International Journal Of Health, Engineering And Technology (IJHET) *E-ISSN 2829 – 8683*
Volume 4, Number 3, September 2025, *Page. 562 – 579*
Email : editorijhess@gmail.com

Comparison Of Flavonoid Content And A-Amylase Inhibition Activity Of Ethanol Extracts From Compound Garlic (*Allium Sativum* L), Black Garlic, And Single Garlic

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Abstract

Diabetes mellitus is a chronic metabolic disease characterized by elevated blood glucose levels due to impaired insulin metabolism, and it remains one of the leading global health problems. One promising therapeutic strategy for diabetes management is the inhibition of digestive enzymes such as α-amylase, which plays a role in carbohydrate breakdown. Flavonoids, as natural bioactive compounds found in plants, have been reported to act as effective enzyme inhibitors. This study aims to compare the flavonoid content and α-amylase enzyme inhibitory activity of ethanol extracts from compound garlic (Allium sativum L.), black garlic, and single garlic. The extraction process was carried out using maceration with 96% ethanol solvent, followed by flavonoid content determination using UV-Vis spectrophotometry, while α-amylase inhibitory activity was tested using the Fuwa method. The results demonstrated that black garlic extract contained the highest flavonoid concentration, reaching 48.874 mg QE/g, compared to single garlic (40.561 mg QE/g) and compound garlic (35.943 mg QE/g). In addition, black garlic exhibited the strongest α-amylase inhibitory activity with the lowest IC_{50} value of 32.390 ppm, indicating a higher potency compared to the other garlic extracts. These findings suggest that the fermentation process in black garlic enhances its flavonoid profile and bioactivity. Therefore, black garlic has significant potential as a natural antidiabetic agent and could be further explored as a functional food ingredient or herbal therapy for diabetes prevention and management.

Keywords: *Flavonoids, α-amylase, Black Garlic, Garlic, Diabetes*

INTRODUCTION

Diabetes mellitus (DM) is a pathological condition that occurs due to a disorder in the pancreas, causing the body to be unable to produce insulin. This condition is characterized by a random blood glucose (RBG) level of ≥ 200 mg/dL and a fasting blood glucose (FBG) level of ≥ 126 mg/dL (Rika Widianita, 2023). Based on data from the International Diabetes Federation (IDF), there are 537 million people with diabetes worldwide, with Indonesia ranking fifth (Wulandari et al., 2024). This increase in prevalence is a serious health problem that requires global attention (Dinnar, 2022; Ghasemzadeh et al., 2022). One therapeutic approach that can be used to manage diabetes is through the inhibition of digestive enzymes, particularly the enzyme α-amylase, which plays a role in breaking down complex carbohydrates into glucose (Dinnar, 2022). Compounds from natural sources, such as flavonoids, have been shown to have α-amylase inhibitory activity, making them potential candidates for antidiabetic treatment (Wulandari et al., 2024).

One plant that is rich in flavonoid compounds is garlic (*Allium sativum L.*). This plant has long been used as a kitchen spice and also as a traditional medicine for various diseases, including diabetes (Wicaksono et al., 2021). The active compounds in garlic, such as allicin and S-allylcysteine (SAC), have been shown to have various pharmacological effects, including antidiabetic and antioxidant properties (Pramitha & Sundari, 2020; Al-Snafi, 2021). Single garlic, or male garlic, is considered to have greater benefits due to its higher allicin content compared to compound garlic (Pramitha & Yani, 2020). In addition, the fermentation process of garlic into *black garlic* can increase the stability and bioavailability of active compounds, such as SAC, which is formed from allicin during heating (Pramitha & Yani, 2020). Studies show that black garlic has strong potential in lowering blood glucose levels (Yuliastri et al., 2020; Kusuma et al., 2022; Amagase et al., 2021).

Comparison of the Antidiabetic Effects of Garlic and Black Garlic

Although garlic is widely known as an antidiabetic agent, there is still uncertainty regarding the comparative effectiveness of compound garlic, single garlic, and black garlic in inhibiting the α-amylase enzyme. Previous studies have shown that black garlic can lower blood sugar levels in diabetic rats (Yuliastri et al., 2020) and has a strong EC50 value in glucose inhibition (Kusuma et al., 2022). However, these studies did not directly compare the antidiabetic activities of the three types of garlic in parallel. The differences in active compound content, particularly flavonoids and allicin, among the three—where single garlic has higher allicin and black garlic has more stable SAC—suggest potential differences in effectiveness (Pramitha & Yani, 2020).

The main issue to be addressed in this study is to determine quantitatively and qualitatively the differences in the antidiabetic potential of these three types of garlic. Although it is known that flavonoids have the ability to bind glucose (Kusuma et al., 2022), there has been no comprehensive comparison of the total flavonoid content in ethanol extracts from each type of garlic. Therefore, this study will measure the total flavonoid content to provide a scientific basis for the antidiabetic activity being tested. In addition, there has been no study that specifically compares the α-amylase enzyme inhibition activity of ethanol extracts of compound garlic, black garlic, and single garlic simultaneously, which is important to determine which is the most effective as a natural antidiabetic agent (Pramitha & Yani, 2020; Jaiswal et al., 2022).

Objectives, Urgency, and Novelty of the Research

Based on the problems identified, this study has several main objectives. First, to measure the total flavonoid content in ethanol extracts of compound garlic (*Allium sativum L.*), black garlic, and single garlic. Second, to evaluate the α-amylase enzyme inhibition activity of the three extracts as antidiabetic agents. This research is urgent given the high prevalence of diabetes in Indonesia and the growing public interest in herbal medicine as a more natural alternative with minimal side effects. The novelty of this research lies in the direct and comprehensive comparison of the three types of garlic, which is expected to provide strong scientific evidence regarding which is the most promising for development as an antidiabetic agent. The results of this study are expected to form the basis for the development of new phytopharmaceutical products that are effective and safe for helping to manage diabetes mellitus (Rika Widianita, 2023; Wulandari et al., 2024; Jaiswal et al., 2022).

RESEARCH METHODS

Research Type and Methods

This study used an experimental method with a quantitative approach, which aimed to scientifically compare the flavonoid content and α-amylase enzyme inhibition activity of three types of garlic extracts. The experimental method allows researchers to manipulate independent variables, namely the type of extract (compound garlic ethanol extract, black garlic, and single garlic), to observe their impact on dependent variables, namely flavonoid levels and α-amylase inhibition activity (Cresswell, 2021; Sudaryono, 2022). This approach was chosen to provide strong and measurable evidence regarding the differences in antidiabetic potential of the three types of samples. The entire research series was conducted in the controlled environment of the Chemistry Laboratory of the Pharmacy Undergraduate Program, Duta Bangsa University Surakarta, to ensure the precision and validity of the data.

Research Population and Samples

The population in this study was all garlic plants (*Allium sativum L.*). The specific samples used were compound garlic bulbs, single garlic bulbs, and black garlic. Compound garlic and single garlic were obtained directly from Nglebak Village, Karanganyar Regency, Central Java Province, with coordinates 7°39'42.8" S and 111°06'48.1" E. This location was chosen based on the availability and quality of garlic that met the research criteria. Meanwhile, black garlic is the result of fermenting compound garlic independently in the laboratory. These three samples will be processed into simplisia and then extracted using 96% ethanol solvent before further testing. The selection of representative samples is crucial to ensure that the research results can be generalized (Sugiyono, 2021).

Research Procedure

The research procedure was divided into several systematic stages. The initial stage was sampling and plant determination, which aimed to ensure the authenticity of the garlic species. This determination process was carried out at the Functional Implementation Unit (UPF) for Traditional Services, Tawangmangu, Karanganyar. Next, *black garlic* was produced through controlled fermentation for 21 days using *a rice cooker* in *keep warm* mode*,* following a previously validated method (Pramitha & Yani, 2020). After that, the three types of samples (compound garlic, single garlic, and black garlic) were processed into uniform dried simplisia through a drying and sieving process. The next crucial stage was the standardization of simplisia and extracts, which included determining the drying shrinkage, moisture content, and ash content to ensure the quality of the raw materials (Indonesian Ministry of Health, 2017).

The process continued with the production of ethanol extracts from the three crude drugs using the maceration method. The maceration method was chosen for its effectiveness in extracting active compounds, especially flavonoids, using 96% ethanol solvent (Chotimah, 2019). The yield of the concentrated extract obtained was then calculated. After that, qualitative phytochemical screening was carried out to identify the presence of bioactive compounds such as flavonoids, alkaloids, tannins, and triterpenoids. This test was carried out using the test tube method and Thin Layer Chromatography (TLC), which have been proven to be reliable in identifying compounds (Kusuma et al., 2022).

Instruments and Data Analysis Techniques

This study used various laboratory instruments to ensure the accuracy of the results. The main instruments used included UV-Vis spectrophotometry for determining total flavonoid content and testing α-amylase inhibition activity (Aminah et al., 2017; Rika Widianita, 2023). Other tools such as a rotary evaporator were used to concentrate the extract, while an analytical balance ensured precise sample weighing. The data analysis techniques used included several calculations, such as linear regression equations to determine total flavonoid content based on the quercetin standard curve and calculation of the α-amylase enzyme inhibition value, which referred to a validated method (Wulandari et al., 2024). IC50 analysis (the concentration required to inhibit 50% of enzyme activity) will also be calculated from a linear regression equation to quantitatively measure the inhibitory potential of each extract (Kusuma et al., 2022). This data analysis procedure allows for an objective and valid comparison between the three types of garlic extracts studied (Emzir, 2021).

RESULTS AND DISCUSSION

Sample Collection and Plant Determination

The samples used in this study were compound garlic and single garlic plants obtained from Nglebak Village, Karanganyar Regency, Central Java. The part of the plant used in the study was the garlic bulb. The compound garlic and single garlic plants were identified by identifying all parts of the plant at the Functional Implementation Unit (UPF) for Traditional Services in Tawangmangu, Karanganyar, Central Java. The identification process was carried out to ensure that the plants used were indeed compound garlic and single garlic with the Latin name *Allium sativum* L (Appendix 1). The results of the identification showed that the plant samples were compound garlic and single garlic, which belong to the *Amarylidaceae* family.

Black Garlic Production

In this study, the process of making black garlic began with selecting fresh, high-quality compound garlic, which was then fermented for 21 days (Pramitha & Yani, 2020) . A total of 1000 grams of compound garlic was used, which was then cleaned without peeling the outer skin. After cleaning, the compound garlic is placed in *a rice cooker* that has been lined with several sheets of tissue paper so that the garlic does not come into direct contact with the hot plate *of the rice cooker.* The heat in *the rice cooker* was set to the *keep warm* mode at a temperature between 60–80°C and was opened occasionally to check whether the garlic had turned black. At a temperature of 60-80°C, during the initial processing period of 7 days, the color of the compound garlic skin changed from white to pale brown and its texture became chewy. During the middle period of processing, which lasted 14 days, the color of the garlic samples changed from light brown to dark brown, and the texture became chewy. Finally, during the final period of processing, which lasted 21– , the color of the garlic samples changed from dark brown to black, and the texture became slightly hard and soft. The results of *black garlic* from garlic bulbs can be seen in Figure 4.1.

(a) (b) (c)

Figure 4. (a) Compound *Black Garlic* Result of Fermentation for 7 days (b) Compound *Black Garlic* Result of Fermentation for 14 days and (c) Compound *Black Garlic* Result of Fermentation for 21 days

The heating process is carried out to remove the water content in garlic, thereby reducing its pungent taste and aroma so that it can be consumed directly. According to research by (Zhang *et* al., 2016) , which states that reducing water content using high temperatures is faster than using low temperatures, the optimal heating temperature for black garlic is between 60°C and 70°C, resulting in a dry structure and an increase in reducing sugar content. The increase in reducing sugars in black garlic can affect color changes in the sample. This process takes place over 21 days or 3 weeks. During the fermentation process, garlic undergoes a color change to blackish brown due to a non-enzymatic reaction called the Maillard reaction. Based on research conducted by (Zhang *et* al., 2016) , at a temperature of 60-70°C for 35 days, the color of the garlic sample changed to a uniform blackish brown, and the reducing sugar content in the sample showed that the formation of reducing sugar was faster than the rate of consumption, so that the very high accumulation of reducing sugar produced an abundant sweet taste.

Based on the results of *black garlic* that has been fermented for 21 days, compound garlic heated using *a rice cooker* undergoes physical changes in the form of changes in texture and color. Before heating, compound garlic has a sharp odor, a hard texture, and a white color. According to research (, Bae *et* al, 2015) , garlic heated at 65°C undergoes a color change to blackish brown, becomes dry, has a slightly harder texture than garlic, and has a slightly bitter aroma. The change in garlic characteristics to black garlic is influenced by several factors, one of which is the heating temperature of 65°C and the duration of heating. Garlic heated for 21 days will produce a dark brown to black color, a slightly bitter taste, and a hard texture compared to garlic heated for 15-20 days, which has a light brown color and a soft texture. The color change in garlic is caused by the Maillard reaction. The Maillard reaction causes the garlic to turn brown. The longer the heating process, the more intense the brown color caused by the Maillard reaction will be, making the garlic appear black (Sailah & Miladulhaq, 2021) . The Maillard reaction is the main reaction between reducing sugars and amino components, the speed of which increases with increasing temperature and decreasing water content (Avianti, 2020) .

Preparation of Simplisia

Compound garlic bulbs and single garlic bulbs were collected and wet sorted, then weighed, yielding a total of 1000 grams. The weighed garlic bulbs were then washed, chopped, and dried. The drying process was carried out using the method of drying under the sun. After drying, the garlic bulbs were weighed and the results were 250 grams of compound garlic simplisia, 201 grams of single garlic simplisia, and 500 grams of black garlic simplisia. The results of drying garlic bulbs and black garlic (*Allium sativum* L) can be seen in Table 3.

Table 3. Calculation Results of the Percentage of Compound Garlic, Single Garlic, and Black Garlic Simplisia.

Simplisia	Wet Weight	Dry Weight	Percentage (%)
Compound garlic	1000 g	250 g	25
Single garlic	1000 g	201 g	20.1
Black garlic	1000 g	500 g	50

Based on Table 3, it can be seen that 1000 grams of dried garlic and black onion bulbs yielded 250 grams of compound garlic, 201 grams of single garlic, and 500 grams of black onion. The percentages of compound garlic, single garlic, and black garlic were then calculated relative to the initial weight of the wet simplisia and obtained as 25%, 20.1%, and 50%. Next, the simplisia was powdered and sieved using a mesh sieve no. 40.

Table 4. Calculation Results of Simplisia Powder Percentage Against Compound Garlic Simplisia, Single Garlic, and Black Garlic .

Simplisia	Dry weight	Powder Weight	Percentage (%)
Compound garlic	250 g	183.97 g	73
Single garlic	201 g	186 g	92.5
Black garlic	500 g	100 g	20

Based on Table 4, it can be seen that the percentage of powder from compound garlic, single garlic, and black garlic is 73%, 92.5%, and 20%, respectively.

Ethyl alcohol extracts of compound garlic bulbs, single garlic bulbs, and black garlic bulbs were prepared using the maceration method. The maceration method was chosen because the procedure and equipment used are simple and do not involve heating, allowing many compounds to be extracted. The maceration process was carried out by weighing 183.97 grams of compound garlic, 186 grams of single garlic, and 100 grams of black garlic. These were then soaked in 1300 ml of 96% ethanol (1:7) for 3 days, stirring occasionally. Maceration was carried out by soaking the maceration residue in 600 ml of water for 2 days with 2 repetitions. Ethanol 96% was used for maceration because ethanol 96% is a polar compound, so the polar flavonoids contained in garlic will dissolve in ethanol 96%.

The maceration and remaceration filtrates were then concentrated using *a rotary evaporator* and evaporated using *a water bath*. From 183.97 grams, 186 grams, and 100 grams of compound garlic, single garlic, and black garlic simplisia powder, 9.68 grams, 20 grams, and 10 grams of concentrated extract were obtained. The results of the ethanol extract yield calculations for compound garlic, single garlic, and black garlic can be seen in Table 5.

Table 5 Yield of Ethanol Extracts from Compound Garlic, Single Garlic, and Black Garlic (*Allium sativum* L.)

Simplisia	Simplisia Powder (grams)	Concentrated Extract (g)	Extract Yield (%)
Compound garlic	183.97 g	9.68 g	5.26
Single garlic	186 g	20 g	10.75%
Black onion	100 g	10 g	10

Based on the calculation of the ethanol extract yield of compound garlic, single garlic, and black garlic, it can be seen that the ethanol extract produced was 9.68 grams, 20 grams, and 10 grams with a yield of 5.26%, 10.75%, and 10% from 183.97 grams, 186 grams, and 100 grams of crude drug powder. This has met the requirements for garlic extract yield, which is less than 26% (Indonesian Ministry of Health, 2017) .

Standardization of Simplisia and Garlic Extract

In this parameter, non-specific parameters are standardized. Non-specific parameters include the determination of chemical, microbiological, and physical aspects that will ensure quality so that consumer safety and product stability can be guaranteed.

1. **Determination of Drying Loss of Simplisia**

   The determination of drying shrinkage is carried out to provide a range or limit on the amount of compound lost during the drying process. The drying shrinkage parameter is basically a measurement of the residue of the material after drying at a temperature of 105°C to a constant weight, expressed as a percentage of the weight before drying (. The results of determining the drying shrinkage of compound garlic, single garlic, and black garlic powder can be seen in Table 6.

Table 6. Results of determining the drying shrinkage of compound garlic, single garlic, and black garlic powder .

Sample	Experiment	Powder weight (g)	Drying loss (%)	Average
Compound onion	Replication 1	2 grams	3.04%	3.37%
	Replication 2	2 grams	3.28	
	Replication 3	2 grams	3.79	
	Replication 1	2 grams	2.83	2.89

Single onion	Replication 2	2 grams	1.84	
	Replication 3	2 grams	4.01%	
Black onion	Replication 1	2 grams	4.66	
	Replication 2	2 grams	2.87%	3.62
	Replication 3	2 grams	3.34	

Based on the results of determining the drying shrinkage of the crude drug, it can be seen that the drying shrinkage of the compound garlic, single garlic, and black garlic crude drugs are 3.37%, 2.89%, and 3.62%, respectively. The drying shrinkage obtained has met the requirements for garlic crude drugs, which is <10% (Indonesian Ministry of Health, 2017) .

2. Moisture Content of Simplisia and Extracts

Moisture content is a standardization aimed at determining the water residue after the evaporation process of the filtrate from maceration into a thick extract. The moisture content parameter is the amount of water contained in a simplisia or extract using *a moisture analyzer* (Indonesian Ministry of Health, 2017) . The results of determining the moisture content of compound garlic simplisia, single garlic, and black garlic can be seen in Table 7.

Table 7. Results of Moisture Content Determination of Compound Garlic, Single Garlic, and Black Garlic .

Sample	Initial weight (g)	Moisture content (%)			Average (%)
		Replication 1	Replication 2	Replication 3	
Compound onion	2 g	5.93%	5.18	5.67	5.59
Single onion	2 g	7.81%	5.47	5.58	6.28
Black garlic	2 g	7.34	6.02%	6.41	6.59

Based on the results of determining the moisture content of the simplisia obtained, it can be seen that the moisture content of the compound garlic, single garlic, and black garlic simplisia are 5.59%, 6.28%, and 6.59%, respectively. The moisture content obtained has met the requirements for garlic simplisia moisture content, which is <10% (Indonesian Ministry of Health, 2017) .

Table 8. Results of Moisture Content Determination of Ethanol Extracts of Compound Garlic, Single Garlic, and Black Garlic

Sample	Initial weight (g)	Final weight (g)	Moisture content (%)
Compound Garlic Ethanol Extract	2 g	1.843 g	8.58
Single onion ethanol extract	2 g	1.851 g	8.81
Black onion ethanol extract	2 g	1.887 g	6.81

Based on the results of determining the moisture content of the extract obtained in Table 8, it can be seen that the moisture content of the compound garlic extract, single garlic extract, and black garlic extract are 8.58%, 8.81%, and 6.81%, respectively. The moisture content obtained has met the requirements for garlic extract moisture content, which is <12% (Indonesian Ministry of Health, 2017) .

3. Ash Content of Simplisia and Extract

Weigh 2 g of the crude drug and extract and place them in a porcelain crucible. The crucible containing the extract sample is then placed in an oven and heated at 105°C for 4 hours. The crucible is weighed and replicated three times, then the percentage is calculated. Calculate the ash content of the material that has been dried in the air. The acceptable requirements for the ash content of simplisia are no more than 3% and the ash content of garlic ethanol extract is no more than 2.7% (Indonesian Ministry of Health, 2017) . The results of determining the ash content of compound garlic simplisia, single garlic, and black garlic can be seen in Table 9.

Table 9. Results of Ash Content Determination of Compound Garlic, Single Garlic, and Black Garlic

Sample	Experiment	Powder weight (g)	Residual ash (g)	Total Ash Content (%)	Average

Compound onion	Replication 1	2 grams	0.044	2.2	2.2
	Replication 2	2 grams	0.045	2.3	
	Replication 3	2 grams	0.042	2.1	
Single onion	Replication 1	2 grams	0.047	2.4	2.4
	Replication 2	2 grams	0.049	2.5	
	Replication 3	2 grams	0.045	2.3	
Black onion	Replication 1	2 grams	0.054	2.7	2.8
	Replication 2	2 grams	0.056	2.8	
	Replication 3	2 grams	0.058	2.9	

Based on the results of determining the ash content of the crude drug, it can be seen that the ash content of the crude drugs of compound garlic, single garlic, and black garlic are 2.2%, 2.4%, and 2.8%, respectively. The ash content of the crude drugs obtained has met the requirements for garlic crude drugs, which is < 3% (Indonesian Ministry of Health, 2017) .

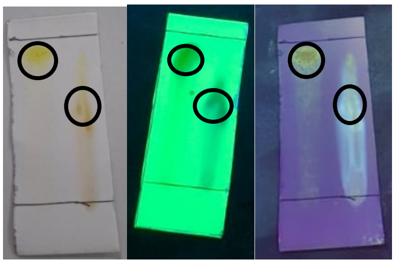
Table 10. Results of Ash Content Determination of Ethanol Extracts of Compound Garlic, Single Garlic, and Black Garlic

Sample	Experiment	Extract weight (g)	Residual ash (g)	Total Ash Content (%)	Average
Compound onion	Replication 1	2 grams	0.034	1.7%	1.8
	Replication 2	2 grams	0.035	1.8	
	Replication 3	2 grams	0.038	1.9	
Single onion	Replication 1	2 grams	0.046	2.3	2.2
	Replication 2	2 grams	0.043	2.2	
	Replication 3	2 grams	0.042	2.1	
Black Garlic	Replication 1	2 grams	0.039	1.95	2
	Replication 2	2 grams	0.041	2	
	Replication 3	2 grams	0.040	2.05	

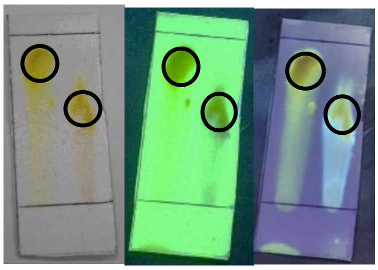
Based on the ash content determination results obtained in Table 10, it can be seen that the ash content of the ethanol extracts of compound garlic, single garlic, and black garlic are 1.8%, 2.2%, and 2%, respectively. The ash content of the extract obtained meets the requirements for garlic ethanol extract because the ash content of the extract obtained is < 2.7% (Indonesian Ministry of Health, 2017) .

Phytochemical Screening

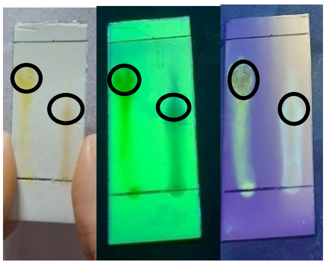
Phytochemical screening is one method used to determine the presence of secondary metabolites in a sample. Some of the compounds examined include flavonoids, alkaloids, saponins, tannins, and steroids/triterpenoids. Phytochemical screening in this study was conducted qualitatively using test tube and thin layer chromatography (TLC) methods. Phytochemical screening using thin layer chromatography (TLC) was performed to identify the presence of flavonoid compounds in ethanol extracts of compound garlic, single garlic, and black garlic using quercetin as a reference. Compound separation was performed using a 2x5 cm silica gel 60 F254 stationary phase with ethyl acetate and methanol p.a. as the mobile phase in a ratio of 7:3. The results were analyzed visually under visible light and UV light at 254 nm and 366 nm. The results of phytochemical testing of ethanol extracts of compound garlic ( ), single garlic, and black garlic using thin-layer chromatography (TLC) can be seen in Table 11 and Figure 5.



(a)



(b)



(c)

Figure 5. KLT results of phytochemical screening (a) observation of flavonoids in compound onions under UV light at 254 nm and 366 nm after spraying with $AlCl_3$ (b) Observation of flavonoids in single garlic under UV light at 254 nm and 366 nm after spraying with $AlCl_3$ (c) Observation of flavonoids in black garlic under UV light at 254 nm and 366 nm after spraying with $AlCl_3$.

Table 11. Results of TLC Test of Ethanol Extracts of Compound Garlic, Single Garlic, and Black Garlic

Figure	Sample	Mobile phase	Phytochemical compounds	Reagent	Rf value	Description
1	Quercetin	Ethyl acetate:Methanol p.a (7:3)	Flavonoid	$AlCl_3$	0,85	Positive for flavonoids, yellow spots become more visible after spraying with $AlCl_3$
	Compound onion extract				0,63	
2	Quercetin				0,77	
	Single onion extract				0,66	
3	Quercetin				0,68	
	Black onion extract				0,57	

Based on the TLC test results, all three extracts contain flavonoid compounds, but with varying intensities and similarities. All three extracts produce yellow spots in positions almost parallel to quercetin. These spots indicate the possible presence of flavonoid compounds in each extract. The yellow color that appears is a positive indication of the presence of flavonoids, which is consistent with the color characteristics of quercetin as a reference compound.

The position of the quercetin spot tends to be higher than that of the extract spot. This occurs because quercetin is a pure compound that moves faster on the TLC plate, while the extract contains a mixture of compounds such as proteins, sugars, and other phenolic compounds that can inhibit the mobility of flavonoids during separation. In addition, the flavonoid content in the extract is relatively lower than pure quercetin, so the intensity and distance of the spots are also smaller.

In both the compound and single garlic extracts, the yellow spots appear more intense and are relatively close to quercetin, indicating that both likely contain flavonoids with a structure and polarity similar to quercetin. Meanwhile, in black garlic extracts, the spots appear dimmer and somewhat faint. This may be due to the fermentation process during black garlic production, which has the potential to cause degradation of flavonoid compounds or transformation into other compounds that are less chromatographically active.

The results of phytochemical screening tests of ethanol extracts of compound garlic, single garlic, and black garlic using the tube test (reagent) method can be seen in Table 12.

Table 12. Results of Phytochemical Screening Tests of Ethanol Extracts of Compound Garlic, Single Garlic, and Black Garlic

Sample	Compound	Reagent	Positive sign	Results	Note
Compound onion	Alkaloid	Mayer	Presence of white/yellow precipitate	Formation of white precipitate	+
		Dragendorff	Presence of orange/brownish-red precipitate	Formation of a reddish-brown precipitate	+
		Wagner	Presence of blackish brown precipitate	Formation of a blackish brown precipitate	+
	Flavonoids	Powdered magnesium and concentrated hydrochloric acid	Color change to red, yellow, or orange	Color becomes yellow	+
	Saponin	Distilled water + 1N HCl	Stable foam is formed	Stable foam is formed	+
	Tannin	$FeCl_3$ 1%	Dark green/blue color formed	Forms a blue-black color	+
	Steroid/triterpenoid	Liebermen Burchard	Brown ring formed	No ring formed	-
Single onion	Alkaloid	Mayer	Presence of white/yellow precipitate	Formation of white	+

				precipitate	
		Dragendorff	Orange/brown-red precipitate	Formation of a brown-red precipitate	+
		Wagner	Presence of a dark brown/ -colored precipitate	No precipitate	-
	Flavonoids	Powder and concentrated HCl	Color change to red, yellow, or orange	Color becomes yellow	+
	Tannin	$FeCl_3$ 1%	Dark green/blue color formed	Formation of a blackish blue color	+
	Saponin	Distilled water + HCl 1N	Stable foam formed	Foam formed	+
	Steroid/triterpenoid	Liebermann-Burchard	Brown ring formed	No ring formed	-
Black onion	Alkaloid	Mayer	Presence of white/yellow deposits	No white/yellow deposits	-
		Dragendorff	The presence of orange/brownish-red deposits	There are reddish-brown deposits	+
		Wagner	Presence of blackish brown deposits	There is a blackish brown deposit	+
	Flavonoids	Powder and concentrated HCl	Color change to red, yellow, or orange	Color becomes red	+
	Tannin	$FeCl_3$ 1%	Dark green/blue color formed	Formation of a blackish blue color	+
	Saponin	Distilled water + HCl 1N	Stable foam formed	Foam present	+
	Steroid/triterpenoid	Liebermann-Burchard	Brown ring formed	No ring formed	-

Based on the results of phytochemical screening tests on ethanol extracts of compound garlic, single garlic, and black garlic using tube tests, it can be seen that the phytochemical compounds contained in garlic ethanol extracts include flavonoids, alkaloids, saponins, and tannins. The phytochemical compound content is similar to that found in studies conducted by (Agustina et al., 2020) , which obtained results in phytochemical tests containing ethanol extracts of garlic and black garlic, namely flavonoids, alkaloids, saponins, and tannins.

Alkaloid testing was performed using Mayer, Dragendorff, and Wagner reagents. Positive alkaloid results were indicated by the formation of a white precipitate for Mayer reagent, a reddish-brown precipitate for Dragendorff reagent, and a blackish-brown precipitate for Wagner reagent. Flavonoid testing was performed by adding mg powder and concentrated HCl solution to the test sample. Positive flavonoid results were indicated by a yellow or red color change. The addition of mg powder and HCl served to reduce the benzopyrome nucleus, thereby forming red or yellow flavilium salts.

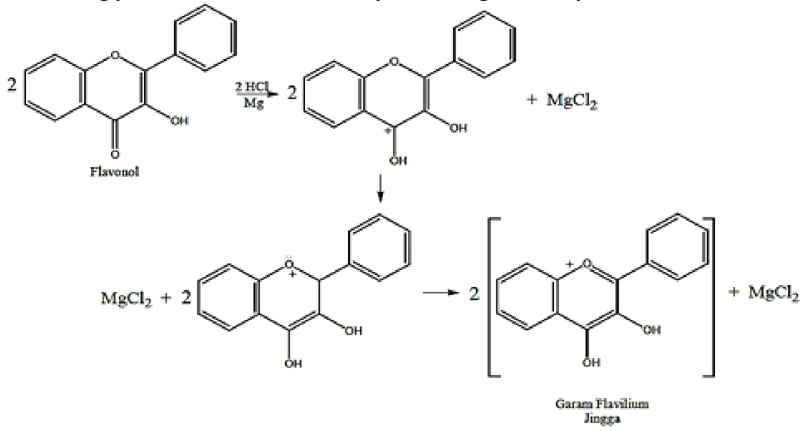


Figure 6 Flavonoid Reaction with Mg+HCl (Ergina *et al*., 2015)

Saponin testing was conducted using distilled water and 2N HCl. Positive results were indicated by the presence of stable foam that did not disappear after the addition of HCl. Foam can form due to the chemical properties of saponin, which is easily soluble in water and produces foam when shaken. Tannin testing was conducted using 1% $FeCl_3$ reagent. The $FeCl_3$ solution is used to determine the presence or absence of phenol groups in the test sample. Tannins (polyphenols) react with Fe^{3+} ions to form a colored complex. Positive results are indicated by the formation of a blue-black color. Steroid testing was performed using the Liebarmann-Burchard reagent. Positive steroid results are indicated by the formation of a brownish ring. However, in this test, the negative results contained steroids. This may be because the ethanol solvent used is a polar solvent, so it tends to extract polar to semi-polar compounds such as flavonoids and phenolics, while steroid and triterpenoid compounds are more non-polar and tend to be more soluble in non-polar solvents such as n-hexane or chloroform. Based on a literature review, garlic is better known as a source of organosulfur compounds (such as *allicin* and *ajoene)*, flavonoids, and phenolic compounds, but not as a major source of steroid or triterpenoid compounds. This is consistent with previous studies that also showed no detection of steroids/triterpenoids in garlic. In the study "*et* al., 2022) , ethanol extracts of black garlic bulbs (*Allium sativum* L.) were found to contain flavonoids, polyphenols, and saponins, which may have antidiabetic potential .

Comparison of Flavonoid Levels in

In this study, the flavonoid content of compound garlic, single garlic, and black garlic ethanol extracts was compared using UV-vis spectrophotometry. The total flavonoid content was compared using a colorimetric method based on the principle of complex formation, resulting in a shift in wavelength toward the visible range, indicated by the solution producing a more yellow color. Qualitative flavonoid extract testing on compound garlic, single garlic, and black garlic was conducted to determine the presence or absence of flavonoid compounds in the extracts. Testing flavonoids with $AlCl_3$ will produce a yellow color if flavonoids are present. This occurs due to the formation of a complex compound between flavonoids and $AlCl_3$ (Marpaung & Wahyuni, 2018).

Before comparing the flavonoid content in ethanol extracts of compound garlic, single garlic, and black garlic, the maximum wavelength, *operating time*, and standard curve for quercetin were first determined. The purpose of determining the maximum wavelength was to determine the measurement wavelength at which the complex between quercetin and $AlCl_3$ provided optimum absorbance at a wavelength of 400-500 nm ( ). The results of the maximum wavelength determination can be seen in Figure 7.

International Journal Of Health, Engineering And Technology (IJHET) E-ISSN 2829 – 8683
Volume 4, Number 3, September 2025, *Page. 562 – 579*
Email : editorijhess@gmail.com

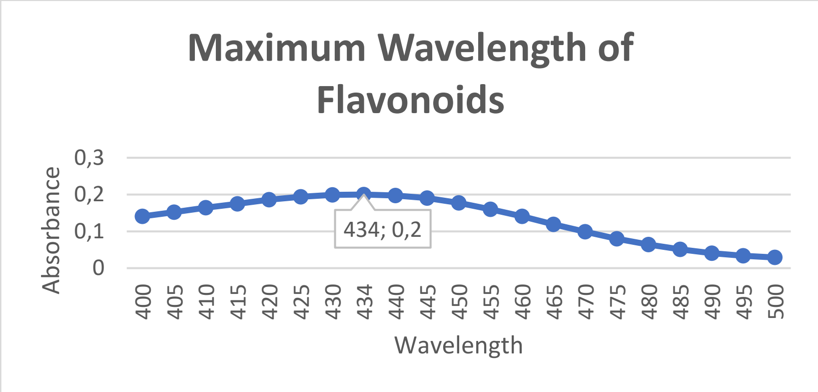


Figure 7 Maximum Wavelength Results in Graph Form

Based on the results of the maximum wavelength determination, it can be seen that the maximum wavelength obtained is 435 nm. The maximum wavelength obtained is then used to determine *the operating time*. The determination *of the operating time* aims to find out the measurement time of the compound obtained when the absorbance is most stable so that this measurement time can minimize errors during measurement. The results of the *operating time* determination can be seen in Figure 8.

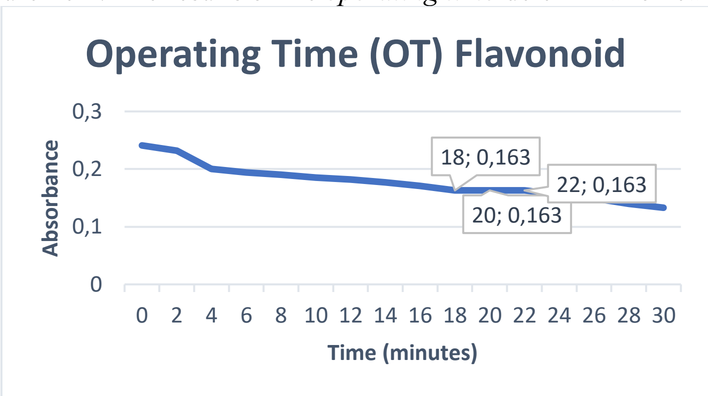


Figure 8. Graph of Flavonoid *Operating Time* (OT) Results

Based on *the operating time* determined from the wavelength of 434 nm, it can be seen that the stable absorbance value is at 20 minutes. This indicates that at 20 minutes, the flavonoid compound has finished reacting with the $AlCl_3$ reagent, as indicated by a stable absorbance reading of 0.163.

The determination of the standard curve for quercetin is used as a standard in determining the content of flavonoids that have a keto group on the C-4 atom and also hydroxyl groups on the C-3 and C-5 atoms, which are adjacent to each other. The determination of the standard curve is related to Labeert-Beer's law. If the standard curve forms a straight line, then Labeert-Beer's law is fulfilled. The results of the determination of the standard curve for quercetin can be seen in Figure 9.

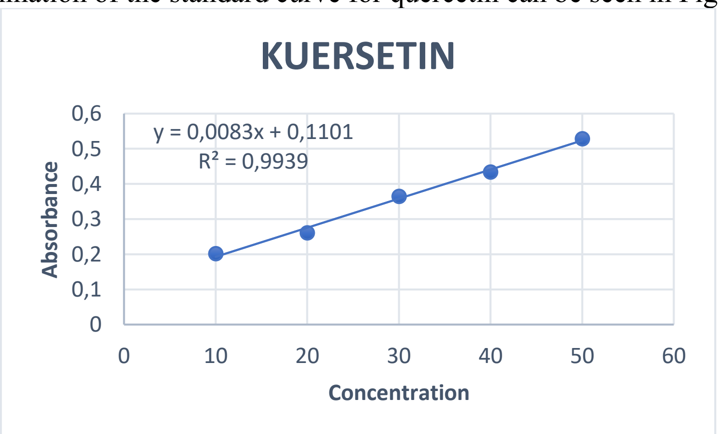


Figure 9. Graph of the Results of the Determination of the Standard Curve for Quercetin

Based on the measurement results obtained, it can be seen that the higher the concentration (ppm), the greater the absorbance produced, as shown in Figure 9. In determining the standard curve of quercetin, the equation y = 0.0083x + 0.1101 was obtained with a correlation coefficient of 0.9939, which was used to calculate the flavonoid content in samples of compound garlic ethanol extract, single garlic, and black garlic. (y) represents the absorbance value of the sample and (x) represents the flavonoid content in the sample. The correlation coefficient (R) value was 0.9939. An R value close to 1 indicates a linear calibration curve and a relationship between the concentration of the quercetin solution and the absorbance value.

The flavonoid content was compared in ethanol extracts of compound garlic, single garlic, and black garlic at a concentration of 1000 ppm. The flavonoid content in the samples is expressed in mg QE/g sample, which is the equivalent value of quercetin in each gram of sample. The results of the comparison of total flavonoid content in ethanol extracts of compound garlic, single garlic, and black garlic can be seen in Table 13.

Table 13. Comparison of Flavonoid Content in Ethanol Extracts of Compound Garlic, Single Garlic, and Black Garlic.

Sample	Replication	Absorbance	Total Flavonoid Content (mg QE/g)	± e Mean SD
Compound onion	I	0.309	35.902	35.943 ± 0,184
	II	0.311	36,143	
	III	0.308	35.782	
Single onion	I	0.351	40,963	40,561 ± 0,364
	II	0.345	40,240	
	III	0.347	40.481	
Black onion	I	0.441	48,192	48,874 ± 0,182
	II	0.419	49,155	
	III	0.420	49.276	

Based on the results of the comparison of total flavonoid content obtained, it can be seen that black onion ethanol extract has a higher flavonoid content of 48.874 mg QE/g compared to single white onion, which has a total flavonoid content of 40.561 mg QE/g, and compound white onion, which has a total flavonoid content of 35.943 mg QE/g. single garlic at 40.561 mg QE/g and black garlic at 48.874 mg QE/g. These results are in accordance with the research by Sukrasno *et al.* (2017), which found that single garlic contains higher flavonoids than compound garlic, due to the growth of a single bulb without division. Additionally, according to (Zhang *et* al., 2016) , high-temperature fermentation at 60-80°C can cause an increase in flavonoid content due to the formation of new compounds resulting from the Maillard reaction.

Alpha Amylase Enzyme Inhibition Test Results

The alpha amylase enzyme inhibition activity test was conducted in vitro. This test was performed to determine the alpha amylase enzyme inhibition activity by observing the intensity of the blue color in the iodine-starch complex due to the reduction of the starch substrate as a result of hydrolysis carried out by the alpha amylase enzyme into mososaccharides (glucose) that do not react with iodine.

The color intensity is measured using UV-Vis spectrophotometry. Measurements are taken on blanks, blank controls, samples, and sample controls. Blank and blank control tests are conducted to determine the activity of alpha amylase in converting starch to glucose. Tests are also conducted on sample controls, which act as a correction factor for the absorbance caused by alpha amylase enzyme activity. The test samples consisted of acarbose, ethanol extracts of compound onions, single onions, and black onions.

Acarbose was used as a comparison because it works as an inhibitor of pancreatic alpha-amylase and membrane-associated intestinal alpha-glucosidase hydrolase. Acarbose slows down glucose absorption, resulting in a decrease in postprandial blood glucose concentration. Starch was used in this study as a substrate for the alpha-amylase enzyme. HCl solution was used to stop the enzymatic reaction because pH is a factor that affects enzyme activity and stability in an enzymatic reaction involving the release or absorption of protons. A pH 6.9 phosphate buffer solution was used to maintain the pH of the alpha-amylase enzyme so that during the process, the enzyme reaction continued to work optimally. Iodine reagent was used as a color indicator because starch reacts with iodine reagent to form a blackish blue or purple color.

Before testing, the maximum wavelength must be measured. The purpose of determining the maximum wavelength is to find out the absorption region under optimal conditions resulting from the absorbance value measured using UV-Vis spectrophotometry. Optimization was carried out in the wavelength range of 400-650 nm, and a maximum wavelength of 582 nm was obtained with an absorbance value of 0.187. The results of determining the maximum wavelength of the alpha-amylase enzyme can be seen in Figure 10.

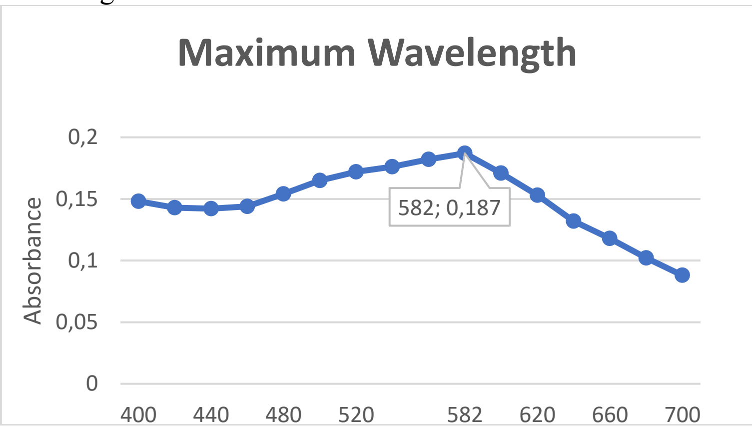


Figure 10 Graph of Maximum Wavelength Measurement of Alpha Amylase Enzyme

After measuring the maximum wavelength, *the operating time* (OT) was determined. *Operating time* is the measurement time taken to determine the stable measurement time. *Operating time* was determined by measuring the absorbance at the previously determined maximum wavelength of 582 nm. The *operating time* measurement results were obtained at 16-18 minutes. *The operating time* (OT) measurement graph can be seen in Figure 11.

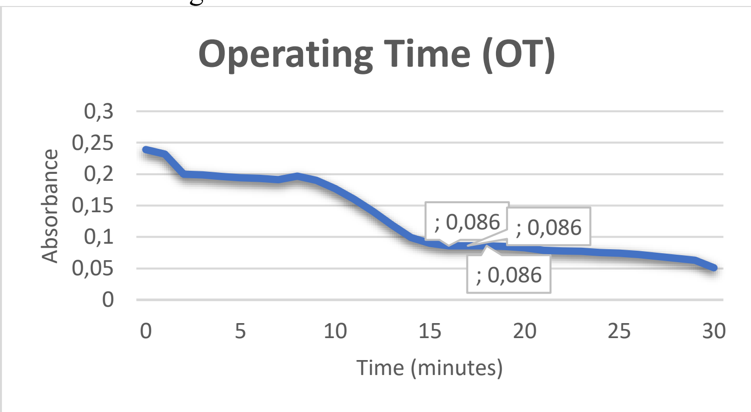


Figure 11. *Operating Time* (OT) Graph

Enzyme alpha-amylase inhibition tests were conducted on samples of acarbose, ethanol extracts of compound onions, single onions, and black onions. Acarbose, used as a comparison, was divided into five concentrations: 0.625 ppm, 1.25 ppm, 2.5 ppm, 5 ppm, and 10 ppm. Meanwhile, the concentrations of the sample extracts used were 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm. There was a difference in concentration between acarbose and the samples because acarbose is an antidiabetic drug with a known mechanism of action as an alpha-amylase enzyme inhibitor, which slows down the breakdown of carbohydrates into sugar, so that blood sugar levels do not rise dramatically. Meanwhile, the phytochemical compounds in the samples that are effective in inhibiting alpha-amylase enzyme activity are not yet known. Therefore, the concentration variation between acarbose as a reference and the samples was made different. All test solutions were measured for their absorption using UV-Vis spectrophotometry to obtain the absorbance value. The absorbance value was then used to calculate the inhibition percentage and IC_{50} value. The sample measurement results can be seen in Table 14.

Table 14. Results of Alpha Amylase Enzyme Activity Test

Sample	Concentration	Sample	Control Sample	(S-K)	% Inhibition	IC_{50}	SD
Acarbose	0.625	0.216	0.020	0.196	30.378	3.553	0.047
	1.25	0.197	0.024	0.173	38.534		

	2.5	0.175	0.026	0.150	46,927		
	5	0.131	0.028	0.103	63,357		
	10	0.086	0.027	0.060	78,842		
Compound Onion Extract	10	0.322	0.071	0.251	11.035	39.447	0.144
	20	0.273	0.073	0.200	29.053		
	30	0.242	0.070	0.173	38,718		
	40	0.217	0.074	0.143	49,133		
	50	0.179	0.076	0.104	63,249		
Single Garlic Extract	10	0.295	0.063	0.232	17.597	33.271	0.178
	20	0.247	0.069	0.178	36.854		
	30	0.227	0.071	0.156	44,553		
	40	0.197	0.073	0.124	55,994		
	50	0.146	0.072	0.075	73,562		
Black Garlic Extract	10	0.266	0.043	0.223	20.903	32.390	0.115
	20	0.235	0.049	0.186	34.017		
	30	0.191	0.051	0.141	50,156		
	40	0.175	0.053	0.123	56,506		
	50	0.127	0.052	0.076	73,225		

***(S-K) = Sample – Control sample**

Based on the test results, ethanol extracts of compound garlic, single garlic, and black garlic have activity in inhibiting alpha-amylase enzymes in vitro. The IC_{50} values obtained from acarbose samples, compound garlic ethanol extract, single garlic, and black garlic were 3.553, 39.447, 33.271, and 32.390, respectively. IC_{50} is the concentration of a compound or extract that has 50% inhibitory activity against the alpha-amylase enzyme. IC_{50} is obtained from a linear regression equation that expresses the relationship between the sample concentration as variable x and the % inhibition as variable y. The smaller the IC_{50} value, the better the inhibitory activity of the compound or extract.

Based on the test results, acarbose showed an IC_{50} of 3.553 µg/mL, which means it has very high effectiveness as an α-amylase inhibitor. Among the three extracts, black onion showed the strongest activity with an IC_{50} value of 32.390 µg/mL, followed by single onion at 33.271 µg/mL and compound onion at 39.447 µg/mL. These results indicate that black onion extract has the strongest potential in inhibiting carbohydrate-digesting enzymes. This is because black onion contains high levels of flavonoids. Flavonoids can lower blood glucose levels with their antioxidant properties. Flavonoids are protective against damage to β cells as insulin producers and can increase insulin sensitivity. The correlation results between flavonoids and %inhibition using SPSS can be seen in Figure 12, and the correlation graph between flavonoids and α-amylase enzyme inhibition activity can be seen in Figure 13.

Correlations

		flavonoid	inhibisi
flavonoid	Pearson Correlation	1	-,841
	Sig. (2-tailed)		,364
	N	3	3
inhibisi	Pearson Correlation	-,841	1
	Sig. (2-tailed)	,364	
	N	3	3

Figure 12 . Correlation Results Between Flavonoids and %inhibition with SPSS

Based on the Pearson correlation analysis results shown in the SPSS table, a correlation coefficient value of -0.841 with a significance value of 0.364 was obtained. This correlation value indicates a strong negative relationship between flavonoid content and alpha-amylase enzyme inhibition activity, showing that in general, the higher the flavonoid content, the lower the percentage of alpha-amylase enzyme inhibition. This result is also reinforced by the correlation graph shown in

Figure 13, which shows that the highest flavonoid content is found in black onion extract at 48.874%, but the enzyme inhibition percentage is lower at 32.39% compared to compound onion, which has a lower flavonoid content of 35.943% but produces the highest inhibition percentage of 39.447%.

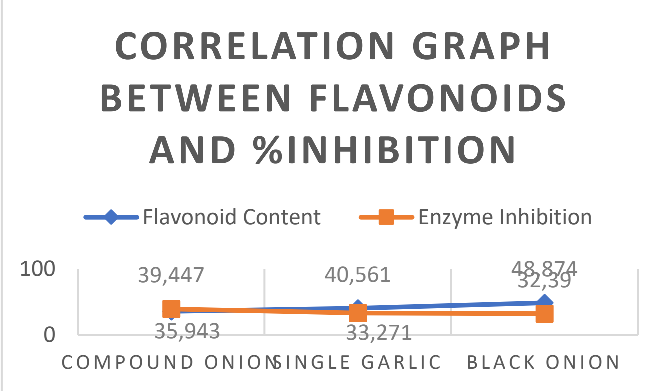


Figure 13. Correlation Graph Between Flavonoids and % Inhibition

Based on the results of the correlation graph between total flavonoid content and α-amylase inhibition activity, it shows that the sample with the highest flavonoid content, namely black onion, has the best inhibition ability (lowest IC_{50} value), and vice versa. The results of the Pearson correlation coefficient also showed a value of $r = -0.841$, indicating a strong negative relationship between flavonoid content and inhibition power, meaning that the higher the flavonoid content, the lower the inhibition. This supports the hypothesis that flavonoid compounds play an important role in the inhibitory activity of this enzyme. Flavonoids such as quercetin are known to have a structure that allows them to interact with the active site of the enzyme, thereby inhibiting the enzyme's activity competitively or non-competitively. Research (Tadera et al., 2015) states that flavonoids can effectively inhibit α-glucosidase and α-amylase, which has positive implications for lowering blood glucose levels.

CONCLUSION

Based on the results of this study, it can be concluded that black onion ethanol extract shows the highest flavonoid content and the strongest α-amylase enzyme inhibition activity with the lowest IC_{50} value of 32.390 ppm, compared to compound garlic and single garlic. This shows that the fermentation process through black onion production is able to increase flavonoid content and strengthen its potential as a natural antidiabetic agent. In addition, there was a strong negative correlation ($r = -0.841$) between flavonoid content and α-amylase enzyme inhibition percentage, which reinforces the important role of flavonoids in the enzyme inhibition mechanism. However, this study has several limitations, such as the use of in vitro methods that have not demonstrated direct effectiveness in human physiological conditions, as well as variations in bioactive compound content influenced by environmental factors and processing. Therefore, further research is recommended to conduct in vivo bioactive activity tests and to examine the effect of the fermentation process on the stability and bioavailability of compounds in black garlic more comprehensively, in order to confirm its therapeutic potential as a safe and effective antidiabetic agent.

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