, with a low binding energy of −8.8 kcal/mol. The hydrophobic interactions contributed significantly to the additional hydrogen bonding and bonding between theaflavins and RdRp and indicated that one of the π cation interactions was formed between theaflavins and Arg553 (Lung *et al*., 2020). CoV-matched drugs included ACE2 receptor entry, major protease (Mpro), and RdRp (Kumar *et al*., 2020). However, targeted drugs show significant side effects and lower potency. Thus far, Mpro is one of the best characterized and most promising drug targets in CoVs. Mpro can process polyproteins from viral RNA transcription. In addition, target Mpro cleaves up to 11 moieties on protein replication (1ab, ≈790 kDa), and Mpro inhibition basically blocks virus replication (Bhardwaj *et al*., 2020; Kumar *et al*., 2020).

Tea leaf (*Camellia sinensis*) has many benefits because it has a high antioxidant content. Currently, several studies are being conducted and have confirmed that the content of tea is more active for anti-SARS-CoV-2 therapy treatment than remdesivir and chloroquine. One of the screenings of the chemical structure that has the potential as an active RdRp inhibitor in SARS-CoV-2 is bioactive compounds, such as oolonghomobisflavan-A, theaflavin-3-O-gallate, and theaflavin (TF) (Bhardwaj *et al*., 2020). These bioactive compounds are components of catechin oxidation, which contribute color, taste, and aroma to black tea. Enzymatic oxidation events in black tea processing have started at the beginning of the mill. This event is a process of oxidation of catechin compounds with the help of polyphenol oxidase enzymes. Oxidation of catechin compounds, especially epigalocatechins and their errors, will produce quinones that will further condense into bisflavanols, theaflavins, oolonghomobisflavan-A, and theaflavin-3-O-gallates. The condensation and polymerization processes proceed to form insoluble substances (Bhardwaj *et al*., 2020; Chandini *et al*., 2013; Lung *et al*., 2020). Many bioactive molecules include a polymerized polyphenol from the tea plant (*C. sinensis* L.) that acts as an effective SARS-CoV-2 Mpro inhibitor (Bhardwaj *et al*., 2020). According to Kanbarkar and Misha (2020), the bioactive compound theaflavin is similar to SARS-CoV-2 drugs and has no side effects. Therefore, this study aimed to analyze *in silico* and find the value of molecular docking simulations for the bioactive compounds oolonghomobisflavan-A, theaflavin-3-O-gallate, and theaflavin (TF). It also aimed to find the levels of selected compounds for analysis using a spectrophotometer and simulating the response surface methodology (RSM).

MATERIALS AND METHODS

Materials

Tea leaves were purchased from Medini Tea producers; distilled water was used as the extraction solvent; and nitrogen was used to support the extraction process. Other chemicals used for the analysis included ethyl acetate p.a. and methanol p.a. (Merck), which were purchased from PT. Kurnia Makmur, Semarang.

Methods

Ligand preparations and molecular docking

Prior to docking, one must pass the macromolecule (target protein) preparation stage obtained from the Protein Data Bank from a web platform. The creation of stable complexes between macromolecules and ligands results in the scoring of binding affinity. This method is widely used for drug design because it can make rational conformational predictions by binding the ligand to the binding site of the macromolecule. This analysis was carried out using the AutoDock Vina software integrated in PyRx 0.8. In addition to bioactive compounds and target proteins, molecular docking is also carried out on target proteins and drug compounds with the same bioactivity that is used for controls to determine the anchoring accuracy of bioactive compounds. The molecular docking results were visualized in 3D and 2D with the BIOVIA Discovery Studio software with the result that amino acid residues and chemical bonds formed were visible, indicating the binding site of a target protein. Residues interacting between the active site and the selected molecule were thoroughly examined using the Discovery Studio Visualizer. In addition, the validation of the docking protocol was confirmed by redocking the early inhibitor of the SARS-CoV-2 protein Mpro and finding 0.00 root-mean-square deviation (RMSD) (Tahir ul Qamar *et al*., 2020).

Production of tea bioactive compounds

Fresh tea leaves must be pretreated to convert the catechins contained in tea leaves into bioactive compounds by fermentation. The obtained tea is aerated; then, the tea leaves are blended until smooth and put into a stirred tank where the fermentation process occurs, which is subsequently supplied with air (O_2) at a flow rate of 17 l/minute. Production of bioactive tea compounds through interfacial activation of tea leaf polyphenol oxidase enzymes accelerates the rate of oxidation of catechins into the contained bioactive compounds.

Analysis of bioactive compounds

The test was conducted using a spectrophotometer by preparing a blank solution (4 ml ethyl acetate and 21 ml methanol). Next, a solution of 50 ml ethyl acetate and 50 ml tea leaf extract was prepared in a 1:1 ratio for analysis. This solution was mixed, put into a separating funnel, and was allowed to stand for 10 minutes after

which two layers were formed. The top layer and 4 ml separated solution were mixed with 21 ml of methanol, and this solution was analyzed with a spectrophotometer at a wavelength of 380 nm and calculated using the following equation (Bhardwaj *et al*., 2020):

$$\%TF \text{ (theaflavin)} = 2.25 \times E_1 \quad (1)$$

where E_1 is absorbance to determine %TF.

Analysis of bioactive compounds using RSM

This study uses a central composite design that provides a response in %bioactive compounds and is processed using statistical software central composite design (Ghosh *et al*., 2012; Yulianto *et al*., 2018).

RESULTS AND DISCUSSION

In silico study

In silico design that uses computational techniques in the drug discovery process is used to streamline and accelerate hit identification and hit-to-lead optimization processes (Ekins *et al*., 2007). One of the *in silico* methods is molecular docking or docking. Docking is a method that predicts the orientation of one molecule to another when bonded to each other to form a stable complex. Docking predicts the binding affinity of a ligand molecule to the target protein molecule (Borman, 1990). Syahputra *et al*. (2014) stated that the more negative the binding affinity value, the stronger and more stable the bond between the ligand and the receptor. Saputri *et al*. (2016) stated that the smaller the binding affinity value, the higher the binding affinity between the receptor and the ligand. Conversely, the larger the value, the lower the binding affinity between the receptor and the ligand. Hydrogen bonds are important for determining the binding affinity because these bond have stronger energy than hydrophobic bonds, with a value of 1–7 kcal/mol to 1 kcal/mol. However, hydrophobic bonds are also important in maintaining bond stability (Hernandez and Rathinavelu, 2006). The molecular docking results were visualized in 3D and 2D with the BIOVIA Discovery Studio software, as shown in Figures 1 and 2. The amino acid residues and chemical bonds were visible, indicating the binding site of a target protein.

Residues interacting between the active site and the selected molecule were thoroughly examined using the Discovery Studio Visualizer. The validation of the docking protocol was confirmed by redocking the early inhibitor of the SARS-CoV-2

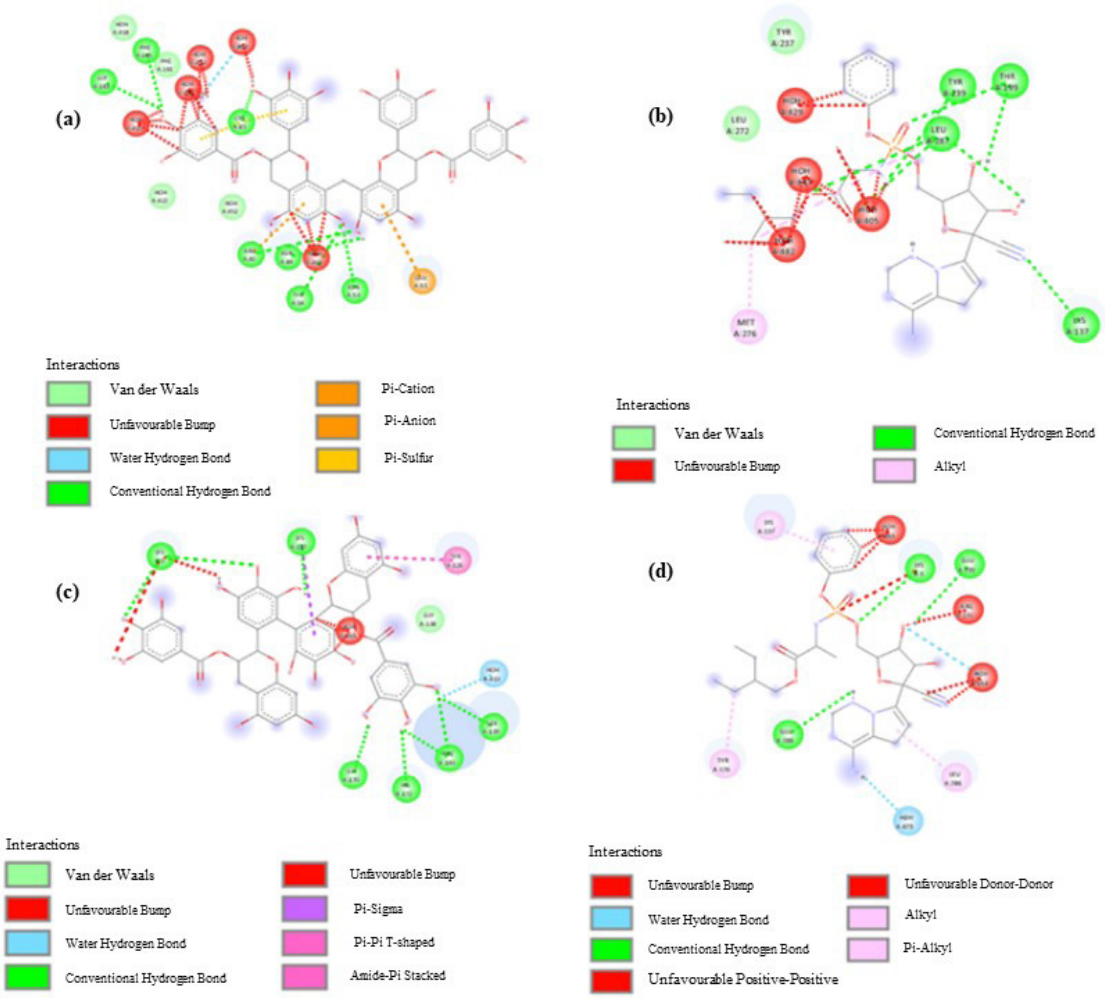

Figure 1. 2D visualization molecular docking: (a) oolonghomobisflavan-A-Mpro, (b) theaflavin-3′-O-gallate-Mpro, (c) theasinensin A-Mpro, and (d) control (remdesivir)-Mpro.

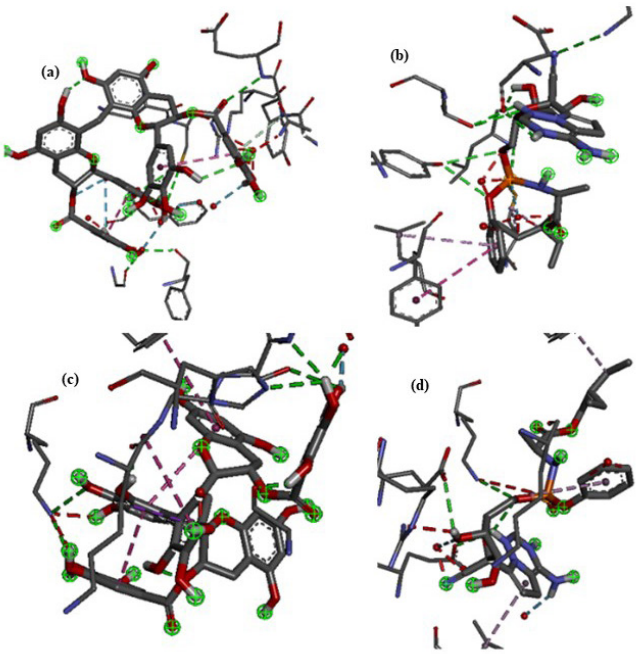

Figure 2. 3D visualization molecular docking: (a) oolonghomobisflavan-A-major protease (Mpro), (b) theaflavin-3′-o-gallate-Mpro, (c) Theasinensin A-Mpro, and (d) control (remdesivir)-Mpro.

protein Mpro and finding 0.00 RMSD. Mpro is one of the best-characterized and most promising drug targets in CoVs. Moreover, Mpro plays a role in the processing of polyproteins, which are products of viral RNA transcription. Mpro targets can cleave up to 11 sites on the large protein replicase (1ab, ≈790 kDa). In addition, Mpro inhibition will block virus replication. There are no known Mpro homologs in humans with identical cleavage specificity. Therefore, its inhibition is unlikely to show adverse toxic results.

Based on the docking results in Table 1, the oolonghomobisflavan compound's binding affinity was −8.0 kcal/mol and that of control docking with Mpro was −5.7 kcal/mol. The binding affinity value is a scoring result of docking in the form of free energy values. The more negative the binding affinity value made, the more stable the binding between macromolecules targets proteins and ligands in bioactive compounds or drugs (Syahputra *et al*., 2014). The binding affinity value of the oolonghomobisflavan compound was higher than the control by showing seven hydrogen bonds at the amino acid residues, namely GlyA:183, PheA:185, CysA:85, ArpA:43, AsnA:86, AsnA:53, and TyrA:54. However, the oolonghomobisflavan compound did not show the same interaction as the control. Oolonghomobisflavan-A is one of the most polymerized polyphenols found in tea. Tea extracts containing oolonghomobisflavan-A or purified compounds can be tested for their inhibitory potential against Mpro SARS-CoV-2, using various *in vitro* and *in vivo* studies. Moreover, the structure of oolonghomobisflavan-A can be further exploited to develop a more potent SARS-CoV-2 Mpro inhibitor (Bhardwaj *et al*., 2020).

The results of docking the theaflavin-3′-O-gallate compound obtained a binding affinity of −6.3 kcal/mol. The docking results showed that the binding affinity value of the oolonghomobisflavan-A compound was higher than the control by showing four hydrogen bonds at the amino acid residues, namely TyrA:239, LeuA:287, ThrA:199, and LysA:137. However, the theaflavin-3′-O-gallate compound showed the same interaction with the control (LysA:137), where the compound formed hydrogen bonds, whereas in the control, only alkyl bond interactions were formed. Amino acids in the docking results between the ligand and the control indicate that the ligand could replace the control compound (Chamata *et al*., 2020).

The results of the docking of the theasinensin A compound obtained a binding affinity of −6.7 kcal/mol. The docking results showed that the binding affinity value of the theasinensin A compound was higher than the control, with six hydrogen bonds at the amino acid residues, namely LysA:5, LysA:187, SerA:139, PheA:140, HisA:172, and GlyA:170. The theasinensin A compound showed the same interaction with the control (LysA:5). In the compound or control, this amino acid formed hydrogen bonds. Amino acids in the docking results between the ligand and the control indicate that the ligand has the potential to replace the control compound (Chamata *et al*., 2020).

Identification and characterization of theaflavin

Theaflavin percentage is obtained from the tea leaf fermentation process using a biofermenter, which uses airflow (O_2). In this analysis, three points where variations are made on the time variable with an oxygen flow rate of 12 l/minute and the percentage of theaflavins produced are shown in Figure 3. In the experiment with a variable oxygen flow rate of 12 l/minute with a duration of 35.85, 50, and 64.14 minutes, the theaflavin content was 0.158%, 0.479%, and 0.078%, respectively. Thus, the longer the fermentation time, the lower the theaflavin percentage because the theaflavin bioactive compounds transform into thearubigin bioactive compounds due to enzymatic and chemical oxidation. The duration of the fermentation process (enzymatic oxidation)

Table 1. Binding affinity of bioactive compounds in tea plants.

No	Ligand-macromolecule	Interaction	Hydrophobic bond			Hydrogen bond
		Van der Waals	π bond	Alkyl bond	Carbon hydrogen bond	Residue
1	Oolonghomobisflavan-A-Mpro (−8.0 kcal/mol)	HohA:418 PheA:181 HohA:422 HohA:452	GluA:55	—	✓	GlyA:183 PheA:185 CysA:85 ArpA:43 AsnA:86 AsnA:53 TyrA:54
2	Theaflavin-3′-O-gallate-Mpro (−6.3 kcal/mol)	TyrA:237 LeuA:272	—	MetA:276	✓	TyrA:239 LeuA:287 ThrA:199 LysA:137
3	Theasinensin A-Mpro (−6.7 kcal/mol)	GlyA:138	TyrA:126	—	✓	LysA:5 LysA:187 SerA:139 PheA:140 HisA:172 GlyA:170
4	Control (remdesivir)-Mpro (−5.7 kcal/mol)	—	TyrA:126 LeuA:286	LysA:137	✓	GluA:288 LysA:5 GluA:290

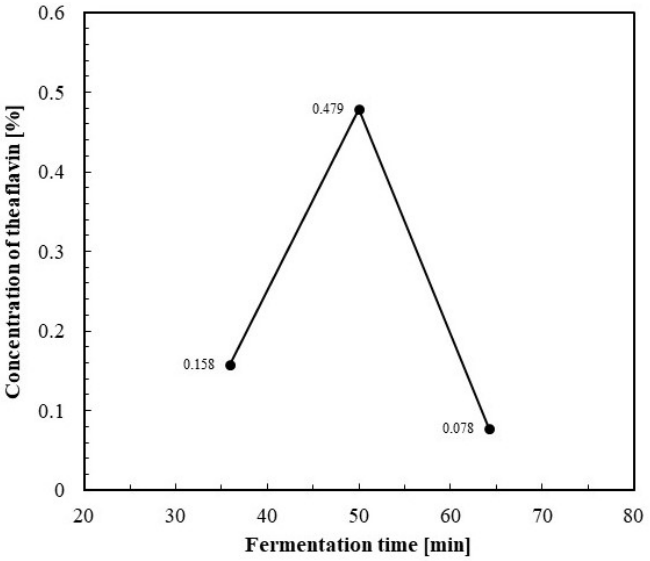

Figure 3. Effect of fermentation time on the concentration of theaflavin.

determines the amount of the theaflavin content. With a longer processing duration, the thearubigin content will be much greater than theaflavin. Figure 4 shows a fluctuating pattern due to the thermolabile nature of the theaflavin bioactive compound (Shabri and Maulana, 2017).

Figure 4 shows that the structure of theaflavins formed through the biochemical synthesis of the catechin precursor Epigallocatechin gallate (EGCG) is initiated when the enzyme polyphenols oxidase (PPO) or peroxidase oxidizes the catechin compounds in quinone. Therefore, with prolonged oxidation time, the theaflavin compounds will be condensed into thearubigin compounds. When the solvent penetrates theaflavins for a long duration, it can thermally degrade theaflavins into thearubigin. Therefore, prolonged contact time between the solvent and tea leaves can cause theaflavins to transform into thearubigin compounds.

Effect of O_2 flow rate on theaflavin percentage

Oxygen flow rate also plays an important role in the formation of theaflavin compounds. The tea leaf fermentation process, commonly called enzymatic oxidation, is supplied with air circulation (O_2) in the fermenter tank. Therefore, in this analysis, three points are considered where the airflow rate (O_2) varies with a fermentation time of 50 minutes, with the result that the theaflavin percentage data is obtained (Fig. 5).

In this experiment, using a fixed time variable for 50 min with a flow rate variation of 6.34315, 12, and 17.65685 l/minute, the theaflavin content was 0.268%, 0.479%, and 0.938%, respectively. The graph 3 shows an increasing trend; thus, it can be interpreted that, with a greater oxygen flow rate that is provided to the fermenter tank, the theaflavin content in the tea leaves will also increase. This is because the increase in airflow in the fermentation process will increase the catechin reaction in the formation of new compounds in the form of theaflavins, resulting in the increase of theaflavin content.

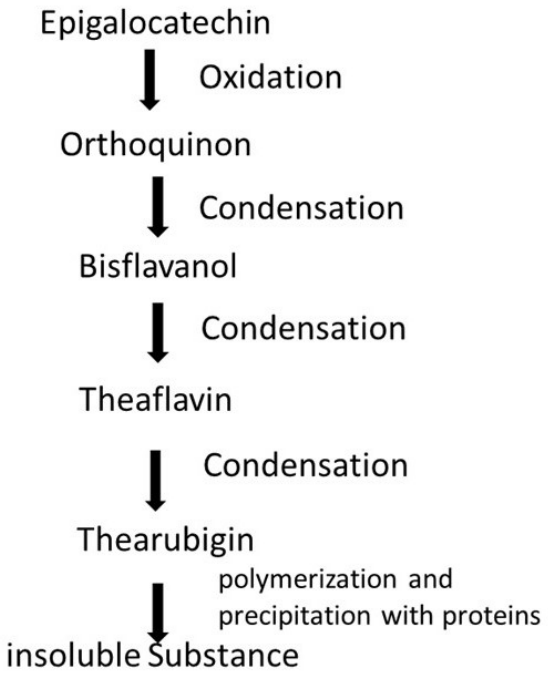

Figure 4. Scheme of enzymatic oxidation in tea leaves.

Shabri and Maulana (2017) explained that the airflow rate used in the enzymatic oxidation process of tea leaves greatly affects the formation of theaflavin compounds. In this study, with a flow rate of 15, 20, and 25 l/minute for 60 minutes, the theaflavin content was 0.88%, 0.97%, and 1.01%, respectively. The increased airflow into the fermenter tank (20 and 25 liters/min) resulted in the increase of theaflavin content to 0.97% and 1.01%, respectively.

Analysis of theaflavin compounds using RSM

Many studies have used the RSM in determining the optimization of a method in design or analysis. The RSM is a design and model that works with various treatments continuously, while finding the optimum value or describing the response according to the objectives (Yulianto *et al*., 2018). The main goal of the RSM is to find the optimal response. In the analysis of theaflavin levels using the RSM to determine the optimal value of the tea leaf fermentation process, this study used a central composite design to respond to the form of theaflavin levels. Tao et al. (2016) stated that oxygen flow rate and fermentation time have an important role in the formation and reduction of theaflavin bioactive compounds in tea leaves with variable oxygen flow rate (l/minute) and fermentation time (min) (Table 2).

In tea leaf fermentation with variable flow rate and fermentation time, the theaflavin content was obtained at a maximum yield of approximately 0.938% with a flow rate of approximately 17 l/minute and a fermentation time of 50 minutes. In comparison, the minimum yield of the theaflavin content obtained was approximately 0.078%, with a flow rate of 12 l/minutes for 64 minutes. Therefore, the highest oxygen flow rate influences the maximum yield of theaflavin content produced. This is because the increased airflow in the fermentation process increases the catechin reaction in the formation of new compounds in the form of theaflavins, resulting in the increase of theaflavin content. While the minimum yield of theaflavin content is influenced by the long fermentation time, the longer fermentation time transforms theaflavin bioactive compounds into thearubigin bioactive compounds. Thus, the theaflavin content decreases when the enzymatic oxidation of tea leaves is considerably long.

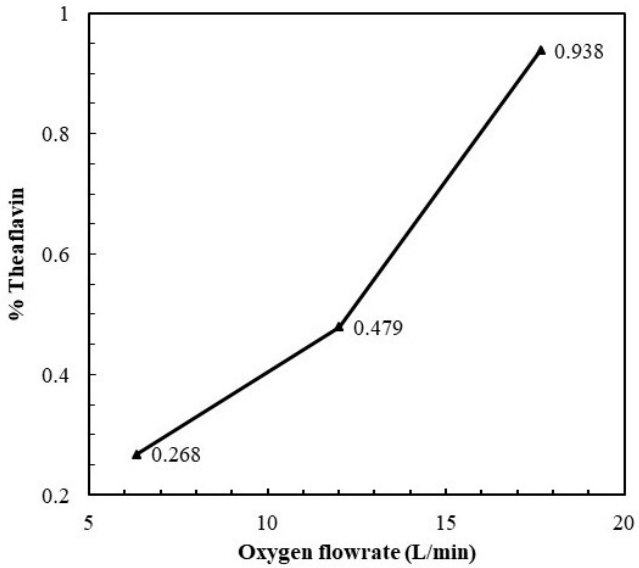

Figure 5. Effect of oxygen flow rate on the theaflavin concentration during fermentation.

Table 2. Concentration of theaflavin during the fermentation process of tea leaves.

Flow rate (l/minute)	Time (minutes)	Theaflavin (%)
8	40	0.265
8	60	0.486
16	40	0.163
16	60	0.34
6.34315	50	0.268
17.65685	50	0.938
12	35.85786	0.158
12	64.14214	0.078
12	50	0.479
12	50	0.479

The effect of the variable on the response can be determined by using a first-order polynomial regression equation. Table 3 shows the estimated effect. The first-order equation provides data on the effect of oxygen flow rate (x) and fermentation time (y); thus, the equation $Z(x,y)$ obtained is as follows:

$$Y = [(0.039764911515348 \times (x)) + (0.0031406250898181 \times (x^2)) + (0.19911071504901 \times y0.0019224993015089) \times (y^2)) - (0.00027499999999995 \times (x) \times (y)) + 0].$$

The regression model above shows that the flow rate (x) has a positive effect on the level of theaflavins. Meanwhile,

Table 3. Estimation effect during fermentation of tea leaves using RSM.

Factor	Estimation of effect								
	Effect	Std. error	*t* (4)	*p*	−95% Cnf. Limt	+95% Cnf. Limt	Coeff.	Std. err. coeff.	−95% Cnf.Limt	+95% Cnf.Limt
Mean/Interc.	0.479000	0.164216	2.91689	0.043378	0.023063	0.934937	0.479000	0.164216	0.023063	0.934937
(1) Flow rate (*L*)	0.174881	0.164216	1.06494	0.346916	−0.281056	0.630818	0.087440	0.082108	−0.140528	0.315409
Flow rate (*Q*)	0.100500	0.217238	0.46263	0.667678	−0.502648	0.703648	0.050250	0.108619	−0.251324	0.351824
(2) Time (*L*)	0.071216	0.164216	0.43367	0.686892	−0.384721	0.527152	0.035608	0.082108	−0.192360	0.263576
Time (*Q*)	−0.384500	0.217237	−1.76995	0.151448	−0.987647	0.218647	−0.192250	0.108619	−0.493824	0.109324
1*L* by 2*L*	−0.022000	0.232237	−0.09473	0.929084	0.666792	0.622792	−0.011000	0.116118	−0.333396	0.311396

the fermentation time (*y*) negatively affects theaflavin levels, as shown in Table 3.

Table 3 shows a closeness between the model and experimental data on the observed and predicted values. The fermentation time is the most influential variable on tea leaf fermentation for theaflavin production. Time plays an important role in forming and reducing the theaflavin content in the tea leaf fermentation process. The relationship between theaflavin levels and time greatly affects the theaflavin content produced. The longer the tea leaf fermentation process, the more the amount of theaflavins that will experience condensation. The tea leaves will also experience a decrease in antioxidant activity. Therefore, in the fermentation process, the time required must be considered. Based on the study of Zhang *et al*. (2019), the time required when fermenting tea leaves to form theaflavins is a maximum of 60 minutes, and if it exceeds this duration, the theaflavin content will decrease because theaflavins will turn into thearubigin compounds. The relationship between theaflavin levels and flow rate greatly increases theaflavin levels because an increase in the airflow rate in the fermentation process will increase the catechin reaction in tea leaves assisted by the PPO enzyme during the enzymatic oxidation process. This results in the rapid formation of theaflavin compounds. From the data that has been obtained and analyzed, a Pareto diagram (Fig. 6) can help identify the significant factors that affect the theaflavin levels resulting from the experiment.

The time factor (Q) has the most significant effect on the levels of theaflavins produced. The variable flow rate (Q) affects the levels of theaflavins produced. A regression analysis on the response surface design compares experimental and predictive data, as shown in Figure 7. In the observed vs. predicted values graph, we can see that the plots are randomly distributed but follow a straight line. Thus, the results obtained are not normally distributed. Therefore, the results obtained are inconsistent with the predicted data.

Figure 8 shows the existence of a three-dimensional response surface on a fixed variable plotted with two independent variables (flow rate and time) whose data has been processed using statistical software central composite design. Figure 8 shows the plot contours of the time and flow rate variables for tea leaf fermentation. At a flow rate of 17.65 l/minutes with a fermentation time of 50 minutes, it produces a maximum theaflavin level of 0.938%, while, at a flow rate of 12 l/minutes with a fermentation time of 64.14 minutes, the lowest theaflavin content produced is 0.078%. Green tea leaves contain a lot of polyphenols in the form of catechins. Thus, during the fermentation process, the tea leaves undergo enzymatic oxidation into theaflavin compounds, marked by changes in the aroma and color of the tea leaves, which are the characteristics of theaflavins, namely, forming a bright color and a fresh, brisk taste. In addition, theaflavins have thermolabile properties where theaflavin compounds will change or be degraded into other compounds in the form of thearubigin. One factor that can cause changes in theaflavin compounds is the time used during fermentation; thus, more accuracy is needed in this study. The accuracy or inaccuracy of the study can cause the theaflavin compounds to decrease because they will turn into other compounds. Therefore, the results obtained are inconsistent with the predicted data.

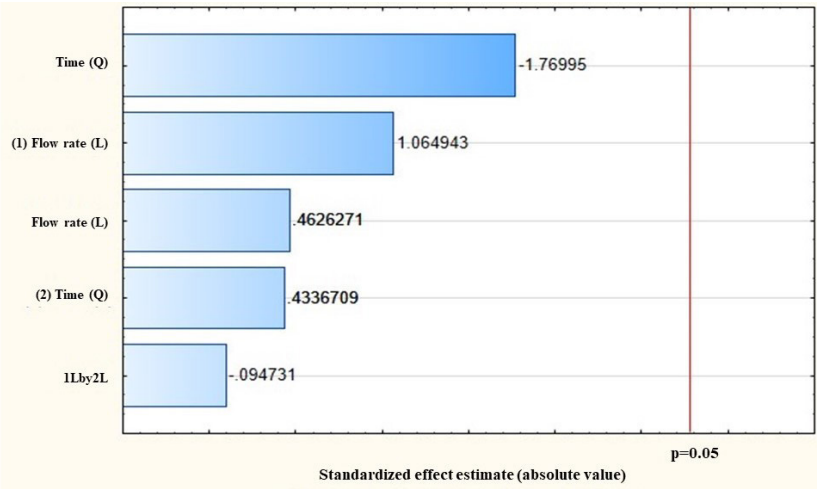

Figure 6. Pareto chart of the standardized effect of theaflavin during fermentation.

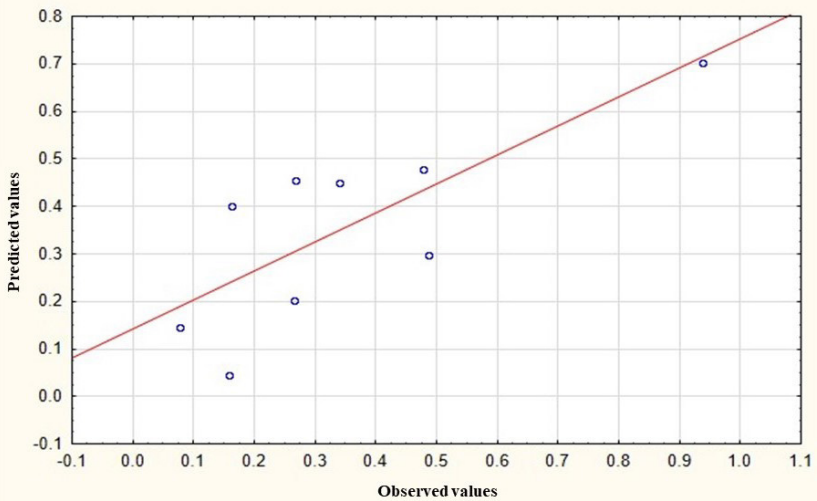

Figure 7. Comparison of observed and predicted values during fermentation of tea leaves.

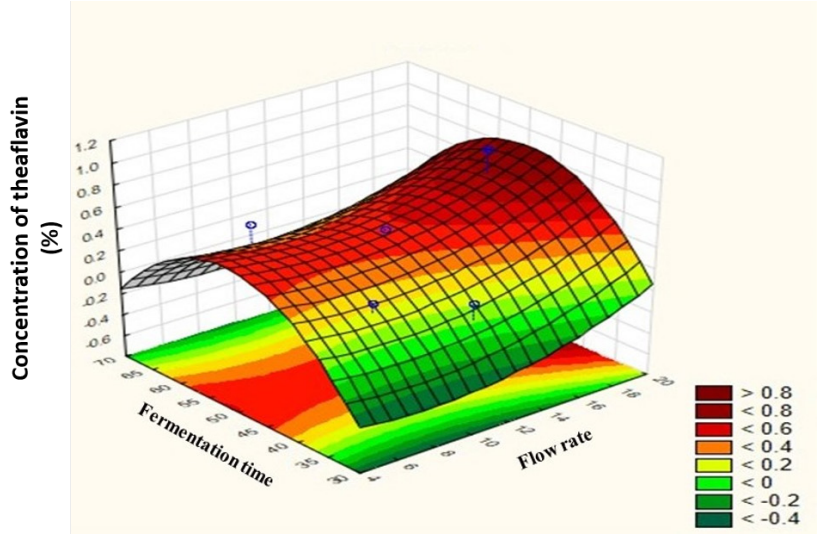

Figure 8. Flow rate and fermentation contour plot in RSM.

CONCLUSION

One of the screenings of the chemical structure's potential as an active RdRp inhibitor in SARS-CoV-2 is bioactive compounds such as oolonghomobisflavan-A, theaflavin-3-O-gallate, and theaflavin (TF). These bioactive compounds are components of catechin oxidation that give color, taste, and aroma to black tea. Based on the results of docking the oolonghomobisflavan compound, the highest binding affinity was obtained, namely −8.0 kcal/mol. However, the oolonghomobisflavan compound did not show the same interaction as the control, while for the docking of theaflavin-3′-o-gallate, the binding affinity was −6.3 kcal/mol and showed the same interaction with the control, namely, LysA:137, where the compound formed hydrogen bonds, and analysis of the selected compound was carried out on the theaflavin-3′-o-gallate compound. The optimal operating conditions for the extraction process were a flow rate of 17.65 l/minutes with a fermentation time of 50 minutes. It produces a maximum theaflavin level of 0.938%. Tea leaves undergo enzymatic oxidation to theaflavin compounds marked by changes in the aroma and color of the tea leaves, which are characteristic of theaflavins, namely, forming a bright color and a fresh, brisk taste. In addition, theaflavins have thermolabile properties where theaflavin compounds will change or degrade into other compounds in the form of thearubigin. One factor that can change the theaflavin compounds is the time used during fermentation, so more accuracy is needed in this study.

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AUTHORS' CONTRIBUTIONS

A. Yuniastuti and V. Paramita analyzed the molecular docking. D. Rohdiana, H.D. Ariyanto, Shabri, and R. Amalia extracted theaflavin and bioactive compounds from tea plants. M.E. Yulianto, Sutrisno, and I. Hartati designed the study. R.D. Nyamiati and S. Rachmawati conducted the experiment and wrote the manuscript. M.E. Yulianto supervised the experiment and reviewed the manuscript.

CONFLICTS OF INTEREST

The authors state no conflicts of interest and have received no payment in preparation of this manuscript.

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ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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