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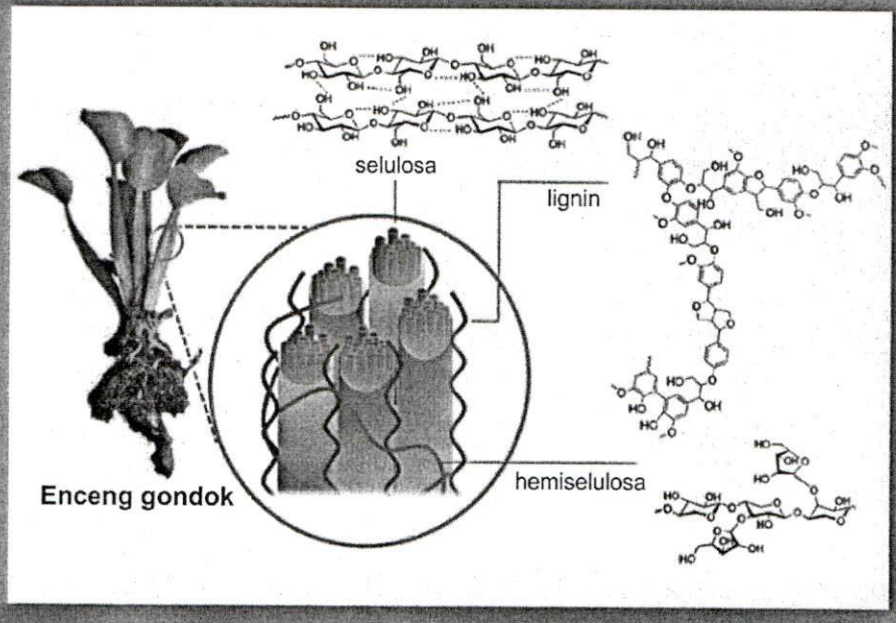

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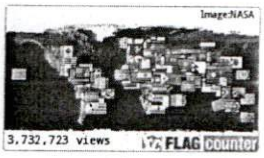

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The Temperature Effect on Ultrasonic-assisted of Synthesis Methyl Ferulate and Its Antiplatelet Assay

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ABSTRACT

Ferulic acid (FA) has been reported to have antiplatelet activity through indirectly inhibiting P2Y12 receptor. One technique to improve the activity of ferulic acid is to increase its lipophility. In this study the carboxylic group of ferulic acid was modified to methyl ferulate through the Fisher esterification reaction. Ultrasonic waves were utilized as source of energy emitted through water as the medium at two various of temperature, i.e., 55 °C and 65 °C. The purposes of this study are to produce methyl ferulate and to determine the reaction constant rate (k) and its energy activation (E_a), at temperature of 55 °C and 65 °C. Moreover, the biological activity as antiplatelet was investigated at dose 20 mg/kg BW. The antiplatelet assay was conducted by clotting time and bleeding time methods. The results were analyzed by one way ANOVA program (P<0.05). The yield of methyl ferulate are 50.3% and 67.1% at 55 °C and 65 °C, respectively. The k value at 55°C is $4x10^{-5}$ $cons^{-1}$ min^{-1}, while that of at 65 °C is $9x10^{-5}$ $cons^{-1}min^{-1}$. The clotting time and bleeding time of methyl ferulate obtained were 265 sec and 175 sec, respectively. The antiplatelet activity of methyl ferulate is better than ferulic acid.

Keywords: antiplatelet, bleeding time, clotting time, ferulic acid, Fisher esterification, methyl ferulate.

INTRODUCTION

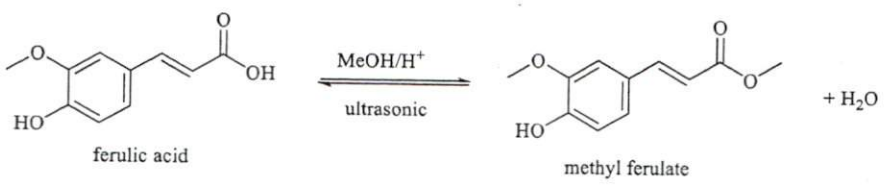
Excessive aggregation of platelets in blood vessels causes thromboembolism. Antithrombotic drugs, which include antiplatelet and anticoagulant therapy, prevent and treat many cases of heart abnormalities. However, some antithrombotic drugs have several pharmacological limitations which produce a variety of antithrombotic effects among patients due to metabolism, and the interactions to the others e.g., other drug interactions, environment, and genetics, or targets of the drug. The rapid knowledge of pharmacological antithrombotic drugs and understanding of the mechanism of thrombosis have triggered the

development of new antithrombotic drugs which has rapid onset of action, smaller interactions, and fewer variations in antithrombotic effects (Mega and Simon, 2015).

Ferulic acid has been reported to have antiplatelet activity by indirectly inhibiting P2Y12 receptor activity (Yang et al., 2013; Zhang et al., 2015). However, a major inconvenience of FA is its disfavored solubility in both oil and aqueous media constraining its application in formulations proposed for pharmaceutical products (Antonopoulou et al., 2017). In an effort to increase the activity of ferulic acid, structural modification is carried out through the esterification reaction of the carboxylic group to produce an ester compound, namely methyl ferulate. The purpose of this esterification is to increase the lipophilicity of the compound, making it easier to enter the cell (Fauchier et al., 2015).

Yang et al. (2013) reported the use of methyl ferulate, in combination as prodrug with dabigatran. The ester bond of dabigatran-methyl ferulate was expected to be hydrolyzed *in vivo* by esterase to release dabigatran and ferulic acid, which has a synergy antithrombotic effect both in vein and arterial thrombosis via two routes, namely antiplatelet and antithrombosis.

Methyl ferulate, as ester compound, can be obtained from carboxylic acid through Fischer esterification reaction (Solomons and Fryhle, 2011), methylation with dimethyl carbonate (Shieh and Repič, 2002), methylation with a mixture of BF_3 and methanol (Demirbas, 2008), and methylation with dimethyl sulfate (Lamoureux and Agüero, 2009). Fischer esterification is the formation of ester compounds from a carboxylic acid by reaction with alcohol and using acid as catalyst (Solomons and Fryhle, 2011). This reaction is reversible, relatively inexpensive and easy to implement due to the use of acid catalysts and the ease in obtaining reagents. In addition, compared to the esterification reaction with a base catalyst, Fischer esterification produces fewer by-products (Pacheco et al., 2014). This reaction also runs very slowly, but can immediately reach equilibrium in a few hours when carboxylic acid and alcohol are refluxed together with sulfuric acid or hydrochloric acid in small amounts.



ferulic acid methyl ferulate

Figure 1. Synthesis methyl ferulate through ultrasonic irradiation

Cravotto et al., (2005) showed that Fischer esterification of cholic acid and methanol assisted by *p*-toluene sulfuric acid as a catalyst was conducted successfully in three methods, namely conventional methods (heating with reflux), microwave irradiation, and ultrasonic waves (Cravotto et al., 2005). The conventional method gave a high yield, which is 93%, in five hours reaction time, while the esterification reaction using microwave and ultrasonic waves provided higher reaction yield (96% with ultrasonic waves and 97% with microwave irradiation) in shorter reaction times (30 minutes for ultrasonic waves and 3 minutes for microwave irradiation). This shows that reactions assisted by microwave and ultrasonic irradiation are more efficient, can obtain higher reaction results, and require shorter reaction times (Cravotto et al., 2005).

This study will evaluate the synthesis of methyl ferulate (Figure 1), and its kinetic and order reaction using ultrasonic waves. Ultrasonic irradiation has been respected as a clean and suitable method in organic synthesis throughout the last three decades, contrasted with conventional methods; the procedure is more accessible. Through the ultrasonic irradiation method, many organic reactions can be prepared in a higher yield, shorter reaction time and milder conditions (Arani and Safari, 2011).

According to Pacheco et al. (2014), the effects of ultrasonic waves play a role in increasing chemical processes, especially in cases where methods require drastic conditions or long reaction times. Ultrasonic waves are emitted through the wave medium through by inducing molecular vibrational movements. Therefore, the distance between molecules can vary to be closer or further due to the movement of the oscillation of the molecule. If the ultrasonic waves in the medium are getting more intense, one point will be reached where the intra-molecular force of the fluid cannot maintain its molecular structure intact. As a result, the molecular structure of the liquid will break and form a cavity. This cavity is a denomination of a bubble cavity, and this process is called cavitation (Pacheco et al., 2014).

In the present research, synthesis of methyl ferulate was conducted by reacting ferulic acid, methanol, and sulfuric acid as catalyst (H_2SO_4) using ultrasonic wave method at 55 °C and 65 °C. This is because the temperature can influence the possibility of collisions between molecules in the reaction. The higher the temperature in the reaction, the higher the kinetic energy produced by compound molecules, thus more collisions occur between molecules. This phenomenon will increase the reaction rate. At the same time, a reaction at a higher temperature will also increase the yield percentage.

To conclude that the reaction at a temperature of 65 °C is greater than the reaction at 55 °C, the determination of the Fischer esterification rate synthesis of methyl ferulate was

evaluated. Theoretically, every 10 °C temperature increase will increase the reaction rate two to three times (Singh, 2006).

METHODS

Ferulic acid (**1**) was bought from Aldrich; dimethyl sulfate, KOH, acetone, chloroform, sodium hydrogen carbonate, H_2SO_4, ether, and methanol were purchased from Merck. The synthesis reaction was conducted by ultrasonic Hwashin Technology Powersonic 400 series and was observed with TLC using UV lamp on $\lambda = 254$ nm to spot detection. Melting points were evaluated by Fischer-John melting point apparatus without correction. UV spectra were achieved by Shimadzu HP 8452 UV-vis spectrophotometer. The concentration of reactant was monitored through Densitometer Shimadzu. IR spectra were presented using a Jasco FT-IR 5300 spectrophotometer. The ^{1}HNMR spectra were obtained from the JEOL JNM-ECS 400 instrument (^{1}H NMR: 400 MHz) using appropriate DMSO-d_6 as a solvent.

Synthesis

A mixture of 400 mg (2.0 mmol) of ferulic acid, 10.0 ml (246.1 mmol) methanol, and 2 drops (1.86 mmol) of concentrated H_2SO_4 in a round bottom flask was shaken homogeneously and reacted using ultrasonic waves at the highest power. This reaction was conducted at temperatures of 55 °C and 65 °C. The completion of the reaction was evaluated by TLC at 0, 30, 60, 90, 180, and 360 minutes reaction time, by using ferulic acid as the reference compound and chloroform: dichloromethane: methanol = 4: 3: 0.2 as the mobile phase. After that, the rest of methanol was evaporated, and the residue was then neutralized by $NaHCO_3$ 5% solution. The desired compound was extracted by chloroform and dried by anhydrous $MgSO_4$. The structure of the obtained compound was confirmed by UV, IR and ^{1}H-NMR spectroscopy.

Determination of Linearity of Densitometry Method

The standard solution was arranged by taking 100 µl, 200 µl, 300 µl, and 500 µl of the stock solution of 40,000 ppm and diluted by methanol (p.a.) until 1000 µl. Each concentration was spotted on the TLC plate and eluted by chloroform: dichloromethane: methanol (4:3:0.2). The area of the resulted spot was determined by a densitometer. The concentration in ppm (as ordinate) and spot area (as abscissa) were then plotted to form a line equation and calculated the linearity by linear equation as equation (1).

$$y = Ax + B \quad (1)$$

The samples of this test were five; the R_{table} of those samples was 0.8054. The curve is linear if $R_{calculated}$ is greater than R_{table}.

Determination of Reaction Order, Rate Constant (k) and Activation Energy (E_a)

Determination of the reaction rate was carried out by the graph method based on the integral equation of the reaction rate between the concentration of ferulic acid (Ct) vs. time (t). The rate constants (k) from each temperature, namely k_{55} and k_{65} were calculated after the rate order was known The reaction rate constant (k) of the two temperatures substituted in the Arrhenius equation as shown equation (2) to determine the energy of activation as shown in equation (3).

$$\log \frac{k_{65}}{k_{55}} = \frac{E_a}{2{,}303\ R}\left(\frac{T_{65}-T_{55}}{T_{65}T_{55}}\right) \quad (2)$$

$$E_a = \left(\frac{2{,}303\ R\ T_{65}\ T_{55}}{T_{65}-T_{55}}\right) \log \frac{k_{65}}{k_{55}} \quad (3)$$

Notes:

k_{65} = rate constant at 65 °C ($cons^{-1}\ min^{-1}$)

k_{55} = rate constant at 55 °C ($cons^{-1}\ min^{-1}$)

E_a = activation energy (kcal/mol)

R = gas constant (1.987 calories/mol.K)

T_{65} = absolute temperature 338 K (65 °C+273)

T_{55} = absolute temperature 328 K (55 °C+273)

***In vivo* Antiplatelet Activity Evaluation**

Bleeding time assay: Twelve healthy adult male white mice aged 8−12 weeks weighing 20−22 g, adapted and fed for one week. The mice were divided randomly into four groups, while each group consisting of three mice. Each mouse was given food in the form of pellets and drinks in the form of water every day in *ad libitum*. Test compounds were given orally at a dose of 20 mg/kg, given for 7 days. On the 8th day, the bleeding time test was carried out in the test animals. Bleeding time measurement was done by lying down the mice on the test table. The tip of the tail was cleaned with alcohol 70% then the tail of the mice was slashed along 2 mm. The dripping blood was placed on a filter paper every 15 seconds to observe whether there is still blood coming out. The duration, started from the first drop of the blood until the blood stops dripping, was determined as the bleeding time. The positive control was given oral aspirin dose of 20 mg/kg body weight (BW) in CMC-Na 0.5%. The negative control group was given oral CMC-Na 0.5%. Other two groups were given oral FA and MF in the same way and dose as that of aspirin. At the same time, the

clotting time was determined from the duration of the first blood drop until the clot formed. This antiplatelet assay method was approved by the Ethical Commission of Airlangga University.

***In silico* test**

The *in silico* test was carried out by docking the molecules against COX-1 (PDB ID 4O1Z) as the target enzyme. 4O1Z is COX-1 crystal structure binding MXM_807 [A] as a native ligand downloaded in a resolution of 2.90 Å from the RCSB Protein Data Bank (www.rcsb.org). The 3D structure of the molecules was imported into workspace and positioned into the cavity aligning to MXM_807 [A]. The molecular docking was performed against the cavity-x using the MolDock SE algorithm with maximum 1500 iteration. The enzyme-ligand affinity was determined from the docking score that expressed as the Moldock Score (MDS). The best docking results must meet the criteria of the lowest energy pose, and the docked molecule was in the same place as the native ligand.

RESULTS AND DISCUSSION

Synthesis

Methyl ferulate (MF) was obtained as white solids, and its melting point was 58 °C. The yield obtained from 55 °C and 65 °C reactions were 50.3% and 67.1%, respectively. The R_f values are shown in Table 1 which display that R_f value of MF is higher than FA. This condition occurred because of a change in lipophilicity of compounds from a hydrophilic acid (FA) to a more lipophilic ester (MF). According to ChembioUltra software, Log P of FA is 1.42 while log P of MF is 1.68.

Table 1. R_f of Methyl ferulate

Eluent	R_f MF	R_f FA
Ethyl acetate : *n*-hexane (1:1)	0.63	0.43
Chloroform : dichlorometane : methanol (4:3:0.2)	0.68	0.31
Acetone : chloroform (1:4)	0.74	0.51

The melting point of methyl ferulate was lower than ferulic acid, which melted at 171 °C. This phenomenon is caused by changes in the hydrogen bonds in these molecules, wherein the molecule of ferulic acid there are hydrogen bond dimers, while in the molecule methyl ferulate there is no hydrogen bond due to the loss of the hydroxy group of the carboxylate in ferulic acid which is replaced by the methoxy group in methyl ferulate.

The spectroscopic data of methyl ferulate were as follow: λ_{max} (nm): 326 nm; IR (ν;cm^{-1}): 1718 (C=O ester), 3406 (O-H), 1642 (-C=C- alkene), 1590 and 1436 (-C=C- aromatic), 1264 (-C-O). ^{1}H-NMR (δ: ppm) (400 MHZ, $CDCl_3$): 3.83 (s, 3H), 3.88 (s, 3H), 6.25 (1H, d, J = 16.0 Hz), 6.40 (1H,s), 6.88 (1H, d, J = 8.4 Hz), 7.00 (2H, d, J = 2.0 Hz), 7.60 (1H, d, J = 16.0 Hz). ^{13}C-NMR (δ: ppm) (100 MHZ, $CDCl_3$): 51.74 (1C), 55.91 (1C), 109.75 (1C), 114.72 (1C), 150.74 (1C), 123.05 (2C), 126.79 (1C), 147.13 (1C), 148.33 (1C), 168.13 (1C).

The profile of IR spectra of the product from the reactions at 65 °C and 55 °C showed that the designed ester was successfully synthesized, based on the stretching band of C=O ester at wavenumber 1718 cm^{-1} that is different from C=O ferulic acid band at 1691 cm^{-1}. In addition, the widening band around 3000 cm^{-1} originating from the OH group in the starting material was no longer found. Comparison between the IR spectra of the product from reactions at 65 °C and 55 °C showed the correlation value of the two compounds was 0.9949. This result shows that the synthesis at both temperatures could produce the same compound of methyl ferulate (Figure 2).

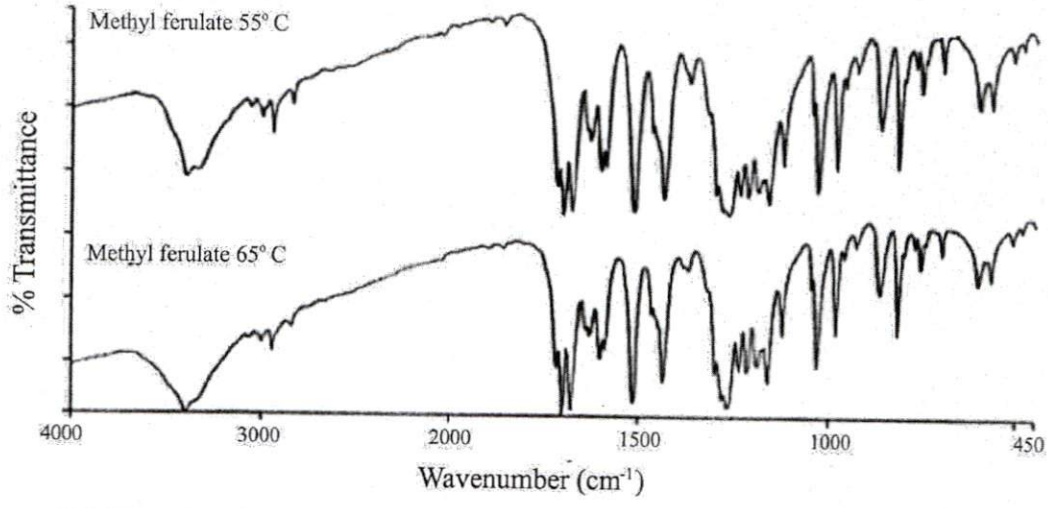


Figure 2. The comparison of IR spectra of methyl ferulate synthesized at 55 °C and 65 °C.

Based on the ^{1}H-NMR spectra, it was known that there were three additional proton from methoxy groups at chemical shift (δ) 3.84 ppm (3H, singlet). There was one proton at δ_H 6.24 ppm (1H, duplet) and one proton at δ_H 7.57 ppm (1H, duplet) which have the same coupling constant J=16 Hz. This value indicated that protons in alkenes have a *trans* configuration (Pavia et al., 2014).

From UV spectral data, it was found that λ_{max} ferulic acid was 322 nm, whereas λ_{max} of methyl ferulate was 326 nm. There was little difference of λ_{max} between two compounds

because the methoxy group in methyl ferulate was not a chromophore group. Moreover, the electronic transition from $\sigma \rightarrow \sigma^*$ in the methyl group required a large amount of energy. As a result, the shift of λ in the methyl ferulate was not significant (Pavia et al., 2014).

All of the spectral data showed that the same compounds from both reactions at 55 °C and 65 °C were successfully synthesized. The higher the temperature, the higher heat energy that is received by the molecule, hence the greater the kinetic energy that can be used by molecules to collide each other in order to initiate a reaction. This theory makes it is easier for chemical reactions and higher probabilities to form new compounds.

The mechanism reaction for the synthesis of methyl ferulate using Fischer esterification method is nucleophilic addition (Figure 3). The reaction began with the protonation of the carbonyl group of ferulic acid by strong acid as catalyst. Protonated carbonyl groups would be attacked by nucleophilic groups from alcohol. The nucleophilic groups of carbonyl would take protons and formed water (H_2O) that would be eliminated as a by-product. The protons in the carbonyl group were then released again to reform a strong acid catalyst (Solomons and Fryhle, 2011).

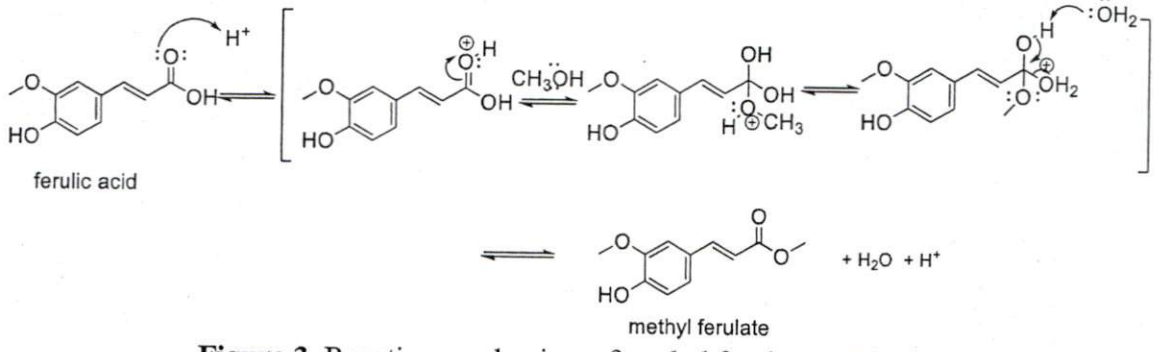


Figure 3. Reaction mechanism of methyl ferulate synthesis

The synthesis in the present study used the ultrasonic wave method, in which the ultrasonic apparatus firstly transmitted the wave through the water as the medium, then the wave propagated to all parts of the solution in the reaction flask. The mechanical activation process, called cavitation, removed the attraction between molecules in the liquid phase. Once small air bubbles were formed inside the cavity, these bubbles would absorb energy from ultrasonic waves and produce the larger cavity.

As the socket grew, the air bubbles inside could no longer absorb ultrasonic energy. Finally, the fluid around the cavity would go inside and broke the cavity. This rupture of the air cavity created favorable conditions for chemical reactions. As a result of the vibrational propagation, kinetic movements of molecules would occur followed by collisions that triggered chemical reactions. In order to obtain the best condition of reactions, the oriented

procedures were conducted at several reaction temperatures. In the condition including temperature at 35 °C and the moderate intensity of ultrasonic wave, the reaction ran slowly and the new compound formed in the 120th minutes, based on the new spot observed on TLC. The next reaction that was carried out at condition of 50 °C in temperature and moderate intensity of ultrasonic wave, the new compound was found after 90 min. This results indicated that the reaction proceeds faster by increasing the temperature.

In a further experiment, the temperature was increased to 65 °C, and the ultrasonic intensity was raised from moderate to high intensity then the new compound was formed after 60 min reaction. Based on these results, temperature of 65 °C and high ultrasonic intensity were selected as the condition of methyl ferulate's synthesis. As a comparison for the study of reaction rates, 55 °C was chosen as reaction temperature with the same ultrasonic intensity. Theoretically, the reaction rate was also influenced by the initial concentration of the reacting compound (Singh, 2006).

To find out whether the TLC-densitometry can be used to monitor the changes of ferulic acid concentration (reactant), a linearity test was carried out between the ferulic acid level (ppm) and the percentage area (%). Data processing result the line equation $y = 0.001x + 3.4833$ with the correlation coefficient (R)= 0.9943. The R_{table} suitable for this experiment using 5 samples is 0.8054. The value of $R_{calculated}$, which is greater than R_{table} indicated that the two variables had significant linear relationship.

Determination of Reaction Order

Determination of the reaction order was done by the graphical method, where the concentration of reactants (ferulic acid) in three data form (C_t, log C_t, $1/C_t$) each was plotted against time (in minutes) to obtained a straight-line. Concentration of ferulic acid during esterification reaction at 55 °C and 65 °C were shown in Figure 4 and 5.

According to Sinko et al. (2006), reaction order can be determined by three methods, namely the graph method, the substitution method, and the half-time method. In this study, we used the graph method. The graph method was conducted by making some plots of C_t, log C_t, and $1/C_t$ each against time (t) and then was compared which plot that produced a linear line. If the graph between concentration (C_t) and time (t) was linear, then the reaction was zeroth order. If the graph between log concentration (log C_t) and time (t) was linear, then the reaction had first order. The reaction order was second if the graph between $1/C_t$ and time (t) was linear (Singh, 2006). The linearity of the graph was determined by the value of R obtained from the regression equation of the line.

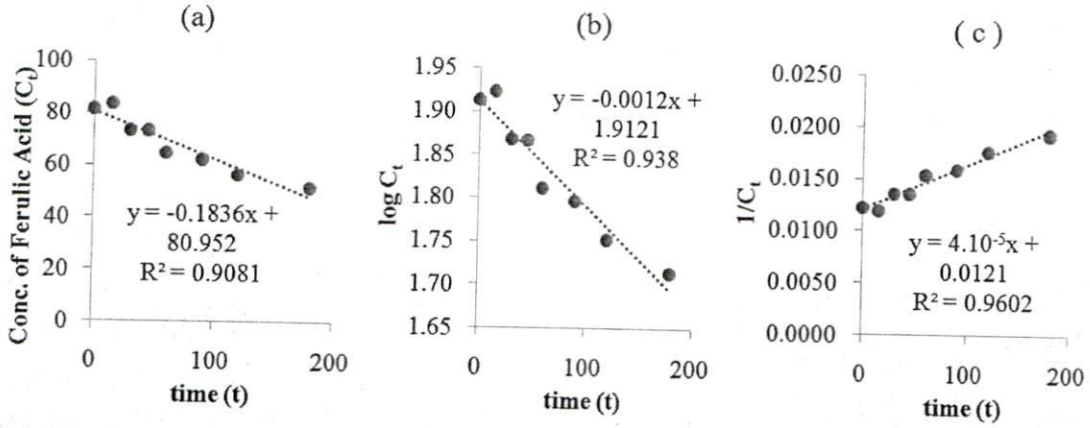


Figure 4. (a) Plot of ferulic acid concentration (ppm) (C_t) vs time in minutes (t) at 55 °C (b) plot of log C_t vs time in minutes (t) at 55 °C (c) Plot of $1/C_t$ vs time in minutes (t) at 55 °C.

Data in Fig 4 and Fig 5 (above) showed that the greatest R^2 value was obtained from the plot of $1/C_t$ vs. time (t). This results showed that the Fischer esterification reaction of ferulic acid to methyl ferulate followed the second-order which has an equation 4.

$$\frac{1}{C_t}=\frac{1}{C_0}+kt \qquad (4)$$

Notes :

C_t = the remaining concentration of the reactant (ferulic acid at time= t minutes)

C_o = initial concentration of reactant (ferulic acid at time= 0 minute)

k = rate constant

t = time of reaction

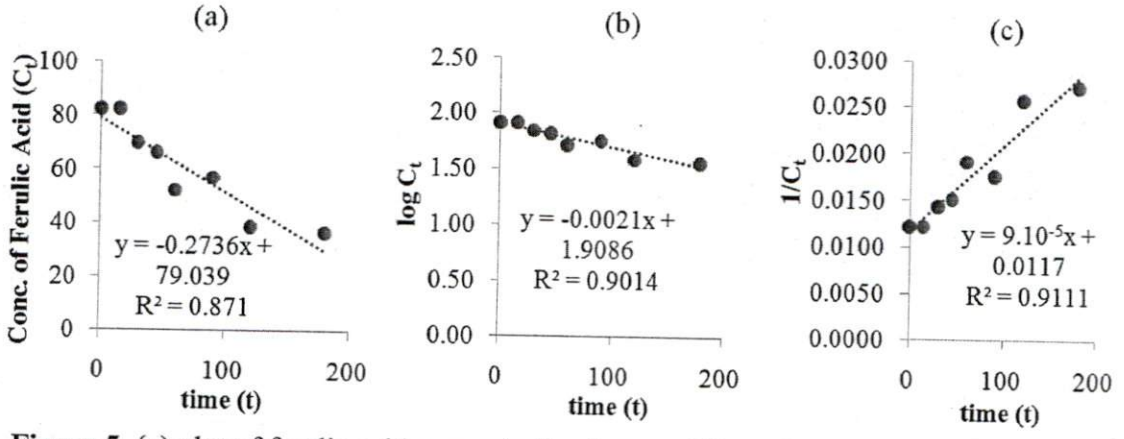


Figure 5. (a) plot of ferulic acid concentration in ppm (C_t) vs time in minutes (t) at 65 °C (b) plot of log C_t vs time in minutes (t) at 65 °C (c) Graph of Plot $1/C_t$ vs time in minutes (t) at of 65 °C.

The equation of second-order reaction (equation 4) means that if the ferulic acid concentration increased twice, then the rate of esterification reaction will rise up 4-fold or increase 2 times exponentially. The changes in reaction conditions such as temperature, solvent or initial compound concentrations will affect the rate constant (k) for the reaction. Results of the rate constant (k) for these experiments are in accordance with the mentioned theory.

Determination of Rate Constant (k) and Energy of Activation (E_a)

After determining the rate order of the reaction and rate constant (k) for each reactions using equation 4., the activation energy (E_a) from the ferulic acid esterification reaction can also be calculated from the two k value of reactions at 55 °C and 65 °C (Figure 6).

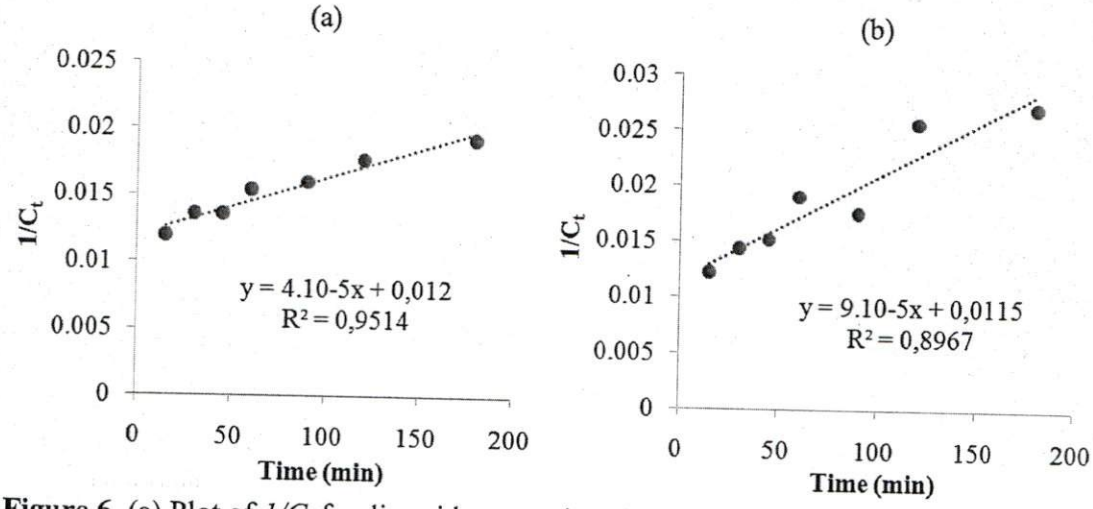


Figure 6. (a) Plot of $1/C_t$ ferulic acid versus time (t) at 55 °C (b) Plot of $1/C_t$ ferulic acid versus time (t) at 65 °C.

The activation energy value (E_a) can be resolved using the Arrhenius equation using data of absolute temperature (T) (equation 2) and rate constant (k) (equation 3). The results of calculation were shown in Table 2.

Table 2. Linear equation and rate constant (k) of both temperatures and activation energy of reaction

Temperature (°C)	Temperature absolute (K)	Line Equation	k (cons^{-1} min^{-1})	Activation Energy (E_a) (kcal/mol)
55 °C	328	$y = 4.10^{-5}x + 0.0120$	4.10^{-5}	17.9
65 °C	338	$y = 9.10^{-5}x + 0.0115$	9.10^{-5}	

The results of data processing denoted that the E_a of the Fischer esterification of ferulic acid was 17.9 kcal/mol. This means that each mole of ferulic acid requires 17.9 kcal energy to carry out an esterification reaction with the help of a catalyst, then the energy needed to react 2.10^{-3} moles of the ferulic acid compound is 0.0358 kcal or equal to 35.8 calories.

Antiplatelet Assay

The result of *in vivo* antiplatelet evaluation of methyl ferulate compared to the negative control, positive control (aspirin) and ferulic acid are shown in Figure 7. Based on the statistical analysis one way ANOVA, it was found that the blood clotting time and bleeding time of the ferulic acid and methyl ferulate respectively were significantly different from negative control (P= 0.000 and 0.002) which means that ferulic acid and methyl ferulate had antiplatelet effects.

According to data in Figure 7, it appears that the change from carboxylic acid to ester group can increases activity 1.6 times more potent based on the blood clotting time, and 1.35 times higher based on bleeding time data. However, the antiplatelet potency of methyl ferulate was same with aspirin according to the capability to prolong clotting time and bleeding time.

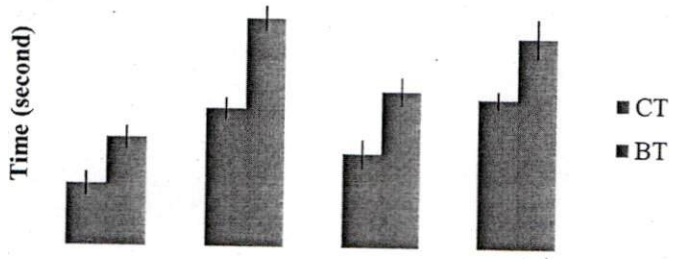


Figure 7. Antiplatelet activity histogram of methyl ferulate (MF), ferulic acid (FA), aspirin (ASP), and control negative (CN). CT = clotting time, BT = bleeding time, (n=5).

The results of docking *in silico* against COX-1 enzyme (PDB ID 4O1Z) indicate that methyl ferulate has greater potency for COX-1 inhibition than ferulic acid. MolDock score of methyl ferulate is 112.223 kcal/mol, while ferulic acid has 106.779 kcal/mol. As observed in the Figure 8 and 9, methyl ferulate and ferulic acid showed the same mode of interaction which involved their same functional groups and the same amino acids, i.e. Ala527 and Met522, in cavity-4 (vol. 76.8; surface 239.36) of COX-1 (X=240.12; Y=99.88; Z=5.09). The possible difference comes from the additional interaction of the hydrophobic and steric interaction of methyl ester moiety with Val349, which are invisible in Figure 9.

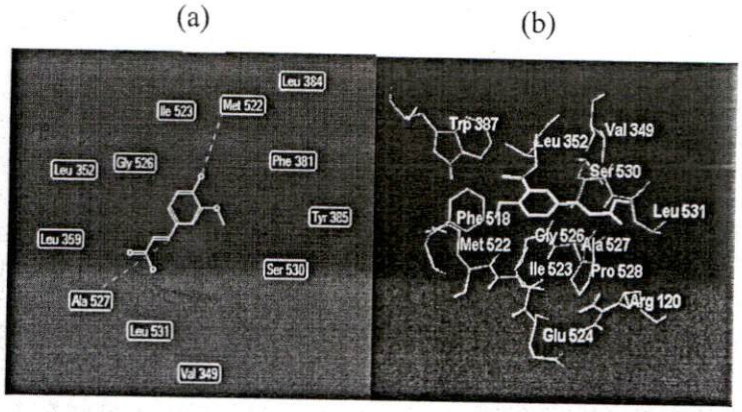


Figure 8. The interaction ferulic acid with amino acids residue of COX-1 in (a) 2D and (b) 3D.

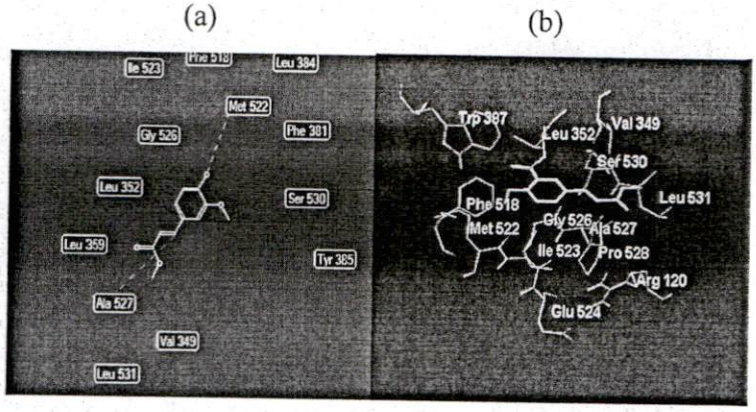


Figure 9. The interaction methyl ferulate with amino acids residue of COX-1 in (a) 2D and (b) 3D.

From *in silico* study on P2Y12 receptors, it was known that MolDock Score of methyl ferulate (-111,142 kcal/mol) was lower than ferulic acid (-100,981 kcal/mol) (Ekowati et al., 2018) which means methyl ferulate was capable of building more strong ligand-receptor interaction than ferulic acid.

The P2Y12 receptor that bound to the Gαi2 protein would encourage platelet activation and aggregation through several intracellular pathways in the downstream receptors. The activation of the Gαi2 protein caused inhibition of adenosine cyclic monophosphate (cAMP) which facilitated platelet activation, by inhibiting cAMP-dependent protein kinase. P2Y12 also strengthened the platelet response by stimulating phosphatidylinositol-3 kinase (PI-3K) activity which leads to continuous aggregation. P2Y12 activated glycoprotein (GP) GIIb/IIIa receptors which in turn bound fibrinogen and connected platelets (Yang et al., 2013; O'Connor et al., 2011). Therefore, if these receptors bind to the test compound then the Gαi2-protein bonding will be inhibited, and further, the activation and aggregation of platelets will be also prevented.

CONCLUSIONS

The temperature effects on ultra sonic assisted synthesis of methyl ferulate are to affect. the reaction rate constant and yield product. The value of reaction rate constant and yield product at temperature of 65 °C are higher than 55 °C. The presence of a methyl group of ester moiety increased the antiplatelet activity. The potency of methyl ferulate as antiplatelet agent was same as aspirin.

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