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Analysis of Phenolic Content, Flavonoids, and α-Amylase Enzyme Inhibition Activity of Ethanol Extracts and Clove Stem Fractions (Syzygium aromaticum L.)

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Abstract

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. The condition is associated with severe long-term complications, including cardiovascular disease, nephropathy, neuropathy, and retinopathy, making effective management strategies crucial. One therapeutic approach is the inhibition of the α-amylase enzyme, which plays a key role in the hydrolysis of complex carbohydrates into glucose, thereby controlling postprandial blood sugar levels. Natural products containing bioactive compounds such as phenolics and flavonoids are increasingly being investigated as safer alternatives to synthetic drugs. Clove stalks (Syzygium aromaticum L.), an underutilized byproduct of the clove plant, are known to be rich in these compounds and may serve as a potential natural enzyme inhibitor. This study aimed to analyze the phenolic and flavonoid content as well as α-amylase inhibitory activity of ethanol extract and its fractions (n-hexane, ethyl acetate, and water) obtained from clove stalks. Extraction was carried out using maceration with 70% ethanol, followed by fractionation and analysis with UV-Vis spectrophotometry. The ethanol extract exhibited the highest phenolic and flavonoid levels (44.581 mg GAE/g and 50.491 mg QE/g, respectively), while the ethyl acetate fraction demonstrated the strongest α-amylase inhibitory activity with an IC_{50} value of 26.303 µg/mL. These findings suggest that the ethyl acetate fraction of clove stalks has promising potential as a natural antidiabetic agent, supporting its development as an alternative functional ingredient in diabetes management.

Keywords: *Clove Stalk, Phenolic, Flavonoid, A-Amylase, Antidiabetic*

INTRODUCTION

Metabolic disorders such as diabetes mellitus are characterized by elevated blood glucose levels arising from impaired insulin production or action, posing high risks for complications when left uncontrolled. One therapeutic strategy for managing type 2 diabetes is inhibition of the enzyme α-amylase, responsible for breaking down complex carbohydrates into simple sugars, thus preventing postprandial glucose spikes. Clinically used drugs like acarbose function as α-amylase/α-glucosidase inhibitors, but their use is often constrained by gastrointestinal side effects such as bloating, diarrhea, and abdominal discomfort (Blahova, et al., 2021; "New Insights into the Latest Advancement in α-Amylase Inhibitors…", 2023). Recent studies also indicate that natural agents rich in phenolic and flavonoid compounds could offer safer alternatives for digestive enzyme inhibition. For example, *Sonchus oleraceus* leaf extract has total phenolic content around 127.66 mg GAE/g and flavonoid content about 74.80 mg QE/g, showing strong antihyperglycemic effects in vivo compared to standard drugs (Salim, Abdel-Alim, Said, & Foda, 2023). Similarly, reviews on α-amylase inhibitors underscore the urgent need for new inhibitors with fewer adverse effects, given the discomfort associated with conventional agents like acarbose (Dietary Natural Products as Inhibitors…, 2024; Recent Advances…, 2025).

One promising natural candidate is clove (*Syzygium aromaticum* L.), known to contain phenolic and flavonoid compounds. Recent investigations show that the flower parts possess strong antioxidant activity, high polyphenolic content, and bioactive compounds such as eugenol which contribute to regulation of oxidative stress and insulin sensitivity (Recent Advances…, 2022; Bioactive Properties of Clove…, 2023). However, research to date has primarily focused on flowers or essential oils, while other

parts, such as clove stems, are less explored despite potentially distinct phytochemical profiles and significant enzyme inhibitory potentials.

Accordingly, this study addresses several research questions: first, what is the total phenolic content in the ethanol extract and fractions of clove stem (*Syzygium aromaticum* L.)? Second, what is the total flavonoid content in those extracts and fractions? Third, do the ethanol extract and stem fractions from clove exhibit α-amylase inhibitory activity relevant to diabetes management? These issues are important because different solvent fractions may vary in bioactivity; quantitative data on phenolics/flavonoids and α-amylase inhibition will help to identify which fraction is most promising.

The aims of this research are to analyze the total phenolic and flavonoid contents of the ethanol extract and stem fractions of *Syzygium aromaticum* L., and to evaluate their α-amylase inhibitory activity as potential natural antidiabetic agents. The urgency of this work stems from the need for safer, natural alternatives in diabetes treatment with minimal side effects, as well as the underutilization of plant parts such as stems. The novelty of this study lies in comparing multiple fractions of the ethanol extract of clove stem for both α-amylase inhibition and phytochemical contents, a relatively underreported area in recent literature.

RESEARCH METHODS

Type of Research and Methodology

This study is a quantitative experimental (laboratory-based) research aimed at measuring and comparing phytochemical content (phenolics and flavonoids) and enzyme inhibition activity (α-amylase) across different extract fractions of clove stem (*Syzygium aromaticum* L.). According to Sugiyono, quantitative research is suitable when the goal is to quantify variables and test hypotheses under controlled conditions. (Sugiyono, 2021) and Creswell & Creswell (2022) argue that experimental methods allow for manipulation of independent variables (e.g., extract and fraction type) and measurement of their effects on dependent variables like enzyme inhibition and compound content.

Instruments and Data Analysis Techniques

The instruments used include UV-Vis spectrophotometer for absorbance measurements, rotary evaporator for solvent evaporation, centrifuge, vortex, hot plate, pH meter, analytic balance, oven, blender, mesh sieve. Standards for quantification include gallic acid (for phenolics), quercetin (for flavonoids), glucose (for the α-amylase inhibition assay). The α-amylase enzyme and buffer solutions are also required reagents. Calibration curves will be constructed (gallic acid, quercetin) according to standard methods. Data analysis will involve calculation of means ± standard deviations, fitting of dose-response curves, linear regression to derive IC_{50} values, and comparison among fractions. Interpretation of IC_{50} values will use criteria similar to those of Oktaviani et al. (2021). Statistical analyses (e.g., ANOVA or t-tests) will be applied to test significant differences among extract fractions. Sugiyono's guidelines on valid and reliable instrumentation and measurement are followed.

Population and Sample

The population for the phytochemical and enzymatic activity analyses is comprised of clove plants (*Syzygium aromaticum* L.). The specific sample is clove stems collected from Desa Gamping, Kecamatan Suruh, Kabupaten Trenggalek, East Java, Indonesia. Approximately 3 kg of clove stems will be harvested from mature buds (flower buds still closed, pink-tinged) in the early morning (07:00-10:00 WIB) to ensure maximal phytochemical preservation, following practices in Mappa et al. (2023). The processed sample will be dried, ground and sieved to uniform particle size (mesh 40) to ensure sample consistency. In terminology of sampling techniques, this is purposive sampling for plant material, with sample size determined by practicality and analytical requirements. Emzir's recommendations on sample preparation and standardization of biological samples are followed. Sudaryono's principles on controlling sample variability (time of harvest, environmental conditions) are implemented.

Research Procedure

1. **Plant Identification (Determinasi)**

   The plant material (*Syzygium aromaticum* L.) will be determined and authenticated at a recognized herbal/traditional medicine unit (e.g., UPF Yankestrad Tawangmangu, RSUP Dr. Sardjito) to ensure species accuracy.
2. **Sample Preparation**

About 3 kg of clove stems are collected as described above. Stems are separated, washed, cut into small pieces, dried under sunlight (approx. 5 days) until moisture is low and brittleness achieved; then ground to powder and sieved (mesh 40), stored in cool, dark conditions.

3. **Standardization of Simplisia**

Tests including moisture content (by drying at 105 °C until constant weight), total ash (incineration at high temperature ~600 ± 25 °C for 3 hours), and determination of extractable yield (rendemen) are carried out. The formulas for moisture loss ("susut pengeringan") and ash content follow standard protocols (Jayani & Handojo, 2021; Utami et al., 2020).

4. **Extraction**

Powdered sample (300 g) is macerated with 70% ethanol (or 96%, according to modified protocol) in a ratio that allows full immersion, for 3 days with periodic stirring. The filtrate is separated, the residue is re-macerated, all filtrates combined, then solvent removed by rotary evaporation at 40-60 °C, then further concentrated if needed via water bath.

5. **Fractionation**

The crude ethanol extract is fractionated sequentially using n-hexane, then ethyl acetate, and the remaining aqueous fraction, employing separatory funnel. Each fraction is collected, solvent removed, and concentrated.

6. **Phytochemical Screening**

Standard qualitative screening tests for flavonoids, alkaloids, tannins, saponins, terpenoids are performed using reagents: $AlCl_3$, $FeCl_3$, Mayer's, Wagner's, Dragendorff's, Liebermann-Burchard, etc.

7. **Determination of Total Phenolic Content**

Using the Folin-Ciocalteu method: sample reagents, gallic acid as standard, measurement of absorbance (e.g., at ~765 nm) after reaction with Folin reagent and Na_2CO_3, incubation for defined time until color stabilization, constructing calibration curve, reporting in mg GAE/g.

8. **Determination of Total Flavonoid Content**

Using aluminium chloride method with quercetin as standard: reaction of sample with $AlCl_3$ and Na acetate, measurement of absorbance (e.g., ~430 nm), calibration curve with quercetin series, calculating mg QE/g.

9. **α-Amylase Inhibition Assay**

Preparation of starch solution (1%), α-amylase enzyme solution in phosphate buffer (pH ~6.9), mixing enzyme with sample or control (acarbose), incubation at 37 °C, adding substrate, stopping reaction with iodine or HCl, measuring absorbance at chosen λmax. Several concentrations of extract/fraction are tested to generate dose response, % inhibition calculated, IC_{50} determined by plotting log concentration vs % inhibition with linear regression as described by Oktaviani et al. (2021).

10. **Data Analysis**

All measurements are done in triplicate. Data are expressed as mean ± standard deviation. Comparisons among fractions for phenolic content, flavonoid content, and α-amylase inhibitory activity are assessed using statistical tests (e.g. one-way ANOVA followed by post hoc tests) at significance level (often $p < 0.05$). Regression analysis used to obtain IC_{50}. Interpretation of IC_{50}: low IC_{50} = strong inhibition, high IC_{50} = weaker inhibition (adapted from Oktaviani et al., 2021).

RESULTS AND DISCUSSION

RESULT

Determinasi Gagang Cengkeh *(Syzygium aromaticum L)*

In this study, a sample of clove *handles (Syzygium aromaticum L) was used.* The purpose of this study was to determine the levels of phenolics, flavonoids and inhibition activity of α-amylase enzymes contained in clove *stalks (Syzygium aromaticum L).* Clove handles were taken from Gamping Village, Suruh District, Trenggalek Regency, East Java.

The determination of clove handles *(Syzygium aromaticum L)* was carried out at (UPF Yankestrad Tawangmangu) Functional Implementation Unit of Tawangmangu Traditional Health Services.

Determination was made to determine the authenticity of the plant samples. Clove stalks *(Syzygium aromaticum L)* that will be used for research to avoid errors and mixing of materials with other plants during sample collection. After determination, the results of the plants used in this study were true clove stalks *(Syzygium aromaticum L).* The results of the determination can be seen in appendix 1.

Sample preparation

In this study, the sample used was clove stalks *(Syzygium aromaticum (L.)* taken from Trengggalek, the sample used weighed 3000g which was then selected a good clove handle and then dried using direct sunlight covered with a black cloth for 5 days. The cover with black cloth aims to protect the active compounds in plants such as phenolics, flavonoids, vitamins and essential oils (World Health Organization, 2019). Then dried simplicia with a weight of 1400 g was obtained with a yield of 46.66%. The simplicia rendition can be seen in table 2 below.

Table 1. Presentation Results of Clove Handle Simplicia Rendemen

Berat Basah (g)	Berat Kering (g)	Nilai Rendemen (%)
3000	1400	46,66%

Clove stalk *(Syzygium aromaticum L.)* that has been dried, then dry sorting is carried out with the aim of separating foreign objects that are still left behind at the time of wet sorting. Then it is refined using a blender. Smoothing is carried out with the aim of minimizing the particles of clove handles *(Syzygium aromaticum L.)* thus making it easier to contact with solvents and refining can take place effectively. The powder is then sifted using a 40 mesh sieve aimed at obtaining a smooth and uniform powder. Because the smaller the simplicia powder, the larger the surface area so that the extraction process in simplicia is more effective and efficient (Syamsul *et al.,* 2020). Clove handle powder *(Syzygium aromaticum L.)* can be found in Table 3 below.

Table 2. Clove Handle Simplisia Powder Weight

Berat Kering (g)	Bobot Serbuk Halus (g)	Rendemen (%)
1.400gram	1.200gram	85,71%

Results of Simplicia Standardization

The clove handle simplicia powder that has been obtained is standardized simplicia such as drying shrinkage test, moisture content test, total ash content test. In this case, the standardization of simplicia aims to include the quality of the simplicia and maintain the stability and consistency of the content of active compounds in simplicia (Courtney, 2017).

1. **Drying Shrinkage Test**

   Weighing 2 g of simplicia is then put in a porcelain cross that has been heated to 105°C for 30 minutes. After that, it is put into the kiln and then weighed. The treatment is repeated several times until the weight is fixed. The determination of the drying shrinkage percentage in simplicia is carried out by means of the initial weight before heating and the final weight after heating. In the Herbal Inodesia II pharmacopoeia in 2017, the requirement for drying shrinkage in simplicia is ≤10% (Courtney, 2017). The results of drying simplicia of clove stalks are shown in the following table:

Table 3. Simplicia Drying Shrinkage Test

Percobaan	Bobot Serbuk (g)	Susut Pengeringan (%)
Replikasi 1	2 gram	6,7
Replikasi 2	2 gram	7,3
Replikasi 3	2 gram	5,7
Rata-rata		6,5%

After being carried out in the drying process of simplicia simplicia *(Syzygium aromaticum L.)* With three experiments, the average result obtained is 6.5%, then in accordance with the good requirements for drying shrinkage is less than 10%. Since the drying shrinkage also represents the evaporated moisture content, the drying shrinkage test results from the three experiments obtained results that meet the requirements of the standard range of drying shrinkage test results (Fadhila *et al.,* 2019).

2. Uji Kadar Air

Determination of simplicia water content using *a moisture balance tool.* The moisture content test on simplicia is carried out with the aim of reducing the moisture content in simplicia to avoid fungal growth. The determination of the moisture content value is very important to provide a maximum limit of water content in a material, because the amount of water is high and can be a medium for the growth of bacteria from fungi In the Indonesian Herbal Pharmacopoeia II in 2017, the drying shrinkage requirement at simplicia is ≤10% (Courtney, 2017). The results of the simplisia simplia test of clove handles are in the following table:

Table 4. Simplisia Water Content Test

Percobaan	Bobot Ekstrak (g)	Kadar Air (%)
Replikasi 1	2 gram	6,81 %
Replikasi 2	2 gram	8,18 %
Replikasi 3	2 gram	10,39 %
Rata-rata		8,46 %

Moisture content testing aims to measure the moisture level in simplicia as well as assess storage quality. High moisture content can lead to microbial growth, decreased material stability, and decreased product quality. In this study, three samples of clove handles, each weighing 2 grams, were analyzed. The results showed a moisture content of 6.81%, 8.18%, and 10.39%, respectively, with an average value of 8.46%.

According to the Indonesian Herbal Pharmacopoeia Edition II (2017, Supplement I 2022), the maximum moisture content limit for common dry simplicia is ≤ 10%, unless otherwise stated in a specific plant simplicia monograph. Based on this reference, the average moisture content of clove handles of 8.46% has met the quality requirements of dried simplisia.

In addition, this data shows that the initial drying process of clove handles is carried out effectively, resulting in a material that is relatively stable and suitable for storage. The consistency of the results between samples also reflects the quality of the raw materials that are homogeneous and ready to be utilized in herbal product formulations or advanced research.

3. Total Ash Level Test

The purpose of testing total ash content is to provide an overview of internal and external mineral content from the initial process to the formation of simplicia (Utami *et al*., 2020). The requirement for the ash content test in simplisia is not more than 7% The results of the simplicia powder ash content test can be seen in the table:

Table 4. Simplicia Total Ash Level Test

Percobaan	Bobot Serbuk (g)	Kadar Abu (%)
Replikasi 1	2 gram	2,8
Replikasi 2	2 gram	2,7
Replikasi 3	2 gram	2,0
Rata-rata		2,5%

The results of the simplicia ash content test resulting from the three experiments produced a value of 2.5% in accordance with the standard ash content parameters, which is no more than 7% (Ministry of Health of the Republic of Indonesia, 2022).

Extract Making Process

The manufacture of clove stalk extract *(Syzygium aromaticum L)* is carried out by the maceration method with 96% ethanol solvent. A total of 300 grams of clove handle powder *(Syzygium aromaticum L)* was extracted with 3000 ml of ethanol. The maceration method was chosen because it is an easy process, does not use high temperatures that may damage the chemical compounds in the clove stalks *(Syzygium aromaticum L),* and is not expensive. 96% ethanol solvent is used because ethanol is a universal solvent that can dissolve polar, semi-polar, and non-polar analytes. Maceration is carried out Tuesday 3 x 24 hours with several stirrings to attract the active substance contained in the clove handle *(Syzygium aromaticum L).* After 3 x 24 hours, filtering is carried out. The maceration filtration residue was then remacerated using 96% ethanol as much as 500 ml for 2 x 24 hours. The results of the remaceration are then filtered and combined with the results of the initial maceration and concentrated using a rotary evaporator at a temperature of 40°C-60°C and heated over a waterbath to obtain a thick extract.

Standardization of Clove Handle Extract

1. **Drying Shrinkage Test**

Weigh 2 g of the extract and then put it in a porcelain cross that has been heated to 105°C for 30 minutes. After that, it is put into the kiln and then weighed. The treatment is repeated three times until the weight is fixed. The determination of the drying shrinkage percentage of the extract is carried out by means of the initial weight before heating and the final weight after heating. In the Inodesia II Herbal Pharmacopoeia in 2017, the requirement for drying shrinkage in the ether is ≤10% (Courtney, 2017). The results of drying clove extract are shown in the following table:

Table 5. Extract Drying Shrinkage Test

Percobaan	Bobot Ekstrak (g)	Susut Pengeringan (%)
Replikasi 1	2 gram	5,8
Replikasi 2	2 gram	5,3
Replikasi 3	2 gram	4,0
Rata-rata		5,0

The determination of drying shrinkage aims to provide the maximum range of compounds lost in the drying process. The drying shrinkage parameter is a non-specific parameter that measures the lack of material residue after drying (Marpaung & Septiyani, 2020). The value obtained from 3 repetitions obtained the average result of shrinkage of drying the extract, which is 5.0%.

2. **Moisture Content Test**

Determination of the moisture content of the extract using the *moisture balance tool.* The moisture content test on the extract is carried out with the aim of reducing the moisture content in the extract to avoid fungal growth. The determination of the moisture content value is very important to provide a maximum limit of water content in a material, because the amount of water is high and can be a medium for the growth of bacteria from fungi In the Indonesian Herbal Pharmacopoeia II in 2017, the drying shrinkage requirement in the extract is ≤10% (Courtney, 2017). The results of the moisture content test for clove handle extract are in the following table:

Table 6. Extract Moisture Content Test

Percobaan	Bobot Serbuk (g)	Kadar Air (%)
Replikasi 1	2 gram	7,32
Replikasi 2	2 gram	7,62
Replikasi 3	2 gram	6,76
Rata-rata		7,27%

Then the results of the moisture content test obtained from the extract of clove stalks *(Syzygium aromaticum (L.)* in the table above through 3 repeated tests of the results obtained are appropriate and stable with the determination of the moisture content requirements that set the concentrated extract to be 5 to 30%. The determination of the moisture content is also related to the purity of the extract. Excessive moisture content (>10%) leads to microbial growth and reduces the stability of the extract (Utami, 2020).

3. Total Ash Level Test

The purpose of testing the total ash content is to provide an overview of internal and external mineral content from the initial process to the formation of the extract (Lestari *et al.*, 2020). The test requirement for the ash content in the extract is not more than 7%. The results of the ash content test of the extract powder can be seen in table 7.

Table 7. Total Ash Extract Test

Percobaan	Bobot Serbuk (g)	Kadar Abu (%)
Replikasi 1	2 gram	4,19
Replikasi 2	2 gram	3,14
Replikasi 3	2 gram	1,04
Rata-rata		2,79%

The results of the total ash content test resulting from the three experiments produced a value of 2.79% in accordance with the standard parameters of total ash content, which is with a value of no more than 7% (Ministry of Health of the Republic of Indonesia, 2022).

Fractionation of Clove Handle Extract *(Syzygium aromaticum L)*

After conducting a phytochemical screening test on clove stalk *extract (Syzygium aromaticum L),* the next stage is the fractionation process. Fractionation is carried out by dissolving 10 g of the extract in 100 ml of water, then stirring until it dissolves completely. The fractionation process uses the liquid-liquid extraction method with the help of a split funnel. The purpose of this fractionation is to separate the group of compounds based on the difference in their polarity level (Melinda *et al.,* 2023) The solvents used in the fractionation of this clove handle extract are water, n-hexane, and ethyl acetate. The first stage of fractionation is carried out by adding 100 ml of n-hexane solvent. After mixing and beating, two layers are formed, the top layer being the n-hexane phase. This process is repeated twice. The n-hexane fraction obtained is then separated and evaporated using a water bath until a concentrated extract is obtained. The second stage of fractionation uses ethyl acetate solvents, which are semi-polar and suitable for dissolving compounds such as flavonoids, tannins, and alkaloids. Since the specific gravity of ethyl acetate is lower than that of the ethanol-water mixture, the ethyl acetate phase will be at the top. The two layers are then separated and fractionation is repeated in the ethanol-water phase twice.

All fractions of the separation result are then separated and heated over a water bath until a thick extract is obtained. The yield results of each fraction can be seen at:

Table 8. Percentage Yield of Clove Handle Fraction Extract *(Syzygium aromaticum L)*

Bobot Ekstrak (g)	Pelarut Fraksi	Bobot Fraksi (g)	Rendemen
10g	Ekstrak Etanol 96%	38,77g	11,75%
10g	Air	21,51g	2,81%
10g	N-Heksan	26,12g	4,77%
10g	Etil Asetat	33,12g	11,37%

Identification of the Chemical Compound Content of Clove Stalks

The purpose of identifying the content of chemical compounds in the sample is to find out the types of compounds contained in clove handle extract (*Syzygium aromaticum L*). This process is known as phytochemical screening, which is a qualitative method used to detect secondary metabolites in the extract. Secondary metabolites tested include flavonoids, alkaloids, tannins, saponins, terpenoids, and

steroids. Phytochemical screening can be done in two ways, namely through test tube tests that observe color changes as an indicator of the presence of compounds, and thin-layer chromatography (KLT) which separates compounds based on polarity and displays color spots on chromatographic plates.

1. **Screening Phytochemistry**

The identification of the content of chemical compounds in the sample aims to find out the type of compound contained in the extract of clove stalks (*Syzygium aromaticum L*). The phytochemical screening process is carried out to qualitatively reveal the secondary metabolites contained in the extract. The secondary metabolites tested included flavonoids, alkaloids, saponins, terpenoids, and tannins. Phytochemical screening can be done by two techniques, namely test tube test (based on color change) and thin layer chromatography (KLT). The test tube test results on clove handle extract showed a discoloration that indicated the presence of these secondary metabolites, so this extract has the potential to be a source of bioactive compounds for further development. The results of the clove handle extract tube test can be seen in table 11.

Table 9. Phytochemical Screening of Clove Stalks *(Syzygium aromaticum L)*

Senyawa	Pereaksi	Hasil Pengamatan	Kesimpulan
Flavonoid	Mg + HCl pekat	Terjadi perubahan warna jingga	(+) (Wahyulianingsih *et al.,* 2019)
Alkaloid	Mayer	Terdapat endapan kuning	(+) (Desi *et al.,* 2023)
	Wagner	Terdapat endapan coklat	(+) (Desi *et al.,* 2023)
	Dragendorff	Terdapat endapan jingga	(+) (Desi *et al.,* 2023)
Tanin	$FeCl_3$ 5%	Terjadi perubahan warna kehitaman	(+) (Pratama et al., 2019)
Saponin	Aquades	Terbentuk busa	(+) (Talahatu & Papilaya, 2018)
Terpenoid	Liberman burchard	Terbentuk cincin berwarna jingga	(+) (Tinggi *et al.*, 2025)

The results of phytochemical tests on clove stem extract showed the presence of flavonoid compounds, alkaloids, tannins, saponins, and terpenoids, while steroid compounds were not detected. Flavonoid detection was characterized by a change in orange color after the addition of magnesium reagents and HCl 2 N. Orange discoloration in flavonoid tests with concentrated Mg and HCl occurred because Mg in an acidic atmosphere reduced the carbonyl group and the conjugated double bond system in the flavonoid nucleus, resulting in the formation of flavilium compounds that have a new chromophore system and produce an orange color (Wahyulianingsih et al., 2019).

Alkaloids give positive results with the appearance of deposits in the three classical reagents, namely Mayer, Wagner, and Dragendorff. In the Mayer test, the reagent used is a mixture of HgCl2 and Kl which will form a potassium tetrajodomercurate (K2[Hgl4]) complex.

When an alkaloid solution is added with a Mayer reagent, the alkaloid that is alkaline will react with the alkaloid-tetrajodomercurate complex ions that are insoluble in water. This reaction causes the formation of yellow deposits as a positive indication of the presence of alkaloid compounds. In the Wagner test, the reagent used was an iodine solution in potassium iodide (l2\Kl). When the Wagner reagent is added to the sample solution containing alkaloids, alkaloids that are alkaline in nature will react with iodine ions to form alkaloids-iodide complex salts that are insoluble in water. This complex will settle. The color of the resulting deposits is generally brown to reddish-brown due to the characteristic nature of the iodine complex formed, the formation of brown deposits in the Wagner test indicates the presence of alkaloid compounds in the sample. In the Dragendorff test, the reagent used was a solution of potassium bismuth iodide (K[Bil4]). This reagent contains bismuth-iodide complex ions that are capable of reacting with alkaloids. When a sample containing alkaloids is added to the Dragendorff reagent, the alkaloids that are alkaline in nature bind to the complex ions and form an alkaloid-bismuth iodide complex salt that is insoluble in water. This complex will settle with a typical orange to brick red color, which is a positive indicator of the presence of alkaloids in the sample. The orange color appears due to the characteristics of the bismuth-alkaloid complex salts that are formed (Desi et al., 2023).

In the tannin test with $FeCl_3$ 5%, Fe^{3+} ions react with phenolic groups (-OH aromatics) in tannins to form a dark iron–phenolic complex. This complex causes a blackish color change, where pyrogalool tannins usually produce a blackish-blue color while catechol tannins produce a blackish-green color (Pratama et al., 2019). Saponins can form foam when shaken with aquades because they have amphipathic properties, which are made up of hydrophilic (water-like) and lipophilic (fat-like) groups in a single molecule. This structure causes saponins to act as natural surfactants that can lower the surface tension of the water. When the solution is shaken, the saponins stabilize the air bubbles that form on the surface of the water so that a long-lasting foam is formed. Therefore, the formation of foam is an indicator of the presence of saponins in the sample (Talahatu & Papilaya, 2018).

In the Liebermann–Burchard test, the reagent used was a mixture of anhydrous acetic acid and concentrated sulfuric acid. When reacting with terpenoid compounds (especially those that have conjugated double bonds or sterol nuclei), these reagents will cause sulfonation reactions and the formation of conjugated carbocations. It is this newly formed conjugated structure that is able to absorb light at a certain wavelength, giving rise to a distinctive color. The color that appears is usually in the form of orange, red, and green rings depending on the type of terpenoids. Thus, the formation of the orange ring in the Liebermann–Burchard test was due to the formation of a conjugated carbocation complex between terpenoids and strong acid reagents (Tinggi et al., 2025).

According to the Indonesian Herbal Pharmacopoeia (FHI Edition II), the identification of secondary metabolite compounds such as flavonoids, alkaloids, and tannins is an important step in the initial screening of herbal ingredients, as these compounds are closely related to pharmacological activities, including as antidiabetics. Flavonoids and tannins are known to have an inhibitory mechanism against the enzyme α-glucosidase and increase insulin sensitivity, while alkaloids also play a role in blood glucose regulation. The content of saponins and terpenoids also supports hypoglycemic activity through antioxidant and protective effects on β-pancreatic cells. With such phytochemical profiles, clove handle extract meets the characteristics of simplicia according to FHI and has the potential to be developed as a candidate for natural antidiabetic agents.

2. Thin-Layer Chromatography (KLT)

The use of the Thin Layer Chromatography (KLT) method in this study aims to determine the profile of secondary metabolite compound content in 96% of clove stem ethanol extract, especially active compounds such as eugenol, flavonoids, and phenolic compounds. The KLT method is used as a separation and initial identification technique (screening) based on differences in the polarity and affinity of compounds to the silent phase (silica gel) and the motion phase (ethyl acetate: n-hexane 7:3), as recommended by the Indonesian Herbal Pharmacopoeia (FHI).

Through the observation of stains under 254 nm and 366 nm UV light, both before and after spraying the visualization agent, KLT provides a qualitative picture of the presence of active compounds in the extract. The Rf values obtained can be compared with the standard literature or pharmacopoeia profile, so that KLT becomes the initial basis for ascertaining the chemical characteristics of clove simplicia, supporting the standardization process, and providing initial validation of the extract's pharmacological potential.

Table 10 KLT Results of Clove Handle *(Syzygium aromaticum L)*

Kondisi Pengamatan	Panjang Gelombang (nm)	Warna Noda	Jarak Migrasi Noda (cm)	Jarak Migrasi Pelarut (cm)	Rf	Keterangan
Sebelum penyemprotan	UV 254	Noda gelap (quenching spot)	2,9	4,0	0,725	Noda gelap mengindikasikan senyawa aromatic seperti eugenol (Mappa *et al.*, 2023)
Setelah penyemprotan	UV 254	Noda gelap lebih kontras	2,9	4,0	0,725	Intensitas meningkat setelah disemprot, menunjukkan reaksi positif visualisasi (Mappa *et al.,* 2023)
Sebelum penyemprotan	UV 366	Noda merah keunguan	2,9	4,0	0,725	Warna tampak menandakan flavonoid atau senyawa fenolik (Mappa *et al.*, 2023)
Setelah penyemprotan	UV 366	Noda merah dan kuning terang	2,9	4,0	0,725	Warna terang menunjukkan keberadaan senyawa aktif seperti flavonoid, senyawa fenolik, eugenol (Mappa *et al.,* 2023)

A Thin Layer Chromatography (KLT) test was performed on 96% ethanol extract from clove stalks to determine the profile of the chemical compound content contained in it. The phase system used is a mixture of ethyl acetate: n-hexane with a ratio of 7:3, as recommended by the Indonesian Herbal Pharmacopoeia (FHI) for the analysis of clove cane extract. The silent phase used is GF254 silica gel plate. Checks were carried out before and after spraying using a stain detection agent, and were observed under UV light of 254 nm and 366 nm.

In the observation before spraying, under 254 nm UV light, there was a single dark colored spot (quenching spot) at a migration distance of about 2.9 cm from the starting line, with a solvent distance of 4.0 cm, so that an Rf value of 0.725 was obtained. The dark color indicates the presence of aromatic compounds or conjugation systems, which are characteristic of eugenol and flavonoid compounds (Wahyulianingsih *et al.,* 2019). On observation under 366 nm UV light, the same stain shows a distinctive purplish-red color, indicating the presence of fluorescent compounds such as flavonoids or phenolic compounds.

After spraying with a reagent (such as vanillin-sulfuric acid), there is a more contrasting stain discoloration below 254 nm UV, and light colors such as red and yellow appear below 366 nm UV, which further confirms the presence of phenolic compounds and essential oils. The Rf value obtained remained consistent at 0.725, which corresponds to the Rf value of the eugenol compound in FHI, and supports that the compound is a major component in clove extract.

Thus, the results of KLT showed that 96% ethanol extract of clove stalks contained active compounds such as eugenol and flavonoids. The stain pattern obtained is in accordance with the characteristics listed in the Indonesian Herbal Pharmacopoeia, so it can be concluded that the extract has an active compound profile that is in accordance with the standard parameters set.

Determination of Phenolic Rate

Determination of total phenolic levels using *the Folin–Ciocalteu method.* This method is the most commonly used method for determining the total phenolic content in plants, considering that it is relatively simple, fast, and accurate. The basic principle of this method is the reaction between phenolic compounds and *Folin–Ciocalteu reagents* in an alkaline atmosphere, which results in a dark blue complex due to oxidation reduction reactions. The intensity of the color formed is proportional to the concentration of phenolic compounds in the sample and can be measured spectrophotometerically.

In this study, the wavelength used was 713 nm, which is the maximum wavelength (λmax) of the reaction complex between the phenolic compounds in clove stem extract and *the Folin–Ciocalteu* reagent. The use of this wavelength provides maximum sensitivity in the detection of phenolic content (Ahmad *et al.,* 2015).

Standard curves are made using a standard solution of gallic acid with stratified concentrations of 10, 20, 30, 40, and 50 ppm. The results of the absorbance measurement resulted in the following linear regression equations:

$$y = 0.0071x + 0.0458 \ (R2 = 0.9915)$$

The value of the determination coefficient (R^2 = 0.9915) indicates that the relationship between the galic acid concentration and the absorbance value is very strong and linear, although not perfect. This still shows that the calibration method used is quite accurate and can be applied to calculate the concentration of phenolic compounds in the sample.

Based on the average absorbance value of clove stem extract samples of 0.390, an equivalent concentration value of gallic acid (GAE) of 32.42 ppm was obtained, which was then converted into a total phenolic content of 4052.5 mg GAE/g extract.

The high phenolic content in clove stem extract is estimated to reach 400–600 mg of GAE/g extract depending on the solvent used (Cortés-Rojas *et al.,* 2019). The phenolic content above shows that this part of the plant has the potential as a source of natural antidiabetic compounds. Phenolic compounds are known as bioactive compounds that have the ability to reduce free radicals, thus playing a role in preventing cell damage due to oxidative stress.

Although the most commonly used part of the clove plant is the flower, the results of this study show that the clove handle, which has tended to be considered as waste, also has a high phytochemical

value. This opens up opportunities for the use of clove stalks as raw materials in the herbal medicine industry, antioxidant supplements, or functional foods. The results of the measurement of the maximum wavelength (λ) of galic acid can be seen in figure 1.

Figure 1. Maximum Wavelength (λ) of Gallic Acid

Table 11. Phenolic Wavelength of Gallic Acid

Wavelength(nm)	Abs
718.0	0.371
717.0	0.372
716.0	0.373
715.0	0.380
714.0	0.375
713.0	0.375
712.0	0.375
711.0	0.375
710.0	0.374
709.0	0.373
708.0	0.372

After obtaining (λ) max, *Operating Time (OT)* is carried out which aims to find out a stable measurement time, namely when the sample reacts perfectly with the reagent and forms complex compounds. *Operating Time* (OT) is carried out using a 50 ppm galic acid solution for 30 minutes. The result of *the Operating Time* (OT) was obtained at 23.5 minutes. The results *of Operating Time* can be seen in table 12.

Table 12. Phenolic Operating Time (OT)

Time(s)	Abs
0.0	0.451
30.0	0.449
60.0	0.450
90.0	0.450
120.0	0.449
150.0	0.449
180.0	0.448
210.0	0.448
240.0	0.448
270.0	0.448
300.0	0.448
330.0	0.448

Based on the measurement results using the UV-Vis spectrophotometer, the determination of the maximum wavelength (λ max) is carried out to determine the wavelength at which the galic acid compound provides the highest absorbance. From the data shown in Table 12, it is known that the wavelength of 714 nm gives the highest absorbance value of 0.380, so the wavelength is chosen as λ max and used for subsequent measurements. After that, *the Operating Time* (OT) is determined which aims to determine the stable measurement time, which is when the reaction between the phenolic compounds in the sample and the reagent has reached stability and forms a complex compound that can be detected optimally (Ahmad *et al.*, 2015). Based on the observations in Table 14, the absorbance value shows a stable tendency ranging from 210 seconds to 330 seconds with an absorbance range between 0.448 to 0.450, so it can be concluded that the optimum time for complex formation occurs at 3.5 minutes or 210 seconds, and this time is used as the ideal measurement time in the spectrophotometric analysis of phenolic levels.

The determination of the standard curve is carried out to determine the relationship between the concentration of the solution and the absorbance value so that the concentration of the sample can be known. In this study, the determination of the raw curve was carried out using a standard solution of quercetin with a concentration series of 10, 20, 30, 40, 50ppm. The measurements produced show that the higher the quercetin concentration, the higher the absorbance value produced, which can be seen in figure 2.

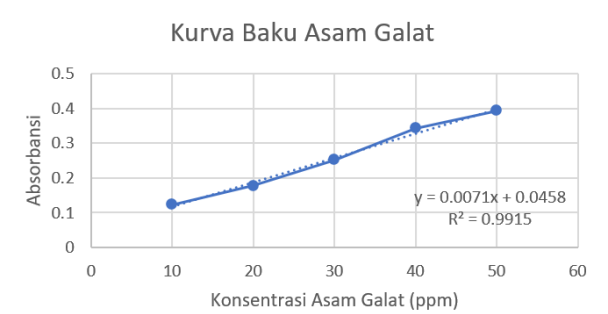


Figure 2. Raw Gallic Acid Curve

Table 13. Phenolic Rate Measurement Results

Ekstrak	Kadar Fenolik (mg QE/g)
Ekstrak etanol	44,535
Fraksi air	33,690
Fraksi etil asetat	42,422
Fraksi n-Heksana	14,112

The measurement of the total phenolic levels in each extract was carried out three times to ensure the validity and reliability of the analysis results. Based on the average of the three measurements, it was obtained that ethanol extract had the highest phenolic content of 44.535 mg QE/g, followed by the ethyl acetate fraction of 42.422 mg QE/g, the water fraction of 33.690 mg QE/g, and the n-hexane fraction with the lowest level, which was 14.112 mg QE/g. The significant difference in phenolic levels between extracts suggests that the type of solvent plays an important role in the extraction process of phenolic compounds. Ethanol and ethyl acetate, which are included in polar and semi-polar solvents, are more effective at extracting phenolic compounds that are also polar, resulting in higher phenolic levels (Desniorita *et al*., 2022). In contrast, the n-hexane fraction as a non-polar solvent has a low extraction ability of phenolic compounds. Three replications were performed to minimize random errors and provide more representative and reliable data. The average values obtained from the three replications also reflect the stability of the measurement method and the accuracy of the tool used.

The high phenolic content of ethanol extract and ethyl acetate fraction suggests that clove stalks have the potential to be a source of bioactive compounds for the development of natural antidiabetic agents. Several phenolic compounds, including galic acid and its derivatives, have been shown to have inhibitory activity against the enzymes α-glucosidase and α-amylase, which play a role in the process of breaking down carbohydrates into glucose in the digestive tract (Pawah *et al*., 2023). By inhibiting these enzymes, phenolic compounds can slow the absorption of glucose into the blood and help control postprandial blood sugar levels. Clove stems, which have been underutilized compared to flower parts, have been shown to contain high amounts of phenolics so that they have the potential to be developed as a natural raw material in type 2 diabetes therapy. These results support the use of clove stalks in phytopharmaceutical formulations or herbal supplements for natural and sustainable glycemia management.

Penetapan Kadar Flavonoid

The determination of flavonoid levels in clove stalk extract *(Syzygium aromaticum L)* was carried out using the UV-Vis spectrophotometry method with quercetin as the raw solution. Quercetin is a flavonoid compound from the flavonol group that has a carbonyl group (keto) in the C-4 carbon atom and a hydroxyl group in the C-3 or C-5 position. The presence of these clusters adjacent to the flavone and flavonol nuclei makes quercetin widely used as a standard in determining flavonoid levels (Sultan M. Almutairi *et al*., 2021).

The selection of quercetin as a raw solution was based not only on its representative chemical structure for the flavonoid group, but also on its ability to form a complex with aluminum chloride ($AlCl_3$), which produces a stable yellow color that can be detected with UV-Vis spectrophotometers. In addition, sodium acetate is used as a shifting reagent which functions to detect the presence of hydroxyl groups at position 7 (7-OH), as well as maintain the stability of absorption wavelengths in the visible spectrum region (Suwarti, 2023).

The initial step in the process of determining flavonoid levels is the determination of the maximum wavelength, optimal working time *(Operating Time),* and the creation of a quercetin standard curve (Nurlinda *et al.,* 2021). The determination of the maximum wavelength aims to obtain the wavelength at which the quercetin-$AlCl_3$ complex provides the highest absorbance value, thus allowing measurements to be made with optimal sensitivity (Suharyanto, 2020).

Observations of maximum wavelengths are made in the range of 400–500 nm. Based on the measurement results, two maximum absorbance peaks were obtained, with the highest peak being at a wavelength of 435 nm and an absorbance value of 0.356. These wavelengths are then used as a basis in the measurement of sample absorbance. The results of the determination of the maximum wavelength are shown in figure 7.

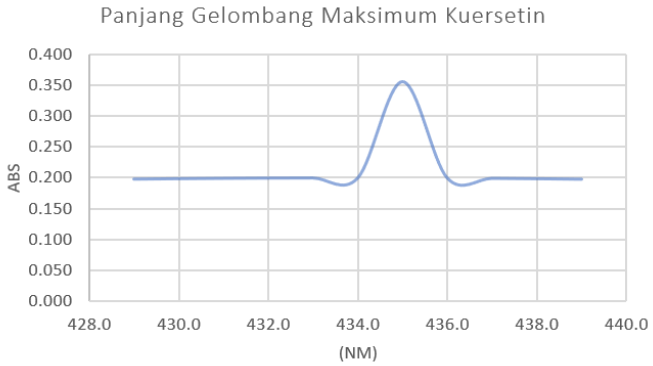


Figure 3. Maximum Wavelength (λ) *of Quercetin*

Table 14. Quercetin Flavonoid Wavelength

Wavelength(nm)	Abs
430.0	0.198
439.0	0.199
438.0	0.199
437.0	0.200

436.0	0.356
435.0	0.200
434.0	0.200
433.0	0.200
432.0	0.199
431.0	0.199
430.0	0.198

After obtaining (λ) max, *Operating Time* (OT) is carried out which aims to determine a stable measurement time, which is when the sample reacts perfectly with the reagent and forms complex compounds. The results *of Operating Time* (OT) can be seen in table 15.

Tabel 15. Operating Time (OT) Flavonoid

Time(s)	Abs
0.0	0.008
30.0	0.009
60.0	0.009
90.0	0.009
120.0	0.009
150.0	0.009
180.0	0.010
210.0	0.011
240.0	0.010
270.0	0.010
300.0	0.010
330.0	0.010
360.0	0.010
390.0	0.010
420.0	0.010
450.0	0.010
480.0	0.010
510.0	0.010
540.0	0.011
570.0	0.011
600.0	0.011
630.0	0.011
660.0	0.011
690.0	0.011
720.0	0.011
750.0	0.011
780.0	0.012
810.0	0.012
840.0	0.012
870.0	0.012
900.0	0.012
930.0	0.012
960.0	0.012
990.0	0.012
1020.0	0.013
1050.0	0.013
1080.0	0.013
1110.0	0.013
1140.0	0.013
1170.0	0.013
1200.0	0.013

1230.0	0.014
1260.0	0.014
1290.0	0.014
1320.0	0.014
1350.0	0.014
1380.0	0.014
1410.0	0.015
1440.0	0.015
1470.0	0.015
1500.0	0.015
1530.0	0.016
1560.0	0.016
1590.0	0.016
1620.0	0.016
1650.0	0.016
1680.0	0.016
1710.0	0.016
1740.0	0.017
1770.0	0.017
1800.0	0.017

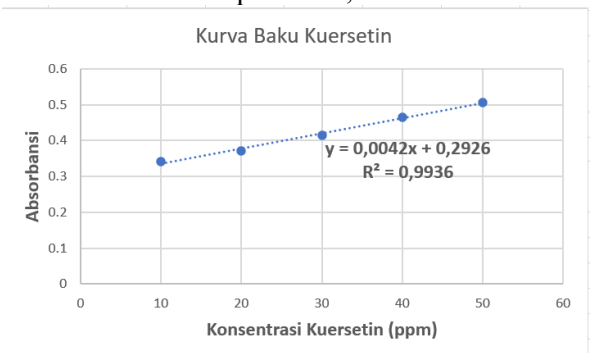
In addition to wavelength, the determination of *Operating Time* (OT) is also important to determine the optimal time for the formation of the quercetin–$AlCl_3$ complex perfectly before absorbance readings. Based on the data in Figure 7 and Table 17, it was obtained that the absorbance value increased gradually from 0 seconds until it reached the maximum point at the 1800th second (30 minutes).

The initial absorbance of 0.008 slowly increased to 0.017 in the 30th minute. This increase suggests that the reaction of the formation of flavonoid complexes takes place gradually. The absorbance value begins to plateau after this time, which indicates that the reaction has reached a *steady state* and the complex has fully formed.

Therefore, the optimum time used as *Operating Time* (OT) is 1800 seconds or 30 minutes. This time is considered the most ideal because it produces a stable and representative maximum absorbance value for quantitative analysis (Khopkar, 2018).

The simultaneous determination of maximum wavelength and *operating time* is important to ensure the reliability and validity of the UV-Vis spectrophotometry method in the analysis of flavonoid content, particularly in clove *stalk extract samples (Syzygium aromaticum L).*

Determination of the standard curve to determine the relationship between the concentration of the solution and the absorbance value so that the concentration of the sample can be known. In this study, the determination of the raw curve was carried out using a standard solution of quercetin with a concentration series of 10, 20, 30, 40, 50 ppm. The measurements produced show that the higher the quercetin concentration, the higher the absorbance value produced, which can be seen in figure 8.



Gambar 4. *Operating Time* (OT) Flavonoid

Table 16. Flavonoid Levels Measurement Results

Ekstrak	Kadar Flavonoid (mg QE/g)
Etanol cengkeh	50,491
Fraksi air	40,253
Fraksi etil asetat	45,650
Fraksi n-heksana	31,237

Figure 4 shows the quercetin standard curve used to determine the total flavonoid levels in the sample. This curve describes the linear relationship between the concentration of quercetin (ppm) and the absorbance value measured using a spectrophotometer at a given wavelength. The regression equation obtained is $y = 0.0042x + 0.2926$ with a coefficient value $R2 = 0.9936$, which shows a very strong and linear relationship between the two variables. Quercetin is used as a standard because it has the ability to form colored complexes with aluminum chloride ($AlCl_3$) reagents, so it can be used in spectrophotometric methods (Chang *et al*., 2018). The regression equation is used to calculate the flavonoid levels in a sample based on their absorbance value, and the results are expressed in mg QE/g (milligrams of quercetin equivalent per gram of sample).

The standard value of flavonoid content in clove handles of ethanol extract ranges from 45–55 mg QE/g (Wahyulianingsih *et al.,* 2019). Based on the results of the calculation of flavonoid levels presented in Table 18, it is known that clove ethanol extract has the highest flavonoid content of 50.491 mg QE/g, followed by the ethyl acetate fraction of 45.650 mg QE/g, the water fraction of 40.253 mg QE/g, and the n-hexane fraction as the lowest of 31.237 mg QE/g. This variation in flavonoid levels is caused by differences in the polarity of the solvents used in the fractionation process. Ethanol as a polar solvent is able to dissolve flavonoid compounds more effectively than non-polar solvents such as n-hexane. This is in accordance with the characteristics of flavonoids which are generally semi-polar, making them easier to extract using polar or semi-polar solvents such as ethanol and ethyl acetate (Maisuthisakul *et al*., 2017). The high level of total flavonoids in clove ethanol extract is generally 45–55 mg QE/g of extract which has the potential to provide pharmacological activity such as antidiabetes, considering that flavonoids can inhibit enzymes such as α-glucosidase and α-amylase which play a role in glucose metabolism (Ghasemi *et al*., 2009). Therefore, clove ethanol extract has significant potential to be developed as a nature-based antidiabetic active ingredient, along with its high flavonoid content and ability to inhibit the activity of enzymes related to glucose metabolism.

Enzyme Inhibition Activity Test

The inhibitory activity test of the enzyme α-amylase is carried out in vitro to evaluate the potential of a compound or extract in inhibiting the action of the enzyme. The basic principle of this method is to observe changes in the intensity of the blue color in the iodine-starch complex. The enzyme α-amylase has the ability to hydrolyze glycosidic bonds in starch molecules, resulting in simpler compounds such as maltose and glucose. The compounds produced by hydrolysis do not react with iodine solutions, so the intensity of the blue color formed will decrease as the enzyme activity increases. Thus, if a sample has inhibitory activity against the enzyme α-amylase, then starch degeacidity will be reduced, and the blue color of the iodine-starch complex will remain concentrated. The stronger the intensity of the blue color formed, the higher the inhibition of the alpha α-amylase enzyme (Putri *et al*., 2021).

The intensity of the blue color of the iodine-starch complex was carried out using the UV-Vis spectrophotometry method. This test includes several treatments, namely blank, control blank, sample, and sample control. The test sample consisted of acarbose, (as a positive control), ethanol extract, water fraction extract, ethyl acetate fraction extract, n-hexane fraction extract. Acarbose is used as a positive control because it has the ability to inhibit the enzyme α-amylase, although it is more commonly known as the inhibitor of the enzyme α-glucosidase. The mechanism of action of acarbose is to inhibit the breakdown of complex carbohydrates such as starch into simple sugars (such as glucose), thereby slowing down the absorption of glucose in the intestines and lowering the spike in postprandial blood glucose

levels. Therefore, acarbose is often used as an adjunct therapy in the management of type 2 diabetes mellitus (PubMed, 2016).

The blank test aims to determine the natural activity of the enzyme α-amylase in hydrolyzing starch into glucose without the addition of inhibitory compounds. Meanwhile, sample control and blank control (to which no enzymes are added) are used as a comparison or correction for the possibility of light absorption by the test compound itself. It is important to ensure that the absorbance obtained is actually derived from enzymatic activity, not from the color or optical characteristics of the sample. With this treatment, the analysis of the inhibition activity of the enzyme α-amylase by each sample can be carried out in a standardized and more accurate manner.

Before testing, it is necessary to determine the maximum wavelength to determine the absorption area under optimal conditions resulting from the absorbance value measured using a UV-Vis spectrophotometer (Sudewi & Pontoh, 2018). Optimization was carried out in the wavelength range of 400-800 nm and a maximum wavelength of 540 nm was obtained with an absorbance value of 0.685. The results of the determination of the maximum wavelength of the enzyme α-amylase can be seen in figure 5.

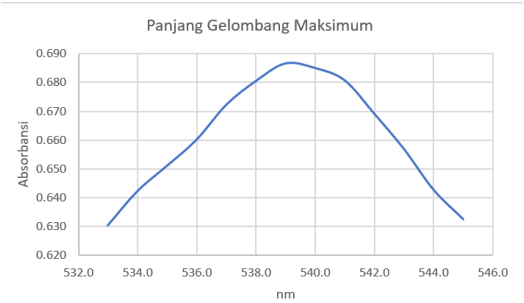


Figure 5. Maximum Wavelength Graph of α-Amylase Enzyme

Table 17. Maximum wavelength of the enzyme α-amylase

Wavelength(nm)	Abs
545.0	0.632
544.0	0.643
543.0	0.657
542.0	0.669
541.0	0.681
540.0	0.685
539.0	0.687
538.0	0.681
537.0	0.672
536.0	0.660
535.0	0.651
534.0	0.642
533.0	0.630

After the maximum wavelength measurement, *the Operating Time* (OT) is determined.*Operating Time* is a measurement time that is carried out to determine a stable measurement time (Salamah & Widyasari, 2018). *Operating Time* is determined by measuring absorbance at a predetermined maximum wavelength of 540 nm, for 20 minutes with a time interval of 2 minutes. The results of *the Operating Time* (OT) measurement were obtained at 10 minutes. It can be seen in table 18.

Tabel 18. *Operating Time* Enzim α-Amilase

Time (s)	Absorbansi
0	0.215
0.03	0.25

0.07	0.398
0.1	0.522
0.13	0.69
0.17	0.678
0.2	0.655
0.23	0.632
0.27	0.621
0.3	0.615
0.33	0.63
0.67	0.63
2	0.63
4	0.63
6	0.63
8	0.63
10	0.63
12	0.63
14	0.63
16	0.63
18	0.63
20	0.63

Operating time (OT) is the optimum time that indicates when enzyme activity reaches its maximum point in catalyzing the substrate. In this test, the activity of the enzyme α-amylase was observed using the DNS method (3,5-dinitrosalicylate) or the spectrophotometric method used to measure the amount of reducing sugars (such as glucose, maltose, etc.) resulting from enzymatic reactions or hydrolysis at a wavelength of 540 nm, which is the maximum wavelength for the detection of reducing sugars (maltose) resulting from starch hydrolysis (Fikriyah & Arfiani, 2024).

Based on the above table of the observation of *the Operating Time of* the enzyme activity α-amylase for 20 minutes, the absorbance value showed a significant increase at the beginning of the reaction, especially between the 0th to the 8th second. This increase reflects that the enzymatic reaction is actively taking place, during which the enzyme begins to work optimally in catalyzing the substrate (N. Hidayah *et al.,* 2023). The highest absorbance value is reached at the 8th second with an absorbance of 0.690, which indicates that at that time the enzyme activity is at its peak.

After reaching the maximum point, the absorbance value began to decline slightly and subsequently tended to stabilize in the range of 0.630. This stability lasted from the 20th second until the end of the observation at the 20th minute (1200 seconds). These circumstances indicate that the system has entered a *steady-state phase,* where most of the substrate has been converted into a product, and the reaction reaches a balanced condition. Thus, it can be concluded that the optimal operating *time of* the α-amylase enzyme occurs in the range of 0–8 seconds. Meanwhile, after this period, the enzyme remained active but did not show a significant increase in its activity, so the reaction lasted constantly until the end of the observation time. After performing the *operating time,* a standard curve was created for the relationship between the concentration of acarbose (ppm) and the absorbance value as an indicator of the activity of the enzyme α-amylase. It can be seen in the image below.

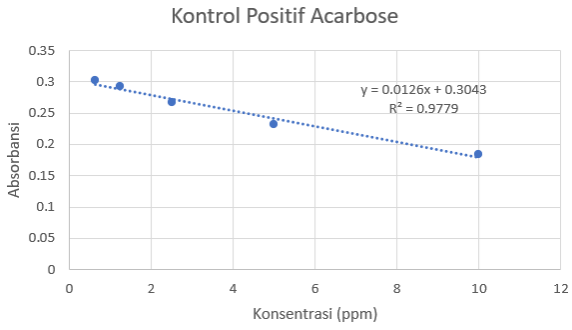


Figure 6. Acarbose Positive Control Chart

Based on the linear regression graph obtained, the line equation shows y = 0.0126x+0.3043 with a determination coefficient value (R^2) of 0.9779. A value of R^2 close to 1 indicates that there is a very strong linear relationship between an increase in the concentration of acarbose and a decrease in the value of absorbance. This decrease in absorbance reflects that the higher the concentration of acarbose, the greater its inhibition against the activity of the enzyme α-amylase. This is in accordance with the mechanism of action of acarbose as a competitive inhibitor that inhibits the breakdown of substrates by enzymes, so that the products of the reaction become less and the resulting absorbance value is also lower. Therefore, acarbose has been shown to be effective as a positive control in α-amylase enzyme inhibition assays.

Inhibition tests of α-amylase enzyme activity were performed on samples of acarbose, ethanol extract and clove handle fractions. The acarbose used as a comparison was divided into five concentrations, namely 0.625 ppm, 1.25 ppm, 2.5 ppm, 5 ppm, and 10 ppm. Meanwhile, the concentration of ethanol extract and fractions used are 12.5 ppm, 25 ppm, 50 ppm, 100 ppm, and 200 ppm. There is a difference in concentration variants between acarbose as a comparison with samples (extracts and fractions) because acarbose is an antidiabetic drug with a well-known mechanism of action as an inhibitor of the enzyme α-amylase, which slows down the breakdown of carbohydrates in food into sugar, so that blood sugar levels do not rise drastically. Meanwhile, the sample is not known for sure the content of phytochemical compounds that are effective in inhibiting the activity of the enzyme α-amylase. Therefore, the variation in concentration between acarbose as a comparator and sample (extract and fraction) is different. Meanwhile, sample testing was carried out with various concentration variants aimed at seeing the effect of sample concentration on the inhibition of α-amylase enzyme activity. All test solutions were measured for absorption using a UV-Vis spectrophotometer until an absorbance value was obtained. The absorbance value is then used to calculate the inhibition presentation value and the IC50 value.

Table 19. Acarbose Positive Control Test Results

Sampel	% Inhibisi	IC_{50}
Acarbose	6.383 11.348 19.149 30.496 50.709	9.596
Ekstrak Etanol	31.206 35.461 43.972 54.965 73.404	87.465
Fraksi Air	45.390 48.936 51.418 56.738 65.248	42.206
Fraksi N-Heksana	33.333 35.816 40.426 52.128 62.057	111.244
Fraksi Etil Asetat	46.099 49.291	26.303

56.738
61.702
78.014

The α-amylase enzyme inhibition activity test aims to evaluate the potential of a compound or extract in inhibiting the breakdown of carbohydrates into glucose. This mechanism is important in managing postprandial hyperglycemia in type 2 diabetes patients. The parameters observed are % inhibition and IC_{50}, which is the concentration required to inhibit 50% of enzyme activity. The higher the % inhibition and the lower the IC50, the stronger the inhibitory activity (Poovitha & Parani, 2016).

Acarbose, as a positive control, showed the lowest IC50 value (9.596 µg/mL) and an increase in % inhibition from 6.383% to 50.709% as the concentration increased. This is in accordance with the mechanism of action of acarbose as a competitive inhibitor of the α-amylase enzyme. This drug has been used clinically and has been proven effective in lowering blood glucose levels (Budianto & Hairullah, 2017).

The ethanol extract had an IC_{50} value of 87.465 µg/mL, which was relatively weak compared to the other fractions. However, the % inhibition value increased significantly from 31.206% to 73.404%. The content of bioactive compounds such as flavonoids, phenols, and tannins in low concentrations may affect its low effectiveness (Rika Widianita, 2023). The water fraction showed better inhibitory activity with an IC_{50} value of 42.206 µg/mL and % inhibition ranging from 45.390% to 65.248%. This activity is associated with the content of polar compounds such as tannins and phenolic acids that are soluble in water. Polar compounds are known to inhibit α-amylase enzyme activity non-competitively (Fikriyah & Arfiani, 2024). The n-Hexane fraction had the lowest activity with the highest IC_{50} value of 111.244 µg/mL and % inhibition ranging from 33.333% to 62.057%. Non-polar compounds such as sterols and lipids in this fraction have low affinity for the α-amylase enzyme, making them less effective in inhibiting the enzyme. The ethyl acetate fraction showed the best potential after acarbose, with an IC_{50} of 26.303 µg/mL and the highest inhibition percentage reaching 78.014%. This high activity is thought to be due to the high concentration of semi-polar compounds such as flavonoids and tannins, which are able to bind to the active site of the enzyme through hydrogen bonds and π–π interactions (Fikriyah & Arfiani, 2024). These results are in line with many studies that the ethyl acetate fraction often contains the highest active compounds.

CONCLUSION

The primary conclusion of this study indicates that the ethyl acetate fraction of clove stalk extract exhibits the most promising potential as a natural α-amylase inhibitor, with an IC_{50} value of 26.303 µg/mL, approaching the effectiveness of the positive control, acarbose. Additionally, the ethanol extract and aqueous fraction showed significant inhibitory activity, supported by high phenolic and flavonoid contents that play crucial roles in enzyme inhibition mechanisms and glucose regulation in type 2 diabetes mellitus. These findings reinforce the potential of clove stalks, often considered waste, as a source of bioactive compounds that could be developed into herbal therapeutic agents for blood glucose management. However, limitations of this research include its in vitro nature, which does not confirm efficacy and safety in clinical settings, nor does it specify the exact chemical composition responsible for the observed activities. Future studies should focus on identifying the active compounds chemically and evaluating their pharmacological effects in vivo to establish therapeutic efficacy and safety over the long term. The practical implication of this research suggests that utilizing clove stalks can optimize the use of what is typically considered waste, transforming it into a natural alternative for blood sugar regulation with fewer side effects.

Furthermore, subsequent research should expand chemical analyses to precisely identify the active constituents and conduct toxicity and efficacy tests in vivo. Advanced techniques such as high-performance liquid chromatography (HPLC) and mass spectrometry could be employed to pinpoint the main bioactive compounds. Additionally, developing stable and effective formulations based on these extracts for blood glucose control is essential to translate these findings into practical applications in pharmacy and traditional medicine. Consequently, clove stalks, traditionally viewed as waste, could emerge as a valuable source of natural therapeutic agents, offering sustainable and safe options for managing diabetes, ultimately benefiting the community through improved health outcomes.

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