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α-amylase inhibition test of ethanol extract colues leaves (*Solenostemon scutellarioides* (L.) Codd) *in vitro*

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Abstract

Background: Hyperglycemia occurs due to an increase in blood glucose levels, making them higher than the normal range, which is a sign of diabetes mellitus. One therapeutic approach for DM is inhibiting enzymes that convert carbohydrates into glucose. Coleus (*S. scutellarioides* (L.) Codd), an ornamental plant, is believed to have antihyperglycemic effects due to its phenolic phytochemicals.

Objective: To determine the phytochemical content and antihyperglycemic activity of 70% ethanol extract of coleus leaves through α-amylase enzyme inhibition.

Method: The leaves were characterized and tested qualitatively for phytochemical content using the test tube method. The extract was prepared using maceration with 70% ethanol, followed by thin-layer chromatography (TLC). The 200-ppm extract was tested for α-amylase enzyme inhibition, with acarbose as a positive control. Statistical differences were analyzed using the t-test method.

Results: The herbarium specimen of coleus leaf was shown to contain flavonoids, tannins, saponins, and steroids. The 200-ppm extract reduced the activity of the α-amylase enzyme by 61.03%, while the positive control (acarbose) reduced it by 92.16%.

Conclusion: The coleus leaf extract contains active phytochemicals that may help lower blood glucose levels. At 200 ppm, the extract demonstrates potential as an antihyperglycemic agent through α-amylase inhibition.

Keywords: coleus leaves, antihyperglycemic, α-amylase enzymes inhibition

1. Introduction

Diabetes mellitus (DM), a serious metabolic disease is characterized by hyperglycemia, a state where blood glucose level is higher than the normal concentrations. It is a metabolic disease initiated by a defect in insulin production or action, where insulin is the hormone that plays a role in regulating blood glucose levels (Kemenkes RI, 2020). Common strategies for managing DM include increasing the sensitivity of insulin receptors, administering insulin therapy, and inhibiting glucose absorption through the digestive system. One therapeutic approach which has been studied extensively concerning the management of DM is the inhibition of digestive enzymes such as α-amylase, which is responsible for the hydrolysis of complex carbohydrates into glucose. By inhibiting this enzyme, intake of glucose in the body post meals can be reduced which may alleviate hyperglycemic conditions on patients with DM (Kemenkes RI, 2020).

The use of natural products as alternative therapies, particularly in the management of chronic diseases such as DM, has received widespread attention. This is because many natural substances contain bioactive compounds like flavonoids, alkaloids, tannins, and saponins that have pharmacological properties, including antihyperglycemic effects (Grover *et al*., 2002). Several studies have reported that these phytochemicals are capable of inhibiting certain digestive enzymes, such as α-amylase, thereby contributing to the reduction of blood glucose levels (Tundis *et al*., 2010). One of

the plants with potential antihyperglycemic effects that is commonly cultivated among the Indonesian communities is the coleus (*S. scutellarioides* (L.) Codd).

Despite being frequently grown as decorative plants, coleus leaves may also have the potential to lower blood sugar levels, which lowers the risk of developing DM (Lestari, 2022). Numerous chemical substances, such as flavonoids, saponins, tannins, and steroids, have been discovered to be present in the herbarium specimen (dried plant material) of coleus leaves (Natasya *et al*., 2023). However, verification of the phytochemical content of plants is trill required, because the presence and concentration of bioactive compounds can be affected by plants parts, growing conditions, and harvest time (Azwanida, 2015; Verpoorte *et al*., 2000). Since there is limited research on the potential of coleus leaves, *in vitro* approaches are also required to evaluate the antihyperglycemic activity of these compounds. Thus, the purpose of this study was to determine which secondary metabolites were found in the herbarium specimen and extracts, as well as to examine the 70% ethanol extract of coleus leaves' ability to inhibit α-amylase enzyme.

2. Method

2.1. Type and design of study

This was an experimental study using a 70% ethanol extract of coleus leaves at a concentration of 200 ppm, with acarbose as the positive control at a concentration of 100 ppm.

2.2. Population and sample

The coleus leaves used in this study were collected from Sukorejo, Gunungpati Regency, Semarang City. The inclusion criteria for coleus leaves were: green and purplish-red coloration, the third to the eighth leaves from the tip, harvested in the morning, fresh, intact without holes or mold, and uniform in size.

2.3. Tools and materials

Tools used in this research included a blender (Philips), glassware (Pyrex), 40-mesh sieve (CBN), test tubes (Iwaki), micropipettes (Socorex), analytical balance (Mettler Toledo, USA), magnetic stirrer (Benchmark), incubator (Fisher), UV-VIS spectrophotometer (Shimadzu), microscope (Olympus, China), microscope slides (Sail Brand), water bath, furnace, rotary vacuum evaporator, sieve, dehydrator, vortex (Boeco V-1 plus), cuvettes, stationery, scissors, gloves, silica gel GF254 plates (Merck), and chromatography chamber.

Materials used included dried coleus leaves from Sukorejo, Gunungpati Regency, Semarang City; distilled water; 70% ethanol (p.a Merck); acarbose (p.a Merck); methanol (p.a Smartlab); chloroform (p.a Smartlab); concentrated HCl (p.a Smartlab); ethyl acetate (p.a Smartlab); acetic anhydride (p.a Smartlab); Mayer's reagent; Liebermann-Burchard reagent; Dragendorff's reagent (p.a Merck); α-amylase enzyme (fermentation product from B. licheniformis); amylase activity assay kit (Sigma-Aldrich); magnesium powder (p.a Merck); sulfuric acid (p.a Tian Jin Shin Damao); ferric chloride (p.a Smartlab); aluminum chloride (p.a Smartlab); piperine; gallic acid; cholesterol; quercetin (p.a Smartlab); water for injection (p.a Smartlab); and aluminum foil (local).

2.4. Plant identification

Coleus leaves were identified by comparing the plant parts to the morphological characteristics of *S. scutellarioides* (L.) Codd. The identification process was conducted at the Laboratory of Ecology and Biosystematics, Faculty of Science and Mathematics, Diponegoro University, Semarang, confirming that the sample was classified as the order Lamiales and the species *Solenostemon scutellarioides* (L.) Codd.

2.5. Preparation of herbarium specimen

A total of 1 kg of fresh coleus leaves was collected, washed thoroughly, and drained. The leaves were then sorted, sliced, and dried using a dehydrator at 40–45°C for 2–3 days until fully dry (crisp-dry). The dried leaves were ground into powder using a grinder and standardized using a 40-mesh sieve, yielding 100 grams of dried powder.

2.6. Characterization of herbarium specimen

2.6.1. Macroscopic examination of fresh coleus leaves

This test aimed to identify the distinctive features of the coleus leaves through direct observation of its characteristics, referring to the morphology of coleus leaves, including shape, odor, and color (Kemenkes RI, 2017).

2.6.2. Microscopic examination of herbarium specimen

A thin transverse section of the coleus leaf was prepared and observed under a microscope by placing the section on a slide with a drop of water, covered with a coverslip. The same method was applied to the coleus leaves powder. The specimens were observed under 40× magnification (Kemenkes RI, 2017).

2.6.3. Ethanol-soluble extract content

Five grams of the coleus leaves powder were macerated for 24 hours in 100 mL of 70% ethanol using a stoppered flask, shaken frequently during the first 6 hours, and then left to stand for 18 hours before filtration. Twenty milliliters of the filtrate were evaporated using a water bath in a pre-heated porcelain dish (105°C) and weighed until a constant weight was achieved (Kemenkes RI, 2017).

2.6.4. Water-soluble extract content

Five grams of coleus leaves powder were macerated in 100 mL of water containing chloroform (2.5 mL of chloroform in 1 L of distilled water) using a stoppered flask, shaken for the first 6 hours, and then allowed to stand for 18 hours before filtration. Twenty milliliters of the filtrate were evaporated using a water bath in a pre-heated porcelain dish (105°C) and weighed until a constant weight was obtained (Kemenkes RI, 2017).

2.6.6. Loss on drying

Two grams of coleus leaves powder were accurately weighed and placed in a porcelain crucible pre-heated at 105°C for 30 minutes and pre-weighed. The crucible was placed in an oven with the lid open and dried at 105°C until constant weight, then cooled in a desiccator and weighed again (Kemenkes RI, 2017).

2.6.6. Moisture content

Ten grams of coleus leaves powder were weighed and placed in a tared container, then 200 mL of saturated toluene was added to the flask containing the test sample and heated. Distillation was carried out at a rate of approximately two drops per second until the water was completely distilled. The volume of distilled water was read using the scale on the distillation apparatus (Kemenkes RI, 2017)

2.6.7. Total ash content

Two grams of coleus leaves powder were weighed and placed in a tared, pre-ignited porcelain crucible. The crucible containing the sample was gradually ignited until the carbon was completely burned off. The ignition was performed at 600°C for 3 hours, then cooled and weighed until a constant weight was obtained (Kemenkes RI, 2017).

2.6.8. Acid-insoluble ash content

The ash obtained from the total ash determination was treated with 25 mL of dilute hydrochloric acid for 5 minutes. The acid-insoluble residue was filtered through ash-free filter paper, washed with hot water, ignited until a constant weight was achieved, and then weighed (Kemenkes RI, 2017).

2.7. Sample extraction

A total of 100 g of dried coleus powder was weighed and macerated in a dark-colored glass container with 70% ethanol as the solvent, using a coleus powder-to-solvent ratio of 1:10. The container was sealed and stored at room temperature, protected from light for 3 × 24 hours, while being stirred frequently during the first 6 hours. Filtration was performed every 24 hours, followed by re-maceration using fresh solvent. The resulting filtrate was evaporated using a rotary evaporator at 40°C until a thick extract was obtained.

2.8. Phytochemical screening of herbarium specimen

2.8.1. Flavonoids

One gram of coleus leaves powder was added to 10 mL of distilled water, boiled for 5 minutes, and then filtered while hot. Five mL of the filtrate was added with magnesium powder and 1 mL of concentrated hydrochloric acid. The presence of flavonoids is indicated by the formation of an orange, red, or yellow color (Farnsworth, 1966).

2.8.2. Terpenoids and steroids

A total of 0.5 grams of coleus leaves powder was macerated with 10 mL of n-hexane for 1 hour, then filtered. Two mL of the filtrate was added with 1 mL of acetic anhydride. The mixture was then carefully layered with 2 mL of concentrated sulfuric acid along the wall of the test tube. A blue, purple, or green color indicates the presence of steroids, while a red or brown color indicates the presence of triterpenoids (Farnsworth, 1966).

2.8.3. Tannins

One gram of coleus leaves powder was added to 10 mL of water, boiled, and filtered. A few drops (1–2) of ferric chloride reagent were added to the filtrate. A blue-black or greenish-black coloration indicates the presence of tannins (Farnsworth, 1966).

2.8.4. Saponins

A total of 0.5 grams of coleus leaves powder was placed into a test tube and added with 10 mL of hot water, then allowed to cool and shaken for 10 seconds. The formation of persistent foam for no less than 10 minutes at a height of 1–10 cm that does not disappear upon the addition of one drop of 2 N hydrochloric acid indicates the presence of saponins (Farnsworth, 1966).

2.8.5. Alkaloids

A total of 0.5 grams of coleus leaves powder was added with 1 mL of hydrochloric acid and 9 mL of distilled water, then heated for 2 minutes, cooled, and filtered. The filtrate was used for alkaloid testing. 0.5 mL of the filtrate was distributed into three separate test tubes. Test tube 1 was added

with Mayer's reagent, test tube 2 was added with Dragendorff's reagent, and test tube 3 was added with Wagner's reagent. The presence of alkaloids is indicated by a white precipitate in test tube 1, a brown precipitate in test tube 2, and a brick-red precipitate in test tube 3 (Farnsworth, 1966).

2.9. Phytochemical screening of the extract

Phytochemical tests using Thin Layer Chromatography (TLC) were carried out on compound groups that showed positive results in the phytochemical screening using reagent-based methods. The stationary phase used was silica gel G60F254/TLC plates.

2.9.1. Identification of flavonoid compounds

The 70% ethanol extracts of the coleus leaves, and a reference standard (quercetin) were spotted using a capillary tube at a distance of 1 cm from the bottom edge of the silica gel GF254 TLC plate, which had been previously heated in an oven at 110°C for approximately 30 minutes. After the spots dried, the TLC plate was placed in a chromatography chamber containing a pre-saturated mobile phase of chloroform:ethyl acetate (8.5:1.5). Elution was carried out until it reached the upper limit mark. The eluted TLC plate was then observed under UV light at λ254 and λ366, both before and after being sprayed with 10% $AlCl_3$. Detected spots were analyzed for their Rf values and observed for their color appearances (Farnsworth, 1966).

2.9.2. Identification of steroid compounds

The 70% ethanol extracts of the coleus leaves, and a reference standard (cholesterol) were spotted using a capillary tube at a distance of 1 cm from the bottom edge of the silica gel GF254 TLC plate, which had been previously heated in an oven at 110°C for approximately 30 minutes. After the spots dried, the TLC plate was placed in a chromatography chamber containing a pre-saturated mobile phase of chloroform:ethyl acetate (8.5:1.5). Elution was carried out to the upper limit mark. The eluted TLC plate was then observed under UV light at λ254 and λ366, both before and after being sprayed with Liebermann-Burchard reagent. Detected spots were analyzed for their Rf values and observed for their color appearances (Farnsworth, 1966).

2.9.3 Identification of Tannin Compounds

The 70% ethanol extracts of the coleus leaves, and a reference standard (gallic acid) were spotted using a capillary tube at a distance of 1 cm from the bottom edge of the silica gel GF254 TLC plate, which had been previously heated in an oven at 110°C for approximately 30 minutes. After the spots dried, the TLC plate was placed in a chromatography chamber containing a pre-saturated mobile phase of chloroform:ethyl acetate (8.5:1.5). Elution was carried out to the upper limit mark. The eluted TLC plate was then observed under UV light at λ254 and λ366, both before and after being

sprayed with $FeCl_3$. Detected spots were analyzed for their Rf values and observed for their color appearances (Farnsworth, 1966)

2.9.4. Identification of alkaloid compounds

The 70% ethanol extract of the coleus leaves, and a reference standard (piperine) were spotted using a capillary tube at a distance of 1 cm from the bottom edge of the silica gel GF254 TLC plate, which had been previously heated in an oven at 110°C for approximately 30 minutes. After the spots dried, the TLC plate was placed in a chromatography chamber containing a pre-saturated mobile phase of chloroform:ethyl acetate (8.5:1.5). Elution was carried out to the upper limit mark. The eluted TLC plate was then observed under UV light at λ254 and λ366, both before and after being sprayed with Dragendorff reagent. Detected spots were analyzed for their Rf values and observed for their color appearances (Farnsworth, 1966).

2.10. Preparation of test solutions

2.10.1. Enzyme stock solution

The enzyme stock solution was prepared at a concentration of 10,000 ppm. α-Amylase enzyme (50 mg) was weighed and dissolved in assay buffer solution up to a volume of 5 mL, then vortexed for 30 seconds until homogeneous. The α-amylase enzyme stock solution at 10,000 ppm is equivalent to 204.20 U/mL of solution.

2.10.2. Acarbose stock solution

Acarbose solution was prepared at a concentration of 1000 ppm. Acarbose powder (50 mg) was weighed, dissolved in 50 mL of water for injection, and vortexed for 30 seconds until homogeneous.

2.10.3. Master mix solution

The master reaction mix was prepared by mixing 25 µL of buffer and 25 µL of substrate, then homogenized using pipetting technique.

2.10.4. Sample stock solution

The extract stock solution was prepared at a concentration of 1000 ppm. The extract (50 mg) was weighed, dissolved in 50 mL of water for injection, and vortexed for 30 seconds until homogeneous.

2.11. Enzyme optimization and determination of operating time

Enzyme optimization was performed by reacting several variations of α-amylase enzyme units with 25 µL of substrate. The optimal enzyme unit was determined based on the amount of

enzyme that could hydrolyze 25 µL of substrate within a specific period. The time required to fully hydrolyze the substrate was used as the operating time for the assay. The optimization test was conducted by reacting the enzyme solution with 50 µL of the master mix (substrate + buffer). The mixture was incubated for 2 minutes, and absorbance was measured at λ 405. The mixture was then incubated at 25°C, and absorbance was measured every 5 minutes using a UV-Vis spectrophotometer at λ 405 until a constant absorbance was reached (Hidayah *et al*., 2023).

2.12. α-Amylase activity assay

2.12.1. Positive control

The positive control test was conducted by reacting 125 µL of α-amylase enzyme solution (25 U/mL) with 75 µL of acarbose solution at a concentration of 100 ppm, followed by the addition of 50 µL of the master mix. The solution mixture was homogenized vertically using pipetting technique. The mixture was incubated for 2 minutes, and absorbance was measured at λ 405. It was then incubated at 25°C and measured again using a UV-Vis spectrophotometer at λ 405. The test was performed in triplicate (Fathiyah, 2023).

2.12.2. Sample

The sample test was conducted by reacting 125 µL of α-amylase enzyme solution (25 U/mL) with 75 µL of the coleus leaves extract sample solution at a concentration of 200 ppm, followed by the addition of 50 µL of the master mix. The solution mixture was homogenized vertically using pipetting technique. The mixture was incubated for 2 minutes, and absorbance was measured at λ 405. It was then incubated at 25°C and measured again using a UV-Vis spectrophotometer at λ 405. The test was performed in duplicate (Fathiyah, 2023).

3. Result and discussion

3.1. Characterization of coleus (S. scutellarioides (L.) Codd) leaves

Macroscopic analysis of the powdered dried coleus leaf reveled that the leaf, which was 9 cm long, was reddish-purple in the middle and green around the edges. The leaf blade had pinnate venation, serrated edges, and a pointed tip. It was ovate in shape. Microscopic examination of the transverse section of the coleus leaf showed the presence of covering trichomes, cuticle, palisade cells, epidermis, glandular trichomes, vascular bundles, and collenchyma. The leaves powder's microscopic analysis indicated the presence of epidermal cells, glandular trichomes, and covering trichomes. **Table 1** displays the findings for the dried coleus leaf powder's moisture content, water-

soluble extractive value, ethanol-soluble extractive value, total ash content, and acid-insoluble ash content.

Table 1. Characterization of dried coleus (*S. scutellarioides* (L.) codd) leaves powder

Parameter	Result (% ± SD)	FHI literature standard (%)
Drying loss	7.53±0.31	≤ 10
Ethanol-soluble extract content	14.83±0.73	≥ 5
Water-soluble extract content	22.91±0.72	≥ 22
Moisture content	5.67±0.58	≤ 10
Total ash content	5.46±0.17	≤ 8
Acid-insoluble ash content	0.61±0.09	≤ 2

According to **Table 1**, the coleus leaves powder's characterization complied with the Indonesian Herbal Pharmacopeia's (FHI) and *Materia Medika Indonesia's* (FHI) standards. The amounts of compounds in the crude drug that can dissolve in ethanol and water were ascertained by testing the coleus leaves powder's contents that were soluble in ethanol and water, respectively. The water-soluble extract content was higher than the ethanol-soluble extract content, indicating that the active compounds in the coleus leaves are better extracted in water than in ethanol (Depkes, 2000).

Moisture content is a necessary parameter to test, as it is related to the quality of the crude drug during storage. Over time, a high moisture content can inhibit the biological activity of the crude drug and encourage the growth of mold (Depkes, 2000). The purpose of total ash content test was to determine the amount of internal and external mineral matter was produce during the processing of crude drugs. Ash can originate from outside pollutants or the plant itself. Finding out how much ash comes from outside source like soil and sand is the goal of the acid-insoluble ash test (Depkes, 2000).

3.2. Extraction of coleus (S. scutellarioides (L.) Codd) leaves

The extraction of the coleus leaves was carried out using 70% ethanol as the solvent. The 70% ethanol solvent aimed to extract both polar and non-polar compounds (Riwanti *et al.*, 2020). The coleus leaves extracted using the 70% ethanol obtained was dark brown in color, with a yield of 13.67 g from 100 g of the sample, resulting in an extraction yield of 13.67%. This yield was higher than that reported in a previous study, which used the same solvent, i.e., 12.97% (Susilawati *et al*., 2016). The difference in yield may be due to differences in the extraction methods. The previous study did not replace the solvent during the maceration process, which led to fewer active compounds being extracted, as the solvent had become saturated.

3.3. Phytochemical content of herbarium specimen of coleus (S. scutellarioides (L.) Codd) leaves

Phytochemical screening aims to identify the content of secondary metabolite compounds in the herbarium specimen. The results of the phytochemical screening can be seen in **Table 2**.

Table 2. Phytochemical content of herbarium specimen of coleus *(S. scutellarioides (L.) codd)* leaves

Compoud	Control	Reagent	Literature	Result	Description
Alkaloids	Piperine	Mayer	White precipitate	Yellow	-
		Dragendorff	Brown precipitate	Black	-
		Wagner	Brick-red precipitate	Black	-
Flavonoids	Quercetin	HCl + Mg	Red, yellow, or orange	Yellow	+
Tannins	Gallic Acid	Water + $FeCl_3$	Blue or dark green	Dark green	+
Saponins	Sapogenin	Air panas	Foam formed	Foam formed	+
Steroids	Cholesterol	Lieberman Burchard	Blue, purple, or green	Green	+

Description:
(+): Contains compound mentioned
(-): Does not contain compound mentioned

The finding of this study's phytochemical content were in line with earlier investigations that discovered secondary metabolites like flavonoids, tannins, steroids, and saponins in the coleus leaves herbarium specimen (Susilawati *et al*., 2016). The natural material's chemical content should be re-examined in light of variations in sample collection locations, harvest times, and plant parts examined, all of which may have an impact on the presence and concentration of the material's bioactive compounds (Azwanida, 2015; Verpoorte *et al*., 2000). To confirm that alkaloid compounds were present in the coleus leaves herbarium specimen, the alkaloid test was carried out three times. Since all three reagents produced negative results, it is likely that coleus leaves's low alkaloid content prevented it from being picked up during the phytochemical screening. The existence of these secondary metabolites raises the possibility that coleus leaves could have pharmacological effects and be used as a medicinal ingredient, especially for antidiabetic purposes. The amylase enzyme is known to be inhibited by flavonoids, tannins, saponins, and steroids (Wahyuntari, 2011; Wakhidah & Silalahi, 2018).

3.4. Identification of secondary metabolite compounds by thin layer chromatography

The identification of secondary metabolite compounds in the 70% ethanol extract of coleus leaves was performed using TLC with a stationary phase of silica gel GF254 and a mobile phase of chloroform:ethyl acetate (8.5:1.5). Based on the eluents, a TLC profile was created using specific spot indicators, namely $AlCl_3$ as a specific spot indicator for flavonoids, $FeCl_3$ for tannins, Lieberman Burchard for steroids, and Dragendorff for alkaloids.

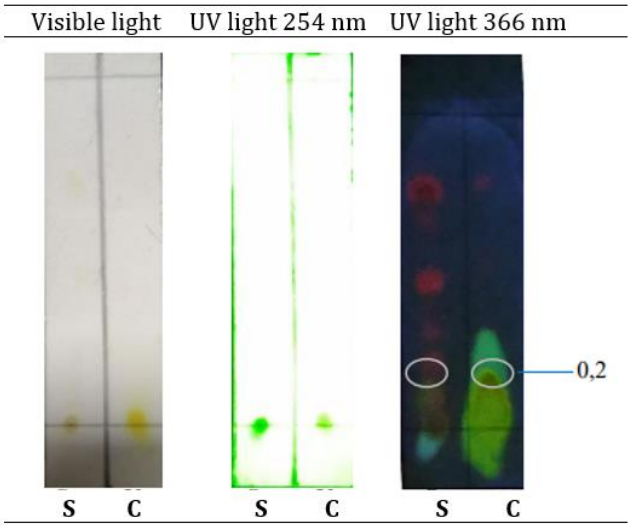

Figure 1. TLC profile of flavonoid compounds in 70% ethanol extract of coleus leaves (*S. scutellarioides* (L.) Codd) after spraying with $AlCl_3$ spot reagent; (S) sample (C) quercetin as a control

Figure 1 shows a yellow-green spot on the sample, indicating the presence of flavonoid compounds in the 70% ethanol extract of coleus leaves with an Rf value of 0.2. This is consistent with a study by (Lisdawati *et al.*, 2012), which also showed the presence of flavonoid compounds in the coleus leaves extract, even though the sample was collected from a different growing location. The use of the specific spot reagent $AlCl_3$ aimed to react with the spot by spraying it onto the TLC plate, making the spot visible under UV light at λ 366. The identification of flavonoid compounds by TLC reinforced the results of the phytochemical screening of herbarium specimen of the coleus leaves.

The presence of tannin compounds in the 70% ethanol extract of coleus leaves was indicated by a yellow-green spot on the sample in **Figure 2**. This finding in line with the previous study, which also showed the presence of tannin compounds in the coleus leaves extract (Lisdawati *et al*., 2012). By spraying the spot onto the TLC plate, the specific spot reagent $FeCl_3$ was used to react with the spot and make it visible under UV light at λ 366. The findings of the phytochemical screening of the coleus leaves herbarium specimen were supported by the TLC identification of tannin compounds.

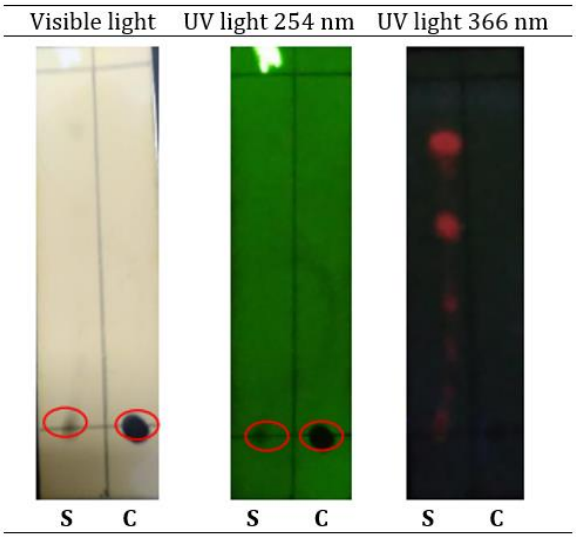

Figure 2. TLC profile of flavonoid compounds in 70% ethanol extract of coleus leaves (*S. scutellarioides* (L.) Codd) after spraying with $FeCl_3$ spot reagent; (S) sample (C) gallic acid as a control

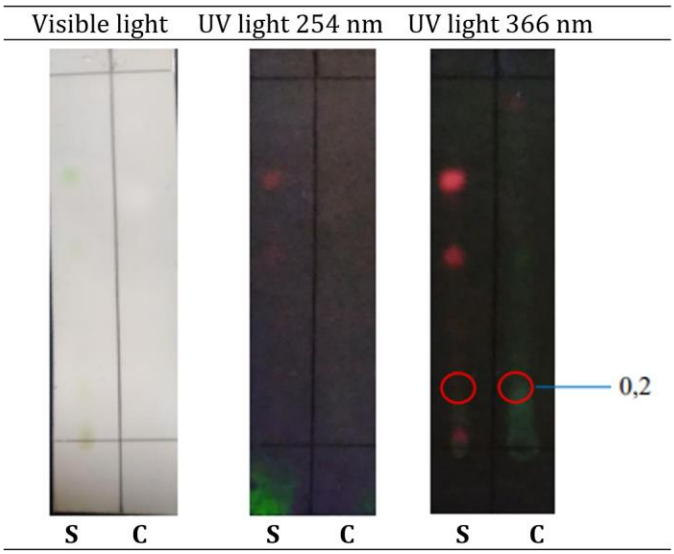

Figure 3. TLC profile of flavonoid compounds in 70% ethanol extract of coleus leaves (*S. scutellarioides* (L.) Codd) after spraying with Lieberman Burchard spot reagent; (S) sample (C) cholesterol as a control

Figure 3 shows a bluish-green spot on the sample, indicating the presence of steroid compounds in the 70% ethanol of coleus leaves with an Rf of 0.2. This is consistent with a study by (Lisdawati *et al*., 2012), which also showed the presence of steroid compounds in the extract of miana leaves. The use of specific spot reagent Lieberman Burchard aimed to react with the spot by spraying it onto the TLC plate, making the spot visible under UV light at λ366. The identification of steroid compounds by TLC strengthened the results of the phytochemical screening of the herbarium specimen of the coleus leaves.

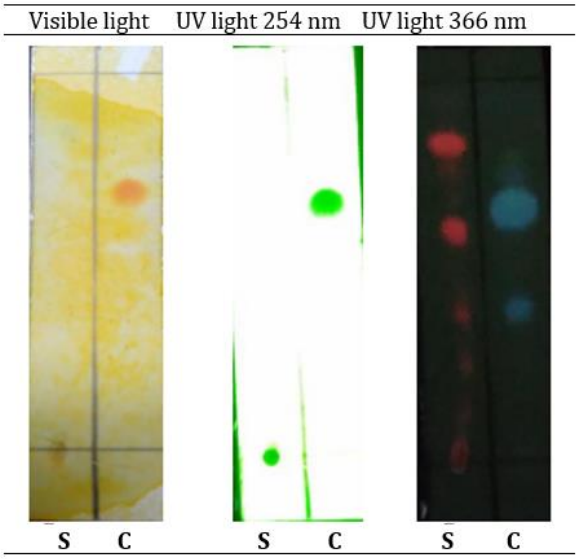

Figure 4. TLC profile of flavonoid compounds in 70% ethanol extract of coleus leaves (*S. scutellarioides* (L.) Codd) after spraying with Dragendorff spot reagent; (S) sample (C) piperine as a control

The lack of yellow spot on the sample in the **Figure 4** suggests that the 79% ethanol of coleus leaves does not certain any alkaloid compounds. This is consistent with previous study, which also demonstrated the absence of alkaloid compounds in the coleus leaves extract (Lisdawati *et al*., 2012). The use of specific spot reagent Dragendorff aimed to react with the spot by spraying it onto the TLC plate, making the spot visible under UV light at λ 366. The alkaloid test by TLC gave a negative result, because the 70% ethanol of coleus leaves contains a very little quantity of alkaloid, making it undetectable. The identification of alkaloid compounds by TLC supported the results of the herbarium specimen of the coleus leaves.

3.5. α-Amylase enzyme inhibition test

The average % inhibition of the α-amylase enzyme by 100 ppm acarbose and 200 ppm of the 70% ethanol of coleus leaves can be seen in **Table 3**.

Table 3. Percentage of α-amylase enzyme inhibition by 70% ethanol extract coleus leaves (*S. scutellarioides* (L.) Codd)

Sample	Average inhibition (%± SD)	Sig. (2-tailed)
70% ethanol extract of coleus leaves at 200 ppm	61.03±1.04	0.043
Positive control (acarbose) at 100 ppm	92.16±0.85	

The 70% ethanol extract of coleus leaves was able to inhibit the α-amylase enzyme with an inhibition percentage of 61.03%. This indicates that coleus leaves extract has potential as an anti-hyperglycemic agent because it can inhibit enzymes that metabolize carbohydrates, with an α-amylase enzyme inhibition percentage exceeding 50%. In the meantime, 92.16% of the α-amylase enzyme could be inhibited by acarbose as the positive control. The t-test method of statistical analysis

revealed that the percentage of enzyme inhibition by the positive control, acarbose, was higher than that of the coleus leaves extract, and that the difference between the two was statistically significant (p<0.05). This implies that in order to attain more ideal enzyme inhibition, a higher concentration of coleus leaves extract might still be required.

In a different *in vivo* study, mice given alloxan showed the greatest decrease in blood glucose percentage when given coleus leaves at a dose of 200 mg/kg BW. Due to the paucity of research on the *in vitro* antihyperglycemic activity of coleus leaves extract, the use of 200 ppm concentration of coleus extract leaves in this study was based on this *in vivo* previous research, which used 200 mg/kg BW *in vivo* (Lestari, 2022). The results of this study indicate that the 200-ppm concentration of coleus leaves extract, which inhibit 61.03% of α-amylase, can still be increased to achieve the optimal inhibition enzyme responsible for carbohydrate lysis.

The 70% ethanol extract of coleus leaves contained flavonoids, tannins, saponins, and steroids, according to the phytochemical screening and TLC profile analysis. These bioactive compounds may be the reason for the inhibition mechanism of coleus leaves on α-amylase because they bind to the enzyme's active site and prevent it from interacting with its substrate, starch, which inhibits the breakdown of starch into glucose and lowers blood sugar levels. These bioactive compound act as competitive inhibitors of the enzyme because they have similar structures to substrate and compete to occupy the enzyme's active site, thereby inhibiting α-amylase (Nafiu & Tom Ashafa, 2017; P *et al*., 2011; Proença *et al*., 2019).

The α-amylase enzyme plays a key role in the early stages of carbohydrate digestion, and its activity determines how quickly and how much glucose is produced from food. The more active the α-amylase enzyme, the faster the conversion of carbohydrates into glucose, the higher the possibility for postprandial blood glucose spikes. Therefore, inhibiting this enzyme's activity is crucial to help control blood glucose levels, particularly after meals in patients with type II diabetes mellitus (Wahyuntari, 2011).

4. Conclusion

The herbarium specimen and 70% ethanol extract of coleus (*S. scutellarioides* (L.) Codd) contain secondary metabolites such as flavonoids, tannins, saponins, and steroids. The 70% ethanol extract of coleus leaves exhibits α-amylase enzyme inhibition activity with an inhibition percentage of 61.03%, although its potential is lower than acarbose as the positive control, which has an enzyme inhibition percentage of 92.16%.

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