HYDROGEN
JURNAL KEPENDIDIKAN KIMIA
https://e-journal.undikma.ac.id/index.php/hydrogen/index

June 2025. 13(3)
e-ISSN: 2656-3061
p-ISSN: 2338-6487
pp. 428-437

Alpha Amylase Inhibitory Activity of Curcumin Analogs and Its Synergy with Ferulic Acid in Vitro

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Article History
Received: 15-05-2025
Revised: 27-05-2025
Published: 30-06-2025

Keywords: α-amylase; curcumin analogs; synergistic; ferulic acid

Abstract

Curcumin analog compounds are α,β unsaturated compounds through simplifying the beta diketo group to monoketo, which has better bioavailability and a more stable structure than curcumin compounds. This study aims to determine the α-amylase inhibitory activity on symmetric curcumin analog compounds, namely the compounds 2,6-bis(3,4-dimethoxybenzylidine)cyclohexanone (A) and 2,6-bis(3,4-dimethoxybenzylidine)cyclopentanone (B). as well as testing its synergistic interaction with ferulic acid in vitro. The α-amylase inhibition test was carried out using an iodine reagent and a starch solution as a substrate. The absorbance value was measured using a UV-vis spectrophotometer (λ 568 nm), and the % inhibition was calculated. The average value of the optimum α-amylase inhibition percentage for compounds A, B, and ferulic acid, respectively is 58.17%; 22.95%, and 93.52%. Based on the synergistic interaction, it was concluded that compounds A and B showed synergistic activity with ferulic acid. The percentage of α-amylase inhibition in the concentration ratio of curcumin analog A: ferulic acid (1:8) was 98.65%, and curcumin analog B: ferulic acid (1:4) was 98.37%. This shows that combining compounds between symmetrical curcumin analogs and ferulic acid can increase the activity of antidiabetic drug candidate compounds compared to single compounds. This study offers a new approach by testing the potential combination of curcumin and ferulic acid analogues as α-amylase inhibitors in vitro, demonstrating a synergy that has not been widely explored and opening up opportunities for developing more effective natural antidiabetic therapies.

How to Cite: Nafillah, K., Ariyani, H., & Asfirah, L. (2025). Alpha Amylase Inhibitory Activity of Curcumin Analogs and Its Synergy with Ferulic Acid in Vitro. Hydrogen: Jurnal Kependidikan Kimia, 13(3), 428-437. doi:https://doi.org/10.33394/hjkk.v13i3.15683

https://doi.org/10.33394/hjkk.v13i3.15683s

INTRODUCTION

According to the International Diabetes Federation (IDF), there are 537 million adults, or 1 in 10 people, suffering from diabetes worldwide. In 2021, Indonesia was ranked fifth with 19.47 million people or 10.6% of the people living with total diabetes (Anonymous, 2021). Hyperglycemia is a sign of a chronic disease known as diabetes mellitus (DM). Two types of diabetes mellitus (DM) are type 1 (T1DM) and type 2 (T2DM). T1DM occurs because damage to pancreatic beta cells causes the pancreas not to produce insulin, while the cause of T2DM is low insulin secretion by the pancreas. One of the therapies for controlling T2DM is to reduce glucose absorption by administering oral antidiabetic drugs such as acarbose, miglitol, and voglibose. This can be done by inhibiting the activity of α-amylase and α-glucosidase, thereby delaying glucose absorption and lowering glucose levels. However, these oral antidiabetic drugs have several adverse effects, such as allergies, diarrhea, and acute hepatitis.

Nampoothiri dkk., (2011) research on alternative compounds as OHDs candidates, but derived from nature, such as curcumin compounds. Important components in turmeric (Curcuma longa) have multi-bioactivities such as antidiabetic, antiviral, antifungal, anticancer, and antioxidant. However, curcumin's limited bioavailability and stability have encouraged the development of various analogues. Meanwhile, ferulic acid has shown the ability to increase insulin sensitivity and suppress oxidative stress. Still, it has not been widely explored synergistically with other compounds against enzymatic targets such as α-amylase.

Based on this, many researchers have conducted studies on modifying the curcumin structure to make it more stable, namely curcumin analog compounds by substituting the β-diketone group with monoketone. The activity test of the curcumin analog compound (2E,6E)-2,6-bis(3,4-dimethoxybenzylidine)cyclohexanone has the potential to be an inhibitor of the α-glucosidase enzyme with an inhibition percentage of 88.53% at a concentration of 7.5 mM (Putri, 2016). The compound 3,3-di(indolyl)indolin-2-ones showed higher α-glucosidase enzyme activity than the standard drug acarbose (Santoso dkk., 2022). A candidate drug compound requires other compounds to reduce toxicity and increase activity (Tallarida, 2001). For example, garlic combined with metformin can reduce drug toxicity and significantly lower blood glucose levels (Gupta dkk., 2017).

The combination of apigenin and scutellarein can synergistically increase the inhibitory effect of the α-amylase enzyme (Wang dkk., 2022). Other studies have shown a synergistic effect between quercetin and quinic acid in improving hyperglycemia, hyperlipidemia, and insulin resistance in diabetic rats with maximum inhibition at a combination dose of 50 mg/kg (Arya dkk., 2014). Administering phenolic and ferulic acid to type 2 diabetic rats can reduce blood glucose levels and increase plasma insulin (Jung dkk., 2007). The inhibitory activity of ferulic acid against rat intestinal α-glucosidase and porcine pancreatic α-amylase in vitro shows that ferulic acid is the most potent inhibitor (Adisakwattana dkk., 2009). The primary focus of this study is an effort to increase the activity of candidate compounds of antidiabetic drugs by synergizing with ferulic acid. Ferulic acid has bioactivities such as antioxidants and antiviruses (Kumar & Pruthi, 2014), anticarcinogenic, antidiabetic, cardioprotective, and neuroprotective, and can be combined with other drugs (Ghosh dkk., 2015).

The research gap this study aims to answer is the absence of a comprehensive study evaluating the synergistic potential between curcumin and ferulic acid analogues on α-amylase inhibition. Most previous studies have only focused on the effects of each compound individually, without examining the possibility of interaction or increased activity when combined. A combinatorial approach can produce stronger or more specific inhibitory effects and reduce the effective dose required for each compound. Thus, this study aims to explore in silico the potential of both individually and in combination of curcumin and ferulic acid analogues as α-amylase inhibitors. This approach is expected to provide new insights into the development strategy of natural compound-based antidiabetic therapy with higher efficacy and lower risk of side effects.

METHOD

Tools and Materials

The equipment used is laboratory glassware, analytical balance (Libror EB330 Shimadzu), thermometer, pH meter, and UV-Vis spectrophotometer (Shimadzu UV-1800). The components used include curcumin analog compounds 2,6-bis (3,4-dimethoxybenzylidine) cyclopentanone (A) and 2,6-bis (3,4-dimethoxybenzylidine) cyclopentanone (B), ferulic acid,

α-amylase enzyme, starch (amylum), K_2HPO_4, KH_2PO_4, KI, KCl, NaOH, HCl, iodine (I_2), ethanol (C_2H_5OH) and distilled water.

Determination of Wavelength (λ_{max})

A total of 10 mL of starch solution (0.5 g/L) was added with 0.1 mL of 0.2% (w/v) iodine solution, then the mixture was measured for absorbance value (λ 400-800 nm).

Preparation of Sample Solutions, Quercetin, and Ferulic Acid

Sample solutions of symmetrical curcumin analog compounds A and B, quercetin (positive control), and ferulic acid were carried out with concentrations of 0.063, 0.125, 0.25, 0.5, and 1 mM and prepared 3.94 mg of compound A, 3.80 mg of compound B, 3.02 mg of quercetin, and 1.94 mg of ferulic acid. Each solution was dissolved in 10 mL of ethanol and stirred until the solid dissolved (solution concentration 1 mM). A total of 0.625, 1.25, 2.5, and 5 mL of each solution were mixed with ethanol in a 10 mL volumetric flask so that the concentration changed to 0.063, 0.125, 0.25, 0.5, and 1 mM.

Inhibitory Activity Test of Compounds A and B Against α-amylase

This inhibition activity test used the modified method of (Xiao dkk., 2006), with the composition of the reagents in the reaction system, namely the S_1, S_0, control and blank systems (**Table 1**). 10 mL of 0.5 g/L starch substrate solution and 1 mL of sample solution with various concentrations of 0.063, 0.125, 0.25, 0.5, and 1 mM. A total of 0.5 mL of enzyme solution (20 U/mL) was added and incubated at the optimum time (37 °C, 10 minutes), then 1 mL of 1% HCl and 0.1 mL of 0.2% iodine were added. The absorbance of the solution was measured at λ_{max}.

Table 1. Composition of Reagents in the Reaction System

	Blanko (mL)	**Control (mL)**	**S_0 (mL)**	**S_1 (mL)**
Starch substrate	10	10	10	10
Sample solution	-	-	1	1
Enzyme	-	0.5	-	0.5
		Incubation 37 °C, 10 minutes		
HCl 1%	-	1	-	1
Iodine 0.2%	0.1	0.1	0.1	0.1

Calculation formula for inhibition (%):

$$\%\ inhibition = \frac{(A_{control} - A_{blanko}) - (AS_1 - AS_0)}{(A_{control} - A_{blanko})}\ x\ 100\%$$

Description :
AS_1 : absorption from the sample and the enzyme system (S_1)
AS_0 : absorption from the sample system without enzyme (S_0)
$A_{control}$: absorption from the control system
A_{blanko} : absorption from the blank system

Synergy Test with Ferulic Acid

Symmetrical curcumin analog compounds A and B were tested for synergy with ferulic acid by comparing concentrations of 1:1, 1:2, 1:4, 1:8, 2:1, 4:1, and 8:1.

RESULTS AND DISCUSSION

Wavelength determination (λ_{max})

The formation of the enzyme-substrate (ES) complex produces a purple complex, so the maximum wavelength (λ_{max}) is determined to obtain the highest absorbance. This shows that the ES complex can more accurately absorb photon energy when measuring the absorbance of the solution to UV-Vis light. Theoretically, this starch-iodine complex provides an absorption peak of around 600 nm, while the addition of amylopectin shows an absorption of around 550 nm. In this study, the starch-iodine complex's maximum wavelength (λ_{max}) was 568 nm with an absorbance value of 0.107 (**Figure 1**) (Sakač dkk., 2020).

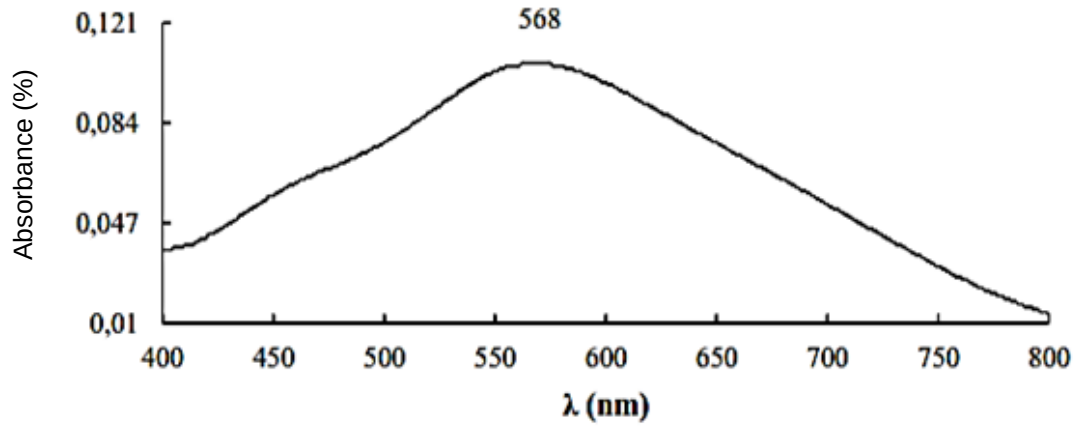


Figure 1. Determination of λ_{max} of starch substrate

α-amylase Enzyme Inhibition Test

The inhibition test of α-amylase is an effort to control type 2 diabetes mellitus (T2DM) by slowing down the hydrolysis of the substrate compound, namely starch (Ponnusamy dkk., 2012). To determine the activity of the α-amylase enzyme, the intensity of the blue color in the starch-iodine reaction is used. Polysaccharides, such as starch, when hydrolyzed, will produce short chains consisting of disaccharides or monosaccharides that cannot form a helical structure and cannot bind iodine. Conversely, polysaccharides (starch) that are not hydrolyzed will react with iodine to form polyiodide chains. The chain can be quantified with a UV-Vis spectrophotometer (λ_{max} 568 nm) because it is a complex compound of starch-iodine with a helical (circular) shape. **Figure 2** shows the process of the α-amylase enzyme inhibition activity test against curcumin analog compounds A and B. Based on the test principle, the higher the α-amylase enzyme activity, the more starch is hydrolyzed, but the less starch can react with iodine, so the absorbance value obtained is lower.

(a) (b)

Figure 2. Inhibition test process of curcumin analogs (a) A and (b) B

Table 2. Inhibitory activity of curcumin analogs A, B, and quercetin

Concentration (mM)	Inhibition (%)		
	A	**B**	Quercetin
0.0625	34.46	15.98	99.52
0.1250	28.95	16.31	90.75
0.2500	38.28	14.38	94.18
0.5000	29.11	21.40	93.51
1.0000	58.17	22.95	99.23

Based on the α-amylase inhibition activity test (Table 2 and Figure 3), it can be seen that compound A shows a trend of increasing inhibition as the concentration increases, with the highest inhibition reaching 58.17% at a concentration of 1.0 mM. Although there are minor fluctuations at low and medium concentrations, compound A generally provides a more significant inhibitory effect than compound B. On the other hand, compound B shows relatively low inhibitory activity and tends to be stable in the range of 14-23%, with no clear increasing trend as the concentration increases. This indicates compound B has a more limited α-amylase inhibitory potential than compound A in the concentration range tested.

Compound A interacts with α-amylase better than compound B, meaning compound A has a higher effect on α-amylase inhibition activity (has the potential as a hypoglycemic compound). This can also be predicted in the curcumin analog compound A, which can bind to the enzyme's active site more stably. The enzyme's active site has aromatic residues containing reactive groups from its amino acids (Ngili, 2013). Compared to curcumin analog compounds A and B, the quercetin compound, or positive control, has higher inhibitory activity. In comparison, quercetin as a positive control compound provides very high inhibitory activity, above 90% at almost all concentrations, with a peak of 99.52% at a concentration of 0.0625 mM. This confirms the effectiveness of quercetin as a very potent α-amylase inhibitor and serves as a standard of comparison for other compounds. The quercetin compound is a flavonoid compound with high antioxidant bioactivity; some researchers state that antioxidant compounds can function as antidiabetic agents. This is because oxidative stress in diabetes mellitus pathology can increase due to the anti-hyperglycemic activity of antioxidants (Annapurna dkk., 2013).

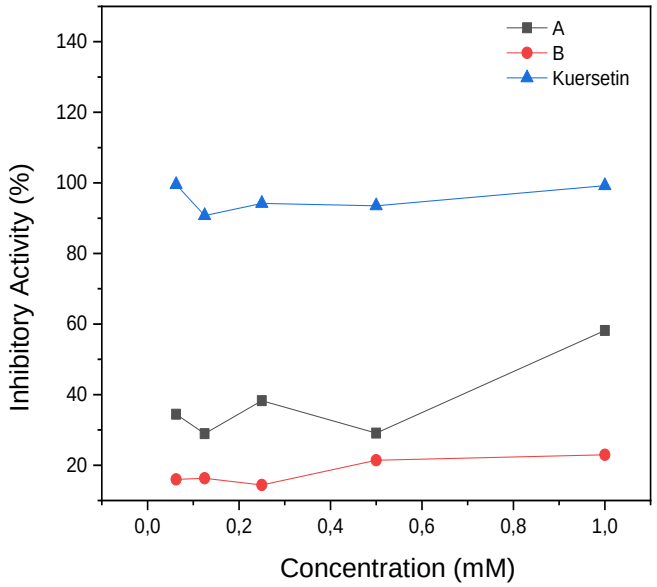


Figure 3. Inhibitory activity of compounds A, B, and quercetin

The data shows that the inhibitor concentration positively correlates with the hydrolyzed starch amount. The higher the inhibitor concentration, the less starch will be hydrolyzed. This is because the inhibitor will stop α-amylase from hydrolyzing starch. Therefore, its inhibitory activity positively correlates with the inhibitor concentration (% inhibition) (Bhosale dkk., 2024).

Synergy test with ferulic acid

Compounds A, B, and AF have optimum inhibition % of 58.17%, 22.95%, and 93.52%, respectively. Furthermore, a test was conducted to determine the effect of the combination of the two samples on the inhibitory activity of α-amylase. The synergy test on symmetrical curcumin analogs and AF was carried out by comparing the concentrations (mM) of 1:1, 1:2, 1:4, 1:8, 2:1, 4:1, and 8:1. Using a reagent consisting of iodine and starch as a substrate, each combination was tested for its inhibitory activity against the α-amylase enzyme. As shown in Table 3, the results of the inhibition calculation were compared with the percentage of inhibition for each sample. The results of the synergy test with ferulic acid are shown in Table 4 and Figure 4.

The data showed that Ferulic Acid had the highest and most consistent inhibitory activity. Starting from 26.42% at the lowest concentration, the effect jumped to 86.26% at 0.1250 mM and continued to increase to 93.52% at 1.0000 mM. This shows a strong response to increasing concentration. Sample A showed a fluctuating pattern. The inhibition was initially high (34.46%), decreased at medium concentration, and then increased significantly to 58.17% at the highest concentration. This means that the inhibitory effect is more pronounced at high doses. Sample B had the lowest and most stable activity, ranging from 14% to 23% without a significant increase. This indicates a weak inhibitory potential, even when the concentration is increased. Overall, Ferulic Acid is the most potential candidate based on its inhibition trend.

Table 3. Inhibitory activity of curcumin analog compounds A, B, and ferulic acid

Concentration (mM)	**Inhibitory Activity (%)**		
	A	**B**	Ferulic Acid
0.0625	34.46	15.98	26.42
0.1250	28.95	16.31	86.26
0.2500	38.28	14.38	87.30
0.5000	29.11	21.40	89.63
1.0000	58.17	22.95	93.52

Table 4. Synergy of samples with ferulic acid

Comparison Concentration (mM)	**Inhibition (%)**	
	A+AF	**B+AF**
1:1	59.29	55.76
1:2	70.92	89.60
1:4	85.41	98.37
1:8	98.65	97.42
2:1	65.20	76.83
4:1	47.18	92.56
8:1	58.43	87.98

Table 4 presents the results of combining Samples A and B with Ferulic Acid (AF) at various ratios. Most of the combinations produced higher inhibitory activity than the single compounds. The principle of synergy means that two compounds combined can create an

effect greater than the sum of their individual effects. This is evident in the combination of B+AF, especially at ratios of 1:4 and 1:8, which achieves inhibition above 97%, significantly higher than B or AF alone. Thus, the combination—especially when accompanied by AF domination—has the potential to produce more substantial biological effects through synergistic mechanisms.

Based on these data, it shows that the highest inhibitory activity in each symmetrical curcumin analog compound is in the comparison of the concentration of compound A: ferulic acid (1:8), which is 98.65%, the highest inhibitory activity among the symmetrical curcumin analog compounds. Meanwhile, compound B: ferulic acid (1:4) is 98.37%. When compared with the individual activity of each inhibitor compound, it shows that higher inhibitory activity is obtained when combined with ferulic acid. This indicates a synergistic effect between the symmetrical curcumin analog compound and ferulic acid as an α-amylase inhibitor.

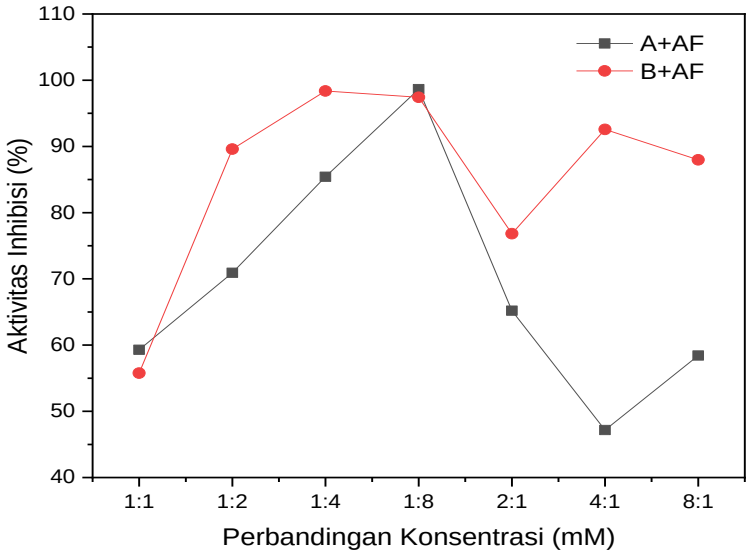
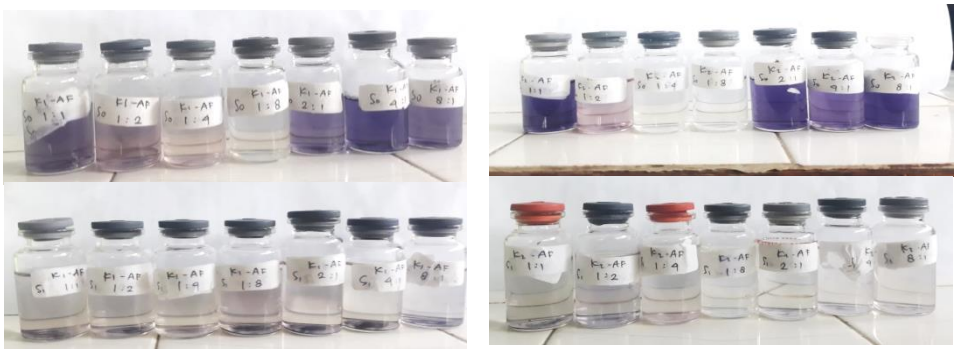


Figure 4. Synergy of compounds A and B with ferulic acid



(a) (b)

Figure 5. Synergy test process of compounds (a) A and (b) B, with ferulic acid

Based on other studies that have been conducted, the synergistic interaction between ferulic acid and hypoglycemic drugs (metformin and THZ) can significantly reduce blood sugar levels in diabetic rats. The combination of oral hypoglycemic drugs can reduce the side effects of the oral drugs. Histological analysis also shows this combination can increase

pancreatic cell regeneration (Prabhakar dkk., 2013). Studies on synergistic interactions with ferulic acid do not show any harmful effects if ferulic acid is used in large concentrations, so it can be a beneficial solution in suppressing the effects of complications from diabetes.

CONCLUSION

The highest percentage of α-amylase inhibition was found in comparing compound A: AF (1:8) concentration, 98.65%, and compound B: AF (1:4), 98.37%. This study proves that compound 2,6-bis(3,4-dimethoxybenzylidine)cyclohexanone (A) and compound 2,6-bis(3,4-dimethoxybenzylidine)cyclopentanone (B) indicated a synergistic effect with ferulic acid. The novelty of this research lies in the identification of a synergistic interaction between these curcumin analogs and ferulic acid, a combination that has not been previously reported for α-amylase inhibition. This provides new insights into the potential development of inhibitors through the synergy of two compounds for the management of type 2 diabetes.

RECOMMENDATIONS

Based on the results of this study, it is necessary to conduct a study to determine the type of inhibitor for AF and curcumin analog compounds against the α-amylase enzyme. In addition, further research is needed on in vivo inhibition tests and computational approaches to see the mechanism of curcumin analog compounds on the active side of the α-amylase enzyme protein molecule.

ACKNOWLEDGEMENTS

The author would like to thank LPDP for funding this research. The author would also like to thank the UGM Organic Chemistry Laboratory.

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