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RESEARCH ARTICLE

Method Development for Determination of Cyanidin-3-O-Glucoside Level in Combination of Roselle and Butterfly Pea Flower Extracts by TLC-Densitometry and its Correlation with Antidiabetic and Antioxidant Activities

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ABSTRACT:
Background: Roselle flowers (*Hibiscus sabdariffa* L.) and butterfly pea flowers (*Clitoria ternatea* L.) are known for their antioxidant and antidiabetic activities. The combination of these flowers has the potential to be developed as a phytopharmaceutical. Cyanidin-3-O-glucoside (Cy3G's), a key compound in both flowers, is believed to play a significant role in these biological activities. However, the development of a reliable analytical method to detect this compound is essential for ensuring the quality of raw materials. **Objective:** This study aims to develop and validate a TLC-Densitometry method for determining Cy3G's levels in a combination of roselle and butterfly pea flower extracts, and to correlate the results with their antioxidant and antidiabetic activities. **Methods:** The research was conducted in three main stages: 1) Extract preparation using the maceration method, 2) Development and validation of a TLC-Densitometry analytical method for Cy3G's identification and quantification, and 3) Antioxidant activity testing using the DPPH method and antidiabetic activity testing based on the inhibition of the alpha-glucosidase enzyme. **Results:** The developed TLC-Densitometry method, using a mobile phase of n-butanol, acetic acid, and water (4:1:5 v/v/v), was validated and met the required parameters of specificity, linearity, accuracy, and precision. Results showed that butterfly pea flower extract had the highest Cy3G's content, followed by the combination extract and roselle extract. The strongest antioxidant activity was observed in butterfly pea flower extract (IC50 0,0979 mg/mL), categorized as strong, while the combination extract showed moderate antioxidant activity (IC50 0,1101 mg/mL). However, antidiabetic activity in all samples was weak. **Conclusion:** The developed TLC-Densitometry method can be used for determining Cy3G's levels in a combination of roselle and butterfly pea flower extracts. Butterfly pea flower extract demonstrated the greatest antioxidant potential, while antidiabetic activity was relatively weak across all samples.

KEYWORDS: Chromatography, Phytochemical, in vitro, DPPH, IC_{50}.

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INTRODUCTION:

Indonesia has a diverse range of plants spread across various regions, which hold great potential to be developed as raw materials for phytopharmaceuticals to support the health sector, especially in addressing degenerative diseases. One of the degenerative diseases that has a high prevalence in Indonesia is Diabetes Mellitus (DM). According to data from the World Health Organization, the number of DM sufferers in Indonesia ranks fourth in the world and is expected to increase to 21.3 million cases by 2030.

Roselle flowers (*Hibiscus sabdariffa* L.) have been widely reported to have antidiabetic activity[1–4]. The administration of roselle flower extract at various doses has been shown to reduce blood sugar levels[2,4] through mechanisms that inhibit intestinal α-glucosidase and pancreatic α-amylase[3]. In addition to roselle flowers, butterfly pea flowers (*Clitoria ternatea* L.) have also been reported to exhibit antidiabetic activity[5–9]. The administration of butterfly pea flower extract at a dose of 500mg/kg BW has been reported to reduce blood glucose levels comparable to metformin at 100mg/kg BW[5]. Both roselle and butterfly pea flowers are also recognized for their antioxidant properties[10,11]. The antidiabetic and antioxidant activities are attributed to the presence of an important marker compound in both flowers, namely the anthocyanin type Cy3G's[12–16]. The combination of roselle and butterfly pea flower extracts has great potential to be developed as a phytopharmaceutical raw material for DM therapy.

In the development of phytopharmaceutical preparations, quality assurance of raw materials through the identification and quantification of marker compound levels is crucial as part of product quality control. Currently, there is no standard method for the identification and determination of Cy3G's levels in roselle and butterfly pea flowers in the Indonesian Herbal Pharmacopoeia (FHI) monograph[17]. Therefore, it is necessary to develop a method for analyzing this compound[18–20]. TLC-Densitometry is a reliable method for analyzing natural materials as it can separate various compounds in the sample based on polarity differences. Consequently, the TLC-Densitometry method was chosen for the analysis of Cy3G's. The validation parameters for the analytical method were assessed in this study to demonstrate that the developed method is accurate, reproducible, and reliable.

In addition to quality assurance of the raw materials, it is also essential to test the activity of these materials to ensure the effectiveness of the phytopharmaceutical product. Therefore, the antidiabetic and antioxidant activity tests of the combination of roselle and butterfly pea flower extracts are necessary to confirm their bioactivity in lowering blood sugar levels and their capacity to counteract free radicals. The findings of this study are expected to serve as a foundation for the development of phytopharmaceutical products for DM therapy using quality-assured raw materials from a combination of roselle and butterfly pea flower extracts.

MATERIALS AND METHODS:

Materials:

Clitoria ternatea and *Hibiscus sabdariffa* were sourced from the Omah Sehat Mandiri Pangan Plantzone plantation in Krakal Klemunan Wlingi District, Blitar, East Java, Indonesia. Cyanidin-3-O-Glucoside Chloride (Sigma US), TLC Silica Gel 60 F254 (E Merck, Darmstadt, Germany), DPPH (Sigma US), and analytical grade ethanol, methanol, quercetin, water, n-butanol, acetic acid, ethyl acetate, toluene, chloroform, formic acid, acetone, and hydrochloric acid (all E Merck, Darmstadt, Germany) were used in the study

Extraction of Butterfly Pea Flower:

Extraction Method:

The extraction method follows the Indonesian Herbal Pharmacopoeia Edition II (2017)[17]. The simplicia of roselle and butterfly pea flowers is sorted and reduced in size. The simplicia is placed into a macerator with a 70% ethanol solvent added at a ratio of 1:10. The simplicia is soaked for 6 hours with occasional stirring and left for 18hours. The macerate is then separated by filtration, and further maceration is conducted. The resultant macerate is evaporated to yield a thick extract.

Extract Characterization:

Characterization of the extract is based on the Indonesian Herbal Pharmacopoeia Edition II (2017)[17] and includes the following parameters:

1. **Description:** Visual and sensory checks on the extract, including texture, color, odor, and taste.
2. **Water Content:** Weigh 10 grams of the sample in a pre-weighed porcelain cup. Dry the sample at 105°C for 5 hours and weigh. Repeat drying and weighing at hourly intervals until the difference between weighings is no more than 0.25%.
3. **Total Ash Content:** Weigh 3 grams of the powdered sample in a pre-weighed silicate crucible, incinerate until the charcoal is completely burned, cool, and weigh.
4. **Acid-Insoluble Ash Content:** Boil the ash obtained from the total ash determination with 25 mL of dilute hydrochloric acid for 5 minutes. Collect the insoluble part and filter through ash-free filter paper, washing with hot water until constant weight at 800±25ºC. Calculate the acid-insoluble ash content as a percentage of the sample weight.
5. **Total Anthocyanin Content (TAC):**
   To prepare a potassium chloride buffer, dissolve 10.465 g of potassium chloride in distilled water and place it in a 250 mL volumetric flask. Continue adding hydrochloric acid until the pH approaches 1. For the sodium acetate buffer, dissolve 8.2 g of sodium acetate in distilled water at pH 4.5 in a 250 mL volumetric flask. Again, add hydrochloric acid until the pH reaches 4.5. The total anthocyanin content was determined using a modified method based on Lee's technique. This method utilizes the differential method between a potassium chloride buffer (pH 1) and a sodium acetate buffer (pH 4.5). Samples were diluted with both buffers prior to analysis, and both buffers were tested against a blank cell filled with distilled water after standing for 60

minutes at 700nm (for haze correction). The following equation was used to calculate total anthocyanin content:

$$Total\ anthocyanin\ \left(\frac{mg}{L}, CyE, ME\right) = \frac{A*MW*DF*10^3}{\varepsilon*1}$$

Where:

A = (A520nm – A 700nm) pH 1.0 – (A520nm – A700 nm) pH 4.5;

MW (molecular weight) = 449.2 g/mol for Cy3G's;

DF = dilution factor established in D;

l = pathlength in cm;

ε = 26900 molar extinction coefficients, in $L \cdot mol^{-1} \cdot cm^{-1}$, for Cy3G's;

and 10^3 = factor for conversion from g to mg.

Preparation of Cy3G's Standard Solution:

Weigh 10mg of Cy3G's standard and dissolve it in 10 mL of ethanol. The standard solution was then diluted to the required concentrations for testing.

Preparation of Sample Solution:

Weigh 300mg of roselle flower extract and butterfly pea flower extract, each dissolved in 5mL of ethanol. For the combination sample, equal amounts of roselle flower extract and butterfly pea flower extract were weighed and homogenized, then dissolved in 5mL of ethanol.

Optimization of TLC-Densitometry Method and Validation of Analysis Method:

Chromatography conditions were optimized to determine the most suitable mobile phase and spotting volume. The mobile phase compositions to be optimized included mixtures of ethyl acetate: toluene: water: formic acid (12: 3: 0.8: 1.2 v/v/v/v) (MP1); n-butanol: acetic acid: water (4: 1: 5 v/v/v) (MP2); and chloroform: ethyl acetate: formic acid (5: 4: 1 v/v/v) (MP3), with spotting volumes varying from 2 to 14 µL. The optimal chromatography conditions were validated according to USP-NF (2024) with Category I parameters, namely specificity, linearity, accuracy, and precision.

Identification and Determination of Cy3G's Levels:

The validated method was utilized for the identification and quantification of Cy3G's levels in samples of roselle flower extract, butterfly pea flower extract, and a combination of both extracts. The F254 silica gel plate was dried in an oven at 105°C for 1 hour, and then the sample and standard solutions were spotted at the optimal volume and eluted using the selected mobile phase. The elution results were read on a densitometer.

Antioxidant Activity Determination of Roselle and Butterfly Pea Flower Extracts Using the DPPH Assay:

The DPPH assay was conducted according to the method of Nurhayati *et al.* (2024)[10]. DPPH was prepared at a concentration of 50ppm. A blank DPPH was prepared by mixing 2mL of DPPH solution with 2mL of ethanol. Quercetin as a standard solution was prepared at a concentration range of 0.5 - 2.5ppm and reacted with DPPH. Samples of roselle flower extract, butterfly pea flower extract, and the combination extracts were prepared at concentrations ranging from 2 - 400ppm and then reacted with DPPH. All solutions reacted with DPPH were measured for their absorbance using a spectrophotometer, and the IC50 value was determined.

Antidiabetic Activity Determination of Roselle and Butterfly Pea Flower Extracts Using In Vitro (α-Glucosidase Inhibition Method):

50µL of 0.1M phosphate buffer saline (pH 6.9) was placed into a plate, followed by the addition of 10µL of 1 ppm glucosidase enzyme and 20µL of sample or standard. Each concentration was placed into 3 wells (triplicates) and incubated for 15minutes at 37°C. After incubation, 20µL of 4-NPP was added, and incubation was continued for another 30 minutes at 37°C. Finally, 50µL of 0.1 M Na2CO3 was added to stop the enzyme reaction. The results were read at a wavelength of 405 nm.[21,22]

RESULT:

Extract Characterization:

Table 1. Results of Extract Characterization Parameters

Parameter	Rosella Flower Extract	Butterfly Pea Flower Extract
Yield weight	65,02% ± 0,0011	53,18% ± 0,0010
Description	Thick extract, red liver color, distinctive odor, sour taste	Thick extract, blackish-purple color, distinctive odor, bland taste
Water content	0,68% ± 0,0002	0,71% ± 0,0004
Total ash content	3,25% ± 0,0017	3,17% ± 0,0010
Acid insoluble ash content	0,09% ± 0,0001	0,14% ± 0,0001

Total Anthocyanin Content (TAC):

The maximum wavelength measurement for anthocyanins was obtained at 543 nm. From these results, the total anthocyanin content of butterfly pea flower extract, roselle flower extract, and the combination of both extracts can be determined.

Table 2. Result of The Total Anthocyanin Content of Extracts

Sample	Anthocyanin Monomer of Extract	Replication	TAC (mgCyE/L)	Mean ± SD
Extract of butterfly pea flower	Cyanidin	1	35,497	35,259 ± 0,259
		2	35,021	
		3	35,438	
Extract of roselle flower	Cyanidin	1	12,519	12,516 ± 0,023
		2	12,492	
		3	12,538	
Combination extract	Cyanidin	1	22,393	22,280 ± 0,148
		2	22,112	
		3	22,335	

Table 3. Results of Optimization of Analysis Method

Sampple	Mobile Phase	Volume of spotting (µL)	Peak Purity*	Peak Shape	Rf	Spectra Correlation**
Extract of butterfly pea flower	MP1	2	0.999	Symmetrical	0,023	0,997
		6	0.999	Symmetrical	0,018	0,996
		10	0.999	Symmetrical	0,013	0,997
		14	0.999	Tailing	0,013	0,998
Extract of roselle flower	MP1	2	0.999	Symmetrical	0,013	0,997
		6	0.999	Symmetrical	0,013	0,991
		10	0.999	Symmetrical	0,013	0,997
		14	0.999	Tailing	0,015	0,997
Extract of butterfly pea flower	MP2	2	0.999	Symmetrical	0,343	0,998
		6	0.999	Symmetrical	0,328	0,998
		10	0.999	Symmetrical	0,328	0,999
		14	0.999	Tailing	0,328	0,999
Extract of roselle flower	MP2	2	0.999	Symmetrical	0,328	0,999
		6	0.999	Symmetrical	0,328	0,999
		10	0.999	Symmetrical	0,328	0,999
		14	0.999	Tailing	0,328	0,999
Extract of butterfly pea flower	MP3	2	0.999	Symmetrical	0,103	0,998
		6	0.999	Symmetrical	0,098	0.997
		10	0.999	Tailing	0,118	0,997
		14	0.999	Tailing	0,110	0,998
Extract of roselle flower	MP3	2	0.999	Symmetrical	0,105	0,994
		6	0.999	Symmetrical	0,110	0,998
		10	0.999	Tailing	0,110	0,999
		14	0.999	Tailing	0,099	0,998

*≥0,999 means pure
** correlation of sample spectra compared with standard spectra, *≥0.990 indicates identical

Method Optimization and Validation of Analysis Methods:

Table 4. Result of Validation of Analysis Method

Parameter	Description
Linearity	r = 0,9844; Vx_0 = 3,23%
Specificity	Peak purity = 0,999; Spectrum correlation = 0,976
Accuracy	% recovery = 101,4 – 102,5%
Precision	% RSD = 3,0%

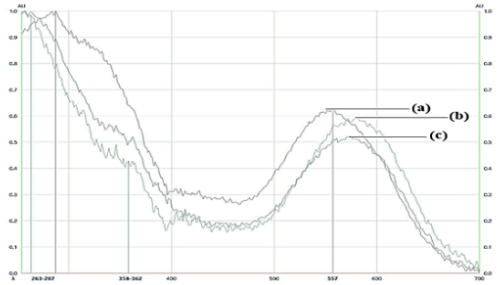

Figure 1. Spectral correlation between (a) Cyanidin-3-O-Glucoside (b) Butterfly pea flower extract (c) Roselle flower extract

Determination of Cy3G's Levels:

The determination of Cy3G's levels was carried out using a regression curve derived from the concentrations of standard solutions ranging from 0.5 – 2.5µL.

Table 5. Result of Cyanidin-3-O-Glucoside Content

Sample	Anthocyanin Monomer of Extract	Replication	Content mg/gram extract)	Mean ± SD
Extract of butterfly pea flower	Cyanidin	1	0,085	0,086 ± 0,001
		2	0,088	
		3	0,086	
Extract of roselle flower	Cyanidin	1	0,016	0,014 ± 0,003
		2	0,015	
		3	0,011	
Combination Extract	Cyanidin	1	0,046	0,044 ± 0,003
		2	0,041	
		3	0,045	

Antioxidant Activity:

Standard curves were obtained from concentrations of 0.5 – 2.5ppm for quercetin, 10 – 120ppm for butterfly pea flower extract, 100 – 350ppm for roselle flower extract and 10 – 120ppm for the combination extract. The results of the linear regression equation were used to determine the IC_{50}

Table 6. Antioxidant Strength of Standards and Samples

Sample	Replication	IC_{50} (mg/mL)	Mean ± SD
Quercetin	1	0,0016	0,0016 ± 0,005
	2	0,0016	
	3	0,0016	
Extract of butterfly pea flower	1	0,0977	0,0979 ± 0,912
	2	0,0971	
	3	0,0989	
Extract of roselle flower	1	0,2519	0,2509 ± 0,921
	2	0,2508	
	3	0,2501	
Combination Extract	1	0,1097	0,1101 ± 0,666
	2	0,1109	
	3	0,1097	

Antidiabetic Activity:

Acarbose, the control extract for butterfly pea flower and roselle flower, produced curves obtained from concentrations of 0.003 – 0.1ppm, 150 – 1250ppm, and 150 – 5000ppm, respectively. The results of the linear regression equation were used to determine the IC_{50}.

Table 7. Antidiabetic Strength of Standards and Samples

Sample	Replication	IC_{50} (mg/mL)	Mean ± SD
Acarbose	1	$0{,}0012.10^{-2}$	$0{,}0012.10^{-2}$ ± 0,149
	2	$0{,}0012.10^{-2}$	
	3	$0{,}0013.10^{-2}$	
Extract of butterfly pea flower	1	0,6430	0,6362 ± 0,060
	2	0,6278	
	3	0,6377	
Extract of roselle flower	1	0,6313	0,6201 ± 0,081
	2	0,6119	
	3	0,6173	
Combination Extract	1	2,4698	2,5614 ± 0,156
	2	2,6106	
	3	2,6068	

DISCUSSION:

Extract Characteristics:

The characterization results of the extracts based on parameters defined by the Indonesian Herbal Pharmacopoeia indicate that all parameters meet the requirements, suggesting that the extracts used are of good quality[17]. Comparing both extracts reveals that the yield of roselle flower extract is higher (6502%± 0.0011) than that of butterfly pea flower (5318%± 0.0010). The color of the roselle flower extract is deep red with a distinctive odor and sour taste, while the butterfly pea flower extract is blackish-purple with a distinctive odor and bland taste. The moisture content and total ash content are relatively similar, although the acid-insoluble ash content is higher in the butterfly pea flower (0.14%±0.0001) compared to roselle (0.09%± 0.0001), indicating a difference in the content of inorganic compounds that are insoluble in acid.

Total Anthocyanin Content (TAC):

The anthocyanin levels were determined using the pH differentiation method, utilizing light absorbance at a visible wavelength of 700nm. The pH values used were 1.0 and 4.5. Absorbance measurements at 700nm serve as a correction factor. The maximum wavelength measurement for anthocyanins was obtained at 543nm. This aligns with research conducted by Harborne, which states that anthocyanin pigments, which exhibit red, purple, and blue colors, have a wavelength range between 515 and 545nm.

Testing on the samples of roselle flower extract, butterfly pea flower extract, and a combination of both indicates that the total anthocyanin levels range from lowest to highest as follows: roselle flower extract, combination extract, and butterfly pea flower extract. This is consistent with findings by Quattrocchio et al. (2005), which showed that butterfly pea flowers contain anthocyanin pigments contributing to their purple color. The total anthocyanin content is also influenced by the compatibility factor between the polarity of the solvent and the solute, allowing anthocyanins to dissolve effectively and produce high anthocyanin levels.[23] In this study, 70% ethanol was utilized to extract anthocyanins, which is effective for polar compounds like anthocyanins. This is supported by the good yield percentage obtained, which was 53.18%[24].

Anthocyanin compounds are stable at acidic pH, so hydrochloric acid was added during buffer preparation. The addition of hydrochloric acid helps hydrolyze anthocyanins usually present in aglycone forms, making them measurable.An increasingly acidic pH (approaching 1) results in a greater amount of colored flavilium or oxonium cations, indicating a higher concentration of anthocyanins and corresponding

absorbance readings. Conversely, at pH 4.5, this cation changes to a more stable semi-concentrated form, reducing color intensity to near colorless, resulting in low absorption readings. This indicates the presence of interfering compounds under testing conditions.

Results indicate that butterfly pea flowers possess higher TAC (35259±0.259mg CyE/L) compared to roselle flowers (12516±0.023mg CyE/L). The combination extract resulted in a lower TAC than the butterfly pea flower alone but still higher than roselle (22280±0.148 mg CyE/L). This suggests that butterfly pea flowers contain a higher concentration of anthocyanins, which are essential as pigments and antioxidants.

Optimization and Validation of Analysis Method:

The optimization results for chromatographic conditions indicate that the mobile phase comprising n-butanol, acetic acid, and water (4:1:5 v/v/v) with a spotting volume of 10µL yielded symmetrical peaks with peak purity above 0.999 for all extracts and provided good Rf values. Other mobile phases yielded relatively low Rf values, indicating suboptimal performance due to the nonpolar nature of the mobile phase, which caused Cy3G's to be retained on the stationary phase. Spotting volumes smaller than 10µL also yielded symmetrical peaks but the desired area was too small, suggesting that the spotting volume affects peak area and shape. Higher concentrations may result in peak tailing. Therefore, the selected optimum conditions comprised a mobile phase of n-butanol, acetic acid, and water (4:1:5 v/v/v) with a spotting volume of 10µL. These results demonstrate that the method can effectively separate components and produce consistent results, making it reliable for further analysis. Validation of the method indicates that it meets all requirements with good linearity (r = 0.9844), accuracy (% recovery 101.4% – 102.5%), and adequate precision (%RSD = 3.0%). This suggests that the method can accurately and precisely quantify the analyzed compounds.

Cyanidin 3-O-Glucoside Level:

Cy3G's is a type of anthocyanin often found in plants, characterized by a chemical structure where the cyanidin molecule is bound to glucose sugar. Cy3G's exhibits strong antioxidant activity and imparts red or purple colors to plants containing this compound. In this study, the determination of Cy3G's level was performed using the TLC-densitometry method with a mobile phase mixture of n-butanol, acetic acid, and water (4:1:5 v/v/v).

Testing of the roselle flower extract, butterfly pea flower extract, and the combination extract reveals that the Cy3G's levels range from lowest to highest as follows: roselle flower extract, combination extract, and butterfly pea flower extract. These findings align with previous tests indicating that butterfly pea flower extract possesses the highest anthocyanin content. The method commonly employed for determining Cy3G's levels in plant extracts is UV-Vis spectrophotometry. Previous studies have indicated that Cy3G's has a maximum absorption wavelength between 520-535nm, making it measurable using a spectrophotometer at this wavelength. The Cy3G's content in the extract can then be calculated based on a calibration curve created from a standard sample of known concentration. The determination of Cy3G's levels indicates that butterfly pea flowers contain higher concentrations (0.086±0.001 mg/gram extract) than roselle flowers (0.014±0.003 mg/gram extract). The combination of both extracts yields lower concentrations (0.044±0.003mg/gram extract) compared to the butterfly pea flower alone but higher than roselle. This indicates that Cy3G's, which functions as an antioxidant, is more abundant in butterfly pea flowers. These results are consistent with findings by Quattrocchio et al. (2005), which indicated that butterfly pea contains higher levels of anthocyanin pigments that contribute to the flower's purple color.[23] This study emphasizes that Cy3G's content plays an important role in antioxidant activity and may also influence antidiabetic effects, although not as strongly as expected.

Antioxidant Activity Measured using DPPH assay:

DPPH is a free radical commonly used in antioxidant research. This method is simple, rapid, accurate, reliable, and practical. The ability to scavenge free radicals correlates with the ability of these compounds to donate electrons to free radicals; these electrons react and decolorize DPPH, changing its color from purple to yellow. The testing process begins by determining the maximum wavelength, which falls within the range utilized for the DPPH assay (500-520nm).[25,26]

In this study, the determination of the maximum wavelength was conducted twice during the antioxidant activity tests on quercetin and the samples, as factors such as temperature, humidity, sample reading time, and light could influence the results. External light entering the cuvette may increase light measurement. Results showed that the IC50 values of quercetin in replicates 1, 2, and 3 had an average of 0,0016mg/mL, categorizing it as a very strong antioxidant based on IC50 value parameters, where values ≤ 50ppm indicate a very strong antioxidant. The butterfly pea flower extract demonstrated IC50 values averaging 0,0979mg/mL, categorizing it as a strong antioxidant. In contrast, the roselle flower extract exhibited IC50 values averaging 0,2509mg/mL, categorizing it as a weak antioxidant, while the combination of both extracts showed IC50 values averaging 0,1101mg/mL, categorizing it as a

moderate antioxidant. Butterfly pea flowers exhibit stronger antioxidant activity compared to roselle flowers, which are significantly weaker. The combination of both extracts shows moderate activity. The higher antioxidant activity in butterfly pea flowers may be attributed to their greater anthocyanin content.

The results of this study differ from research conducted by Andriani and Murtisiwi (2020), which reported the antioxidant activity of butterfly pea flowers using 70% ethanol solvent with an IC50 value of 41.36±1.191 µg/mL, categorizing it as very strong.[27] This difference may be due to variations in altitude where the plants are grown, as well as differences in the concentration of ethanol used for extraction. The phytochemical content of secondary metabolites such as flavonoids can vary by region, influenced by environmental factors including light, temperature, pH, altitude, and humidity. The antioxidant activity in butterfly pea flowers is likely influenced by the activity of secondary metabolites in the extract, particularly flavonoids. The results indicate that the ethanol extract of butterfly pea flowers possesses higher antioxidant power than both roselle flower extract and the combination of extracts. This aligns with the principle of solubility, where flavonoids are polar and thus dissolve better in polar solvents like water, while ethanol is a semi-polar solvent. Consequently, the water solvent is better at extracting flavonoids than ethanol. This is consistent with previous test results indicating that butterfly pea flower extract contains the highest anthocyanin and Cy3G's levels compared to roselle flower extract and their combination.

Antidiabetic Activity:

Antidiabetic activity was measured using IC50 values, indicating that acarbose as a control exhibited very strong activity (IC50 $0.0012.10^{-2}$±0.149 mg/mL). In contrast, both butterfly pea and roselle flowers displayed very weak antidiabetic activity (IC50 values around 0,6362 ± 0.060 and 0,6201±0.081 mg/mL, respectively). The combination of both extracts also demonstrated weak activity (IC50 0,2561±156mg/mL), indicating that these extracts are less effective as antidiabetic agents compared to the control. In the study by Andriani and Murtisiwi (2020), which also evaluated the antidiabetic activity of butterfly pea using the DPPH method, it was found that the extract had lower effectiveness compared to the metformin control.[27] These results align with this study, which indicates that although butterfly pea has high levels of anthocyanins and Cy3G's, its antidiabetic activity remains weak.

Correlation Between Cy3G's Levels and Antioxidant and Antidiabetic Activities:

The stronger antioxidant activity observed in butterfly pea flowers can be correlated with the higher content of Cy3G's, as this compound acts as a potent antioxidant. In other words, higher Cy3G's content corresponds to greater antioxidant capacity of the extract. However, despite butterfly pea flowers containing higher Cy3G's levels and demonstrating stronger antioxidant activity, this does not directly enhance their antidiabetic activity, which remains weak compared to acarbose as a control. This suggests that Cy3G's may not significantly contribute to the mechanism of inhibiting the alpha-glucosidase enzyme, which is associated with antidiabetic activity. Strong antioxidant activity, as seen in butterfly pea flowers, does not necessarily correlate with strong antidiabetic activity. This indicates that both activities are governed by different mechanisms, and a compound effective as an antioxidant may not be effective as an antidiabetic agent. Other factors, in addition to Cy3G's content, may play a role in antidiabetic activity, resulting in the weak inhibition of alpha-glucosidase by both butterfly pea and roselle flowers.

CONCLUSION:

Butterfly pea flowers exhibit greater potential than roselle flowers in terms of anthocyanin content, antioxidant activity, and Cy3G's levels. However, both flowers display relatively weak antidiabetic activity. The combination of both extracts does not always enhance the effects compared to the individual extracts, although it provides satisfactory results in some parameters. The high Cy3G's content in butterfly pea flowers indeed increases antioxidant activity but does not sufficiently enhance antidiabetic activity. This highlights the importance of other compounds in determining antidiabetic potential and indicates that, while antioxidants like Cy3G's are beneficial, they may not always be effective across all types of biological activities, such as antidiabetic activity. This study highlights the importance of further testing to understand the mechanisms of action of these compounds and their potential in treating degenerative diseases such as diabetes.

CONFLICT OF INTEREST:

There is no conflict of interest in this research.

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