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Utilization of dried butterfly pea flower (*Clitoria ternatea* L.) as a source of natural antioxidants: determination of total flavonoid content and activity test with dpph method

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Abstract : Butterfly pea flower (Clitoria ternatea L.) is a herbaceous plant known for its high content of bioactive compounds, particularly flavonoids, which serve as natural antioxidants. Commonly used in traditional medicine and functional foods, this flower's ability to neutralize free radicals makes it a valuable natural resource. This study evaluates the antioxidant potential of dried butterfly pea flowers by determining their total flavonoid content and assessing antioxidant activity using the DPPH method. A key novelty of this research lies in the use of controlled oven drying at 50°C, which differs from previous studies employing sunlight drying. This method preserved flavonoids more effectively, resulting in a higher flavonoid content (40.60 mg/100 mg extract) compared to prior reports. Phytochemical screening also detected the presence of saponins and tannins, broadening the understanding of its bioactive profile. Antioxidant activity was evaluated using the DPPH method, yielding an IC50 value of 36.06 ppm, indicating very strong activity. Comparatively, vitamin C as a positive control showed an IC50 value of 2.13 ppm. These findings underscore the advantages of optimized drying and extraction methods, highlighting dried butterfly pea flowers as a promising source of natural antioxidants for applications in functional foods and health products.

Keywords : Butterfly pea flower; Clitoria ternatea L.; flavonoids; antioxidant activity; DPPH method.

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Introduction

Butterfly pea flower (*Clitoria ternatea* L.) is an herbaceous plant known to be rich in secondary metabolites with potential as a source of natural antioxidants. The secondary metabolites found in butterfly pea flowers include flavonoids, tannins, anthocyanins, phenolics, and saponins (Cahyaningsih et al., 2019) (Yumni et al., 2022). Among these, flavonoids are the most dominant, contributing significantly to the plant's biological activities, particularly its antioxidant properties. The dried form of butterfly pea flowers is widely used in traditional medicine and functional foods due to its high content of bioactive compounds and ability to scavenge free radicals effectively.

Antioxidant activity is crucial for preventing oxidative damage caused by free radicals, which can trigger various diseases such as cancer, cardiovascular diseases, diabetes, and premature aging (Syauqul Jannah, Dika Rizki Kurniawan, 2022). Therefore, research into the antioxidant activity of medicinal plants like butterfly pea flower is vital. The DPPH (*2,2-diphenyl-1-picrylhydrazyl*) method is widely used to assess antioxidant activity, measuring a compound's capacity

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to neutralize free radicals (Sumartini & Ikrawan, 2020) (Syauqul Jannah, Dika Rizki Kurniawan, 2022).

Previous studies have shown that butterfly pea flowers have significant antioxidant activity. Several studies report IC_{50} test results, which represent the concentration required to inhibit 50% of DPPH free radical activity. A lower IC_{50} value indicates stronger antioxidant activity. According to research, the aqueous extract of butterfly pea flowers has an IC_{50} value of 259.84 ± 0.50 ppm. The ethanol extract demonstrated a stronger antioxidant activity with an IC50 value of 87.86 ppm (Cahyaningsih et al., 2019). Additionally, herbal tea made from butterfly pea flowers has an IC_{50} value of 13.72 ppm when extracted at 70°C for 5 minutes, indicating high antioxidant activity (Aqila et al., 2023).

Moreover, a functional beverage formulated with butterfly pea flower showed high antioxidant activity with an IC50 value of 80.14 ppm (Kurniadi et al., 2024). The study identified the optimal concentration of butterfly pea extract for functional drinks, with 15 mL being the best concentration for desirable taste, aroma, color, and significant antioxidant activity.

Further research on the fractionation of butterfly pea ethanol extract revealed significant flavonoid content. Tests on the aqueous and ethyl acetate fractions of the ethanol extract showed total flavonoid levels of 10.9 ± 2.029 mgQE/gram and 47 ± 3.026 mgQE/gram extract, respectively. Another study reported total flavonoid content of butterfly pea flowers from North Lombok and Wonosobo to be 59.37 and 63.09 mgEQ/g of dry extract. These flavonoid levels indicate the flower's strong potential as a natural antioxidant source (Yumni et al., 2022).

Besides flavonoids, butterfly pea flowers contain large amounts of anthocyanins, which serve as pigments and have strong antioxidant activity (Ramdan, 2024) (Sumartini & Ikrawan, 2020). An analysis using the LC-MS/MS method identified that the butterfly pea extract contains 12 types of anthocyanins, 18 flavonols, and 11 flavones. These secondary metabolites contribute to the flower's strong antioxidant activity. The antioxidant activity of butterfly pea flowers can be influenced by factors such as pH variation, extraction temperature, and processing methods, which affect the active compound content (Sumartini & Ikrawan, 2020).

Studies have demonstrated that butterfly pea flowers, in both fresh and dried forms, possess great potential as a source of natural antioxidants. Hence, further research into determining total flavonoid content and assessing antioxidant activity using the DPPH method in dried butterfly pea flowers is crucial to maximize their use as functional food ingredients and a safe and effective antioxidant source for health (Kurniadi et al., 2024).

The key novelty of this research lies in the use of controlled oven drying at 50°C, a technique that differs from conventional drying methods used in previous studies. This controlled drying method minimizes the degradation of heat-sensitive bioactive compounds, such as flavonoids and anthocyanins, which are critical for antioxidant activity. By comparing IC50 values and total flavonoid content obtained under this method to those reported internationally, this study seeks to provide insights into optimizing butterfly pea flower processing for enhanced antioxidant potential.

Furthermore, leveraging a broad and comprehensive set of references, this research highlights existing gaps in the literature concerning drying techniques, bioactive compound preservation, and antioxidant activity assessment. The findings aim to contribute to the growing body of knowledge on butterfly pea flowers as a functional food ingredient and a safe, effective source of antioxidants for health applications. This study also builds on and supports international research by offering a deeper understanding of the effects of controlled drying methods on the antioxidant activity of butterfly pea flowers.(Aqila et al., 2023) (Permata Kumala Sari, Yupink Aprillya Santo, 2024).

Materials and Methods

Materials

Simplicia of butterfly pea flower (Clitoria ternatea L.), ammonia, chloroform, hydrochloric acid (1:10), Dragendorff's reagent, Mayer's reagent, concentrated hydrochloric acid, magnesium powder, amyl alcohol, distilled water (aquadest), ethanol 70%, iron (III) chloride solution (1%), Stiansny's reagent, sodium hydroxide 1 N, ether, anhydrous acetic acid, sulfuric acid P, petroleum ether, sodium carbonate, Liebermann-Burchard reagent, gelatin solution (1%), DPPH reagent (2,2-diphenyl-1-picrylhydrazyl), vitamin C, methanol, BP quercetin, aluminum (III) chloride, and sodium acetate.

Equipment

Glassware, analytical balance, evaporating dish, watch glass, water bath, oven, micro balance, pipettes, micropipettes, UV-Vis spectrophotometer, aluminum foil, mortar and pestle, weighing bottle, filter paper, funnel, cotton, spectrophotometer, and refrigerator.

Methods

Plant Determination

The plant determination was conducted to confirm the identity of the specimen by the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Indonesia, Depok, West Java (-

6.368708691614558, 106.82746384813458). The determination process involved identifying the butterfly pea flower based on its morphological characteristics and comparing these features with the species description of *Clitoria ternatea* L. (Laia et al., 2023).

The material used in this study was butterfly pea flowers (*Clitoria ternatea* L.), which were obtained and collected from East Java. The collected materials were stored at room temperature (25-30 ^{o}C) in airtight containers to maintain their quality and prevent contamination. (Waruwu et al., 2023).

Examination of Foreign Organic Matter (FOM)

The purpose of examining foreign organic matter is to gather high-quality and appropriate research materials. The butterfly pea flowers were dried in an oven at 50°C until the moisture content was minimized. After drying, the flowers were stored in a closed container and protected from light to prevent degradation of active compounds (Andriani & Murtisiwi, 2020).

Preparation of Butterfly Pea Flower Extract

The flowers were washed, chopped (1–2 mm), and dried in an oven at 50°C for 8 hours. About 400 grams of dried sample was brewed with 1 L boiling demineralized water, stirred, and cooled to room temperature. The mixture was filtered, and the filtrate was concentrated using a rotary evaporator (40°C, 72 mbar), followed by evaporation of residual water in a water bath. (Hafizah et al., 2024) (Waruwu et al., 2023).

Phytochemical Screening of the Extract
Alkaloid Identification

The processed 0.2 g of the extract with 30% ammonia and chloroform, obtaining a filtrate (solution A). Solution A was partially extracted with HCl to produce an upper layer (solution B) for alkaloid testing. Alkaloids were identified by a red or orange color with Dragendorff's reagent on solution A or by brick-red (Dragendorff) and white precipitates (Mayer) in solution B.(Indriasari et al., 2023)

Flavonoid Identification

0.2 g of the extract boiled with hot water, filtered it, and used the filtrate as a test solution. Flavonoids were detected by adding magnesium powder, concentrated HCl, and amyl alcohol to the solution, with the appearance of color in the amyl alcohol layer confirming their presence. (Indriasari et al., 2023).

Saponin Identification

The authors tested for saponins by shaking 10 mL of the solution vertically, allowing it to stand, and observing the formation of stable foam that remained after the addition of 1% HCl. (Aulia Rahmi Azizah et al., 2024).

Tannin Identification

Boiled 0.2 g of the extract with water, cooled, and filtered it to obtain two filtrates. The first filtrate, treated with 1% $FeCl_3$, showed a dark blue or greenish-black color, indicating the presence of tannins. The second filtrate, heated with Stiasny's reagent, produced a pink precipitate, confirming catechuic tannins. Saturating the filtrate with sodium acetate and adding 1% $FeCl_3$ resulted in an ink-blue color, indicating gallic tannins. (Aulia Rahmi Azizah et al., 2024).

Quinone Identification

To 5 mL of the test solution (from experiment b), a few drops of 1N NaOH are added. An intense red color indicates the presence of quinones (Andriani & Murtisiwi, 2020).

Steroid and Triterpenoid Identification

Extract was macerated 0.2 g of the extract with ether, filtered it, and evaporated the filtrate to obtain a residue. The residue was treated with acetic anhydride and concentrated H_2SO_4 using the Liebermann-Burchard reagent, where the appearance of green or red color indicated the presence of steroids or triterpenoids. (Hafizah et al., 2024).

Coumarin Identification

Identified coumarins by mixing 0.2 g of the extract with chloroform, heating it, filtering the mixture, and evaporating the filtrate. The resulting residue was dissolved in hot water, cooled, and treated with ammonia. Under UV light (366 nm), the presence of coumarins was confirmed by green or blue fluorescence. (Laia et al., 2023)

Essential Oil Identification

Identified essential oils by mixing 0.2 g of the extract with petroleum ether, heating it, and evaporating the filtrate. The residue was dissolved in alcohol, and the detection of an aromatic residue confirmed the presence of essential oils. (Clarista Eloydia Vintari, Eny Yulianti, 2022).

Determination of Total Flavonoid Content (Aulia Rahmi Azizah et al., 2024) (Clarista Eloydia Vintari, Eny Yulianti, 2022)

Preparation of Quercetin Standard Solution

Prepared a quercetin stock solution at a concentration of 250 ppm by dissolving 2.5 mg of quercetin in 10 mL of methanol. Standard solutions with concentrations of 10, 20, 30, 40, and 50 ppm were prepared by pipetting 0.4, 0.8, 1.2, 1.6, and 2.0 mL of the

stock solution, respectively, and diluting each to 10 mL with distilled water.

Preparation of 1M Sodium Acetate Solution

1 M sodium acetate solution was prepared by dissolving 0.98 g of sodium acetate powder in 10 mL of distilled water.

Preparation of 10% Aluminum Chloride Solution

10% aluminum chloride solution by dissolving 1 g of aluminum chloride powder in 10 mL of distilled water.

Optimization of Maximum Wavelength

The authors prepared a 250 ppm quercetin stock solution by dissolving 2.5 mg of quercetin in 10 mL of methanol. From this solution, 1 mL was mixed with methanol, aluminum chloride, sodium acetate, and distilled water. The mixture was homogenized, incubated, and analyzed with a UV-Vis spectrophotometer over 350–500 nm to determine the maximum wavelength.

Optimization of Incubation Time

Prepared a 250 ppm quercetin stock solution by dissolving 2.5 mg of quercetin in 10 mL of methanol. A 1 mL aliquot of the stock solution was combined with methanol, aluminum chloride, sodium acetate, and distilled water. The mixture was homogenized, and its absorbance was measured at 427 nm over 60 minutes, with readings taken every 5 minutes, until a stable absorbance was observed.

Preparation of Quercetin Calibration Curve

Prepared quercetin solutions in methanol with concentrations of 10, 20, 30, 40, and 50 µg/ml. From each solution, 1 mL was mixed with methanol, aluminum chloride, sodium acetate, and distilled water. The mixtures were incubated for 30 minutes at room temperature, and their absorbance was measured at 427 nm using a UV-Vis spectrophotometer. A calibration curve was constructed by plotting quercetin concentration (ppm) against absorbance.

Preparation of Butterfly Pea Flower Extract Solution

The authors prepared a butterfly pea flower extract solution by dissolving 10 mg of the extract in 10 mL of methanol to obtain a 1000 ppm concentration. A 1 mL aliquot of this solution was mixed with methanol, aluminum chloride, sodium acetate, and distilled water. The mixture was incubated for 30 minutes at room temperature, and its absorbance was measured at 427 nm. The total flavonoid content was calculated and expressed as a percentage (%).

Calculation of Total Flavonoid Content

Calculate the total flavonoid content using the calibration curve equation: Y = a + bx, where y is the absorbance, a is the intercept, b is the slope, and x is the concentration. Convert the result into a percentage of flavonoid content using the appropriate formula.

$$\text{Flavonoid Content (\%)} = \frac{\text{V}\times\text{X}\times\text{Fp}}{BS} X\ 100\% \quad \ldots\ldots(1)$$

Description:

V : Volume (mL)
X : Concentration (ppm)
Fp : Dilution factor
BS : Sample weight (g)

Antioxidant Activity Test Using the DPPH Method (Susiloningrum & Mugita Sari, 2021) (Andriani & Murtisiwi, 2020) (Clarista Eloydia Vintari, Eny Yulianti, 2022)

Preparation of DPPH Solution (0.4 mM)

Prepared a 0.4 mM DPPH solution by weighing approximately 4 mg of DPPH (Molecular Weight: 394.32) and dissolving it in methanol to a final volume of 25 mL. The solution was stored in a dark bottle to protect it from light exposure.

Preparation of Blank Solution

Prepared a blank solution by pipetting 1 mL of a 0.4 mM DPPH solution into a 5 mL volumetric flask and diluting it with methanol to the calibration mark. The mixture was homogenized, and the flask was covered with aluminum foil to protect it from light exposure.

Preparation of Vitamin C Solution (Positive Control)

Prepared a 1000 µg/ml stock solution of vitamin C by dissolving 10 mg of vitamin C in 10 mL of methanol. From this stock solution, working solutions with concentrations of 0.5, 1, 1.5, 2, and 2.5 µg/mL were created by pipetting 2.5, 5, 7.5, 10, and 12.5 µL of the stock solution into separate 5 mL volumetric flasks. Each flask was filled with 1 mL of 0.4 mM DPPH solution and methanol to the calibration mark, homogenized, and protected from light with aluminum foil.

Preparation of Test Solution

Prepared a 1000 µg/mL stock solution of the extract by dissolving 25 mg of the sample in 25 mL of methanol. Test solutions with concentrations of 10, 20, 30, 40, and 50 µg/mL were then prepared by pipetting 50, 100, 150, 200, and 250 µL of the stock solution into separate 5 mL volumetric flasks. Each flask was supplemented with 1 mL of 0.4 mM DPPH solution and methanol up to the calibration mark, homogenized, and shielded from light with aluminum foil.

Absorbance Measurement

The test solutions and positive control solutions were incubated at 37°C for 30 minutes. Absorbance measurements were then taken at a wavelength of 515 nm using a UV-Vis spectrophotometer.

Calculation of Inhibition Percentage

Calculate the percentage of free radical scavenging using the formula:

$$\text{Free radical scavenging } (\%) = \frac{\text{Ab}-\text{As}}{Ab} X\ 100\% \ldots(2)$$

Description:
Ab :Absorbance of DPPH blank solution in methanol
As : Absorbance of solution after reacting with the sample

Determination of IC50 Value

Determined the IC50 value, defined as the concentration of the extract required to inhibit 50% of free radicals. This was achieved by plotting a curve of the percentage of inhibition against the concentration of the extract. The IC50 value was obtained from the point of intersection where the curve reached 50% inhibition.

Result and Discussion

The material used in this study was the butterfly pea flower (*Clitoria ternatea* L.) obtained from East Java. The selected flowers had a harvesting age of 2 months, were fresh, blue in color with white petals in the center, and free from caterpillar damage. The dark blue flowers were chosen as they are presumed to contain higher flavonoid levels compared to other color variants (Angelina & Syuhada, 2023). Butterfly pea flowers used as research materials are generally collected from locations with optimal growth conditions, such as rural areas with suitable soil (Anwar Fauzi et al., 2022). The selected flowers exhibit distinctive characteristics, including a deep blue color and no signs of damage or pest bites, which are indicative of high flavonoid content and other bioactive compounds. Prior to use, the butterfly pea flowers were dried using an appropriate method to preserve their bioactive compounds and ensure the quality of the raw material (Manurung et al., 2023).

The determination of butterfly pea flowers was carried out to confirm the authenticity of the plant species used. This process was conducted at the Department of Biology, Faculty of Mathematics and Natural Sciences, University Indonesia, Depok, West Java. The determination results confirmed that the plant is butterfly pea (*Clitoria ternatea* L.) from the Fabaceae family. The identification of morphology and species is crucial to ensure the authenticity of the material, as the correct species will influence the content of active compounds and the efficacy of the raw material. (Manurung et al., 2023).

Table 1. Butterfly pea flower extract results

Parameter	Description
Weight of butterfly pea flowers (g)	300
Amount of thick extract (g)	60.5
DER-*native*	4.96
Yield (%)	20.17

The extraction process of butterfly pea flowers was conducted using an infusion method aimed at extracting the secondary metabolites from the flower simplicia. The extraction was carried out stepwise by brewing 300 grams of butterfly pea flowers, where each 100 grams of flowers were brewed with 1 liter of water, and this process was repeated three times. This method is a simple technique in which distilled water (aquadest) is used as the solvent. After brewing, the liquid was filtered using Whatman filter paper and cotton to separate the extract from the residue. The brewing process began by soaking the flower simplicia in water until reaching the boiling point. The solvent penetrates the cell walls of the flower and enters the cell cavities containing active compounds, allowing these compounds to diffuse out during maceration. The resulting filtrate or liquid extract was then concentrated using a vacuum rotary evaporator. This equipment was operated at a temperature of 40°C, pressure of 175 mbar, with a speed of 60 rpm, and a condenser set to 10°C. The temperature and pressure conditions were controlled to minimize heat exposure to the active compounds, ensuring that the bioactive contents remained intact and undamaged during the concentration process. The thick extract of butterfly pea flowers obtained is shown in the table. (Handayani & Kumalasari, 2022) (Anwar Fauzi et al., 2022).

Table 2. Phytochemical screening butterfly pea flower results

No	Compound	Result
1	Alkaloids	-
2	Flavonoids	+
3	Saponins	+
4	Quinones	-
5	Tannins	+
6	Steroids/Triterpenoids	+
7	Coumarins	-
8	Essential Oils	-

This study conducted a phytochemical screening of the aqueous extract of butterfly pea flowers (*Clitoria ternatea* L.) to identify the secondary metabolites

contained within. The screening results revealed that the aqueous extract of butterfly pea flowers tested positive for flavonoids, saponins, and tannins but did not contain alkaloids (Putri & Mahfur, 2023) (Angelina & Syuhada, 2023). The absence of alkaloids can be attributed to various factors, including soil fertility, growing conditions, climate, processing methods, plant age, and sample size (Anwar Fauzi et al., 2022).

Flavonoids were detected by reactions producing red, yellow, or orange colors in the amyl alcohol layer. These compounds act as antioxidants capable of transferring electrons to free radicals and forming complexes with metals. This mechanism provides cellular protection effects, such as inhibiting lipid peroxidation and preventing tissue damage caused by free radicals (Nur et al., 2019).

Saponins, known as surface-active compounds, were detected by their ability to form stable foam after shaking. The glycoside component in saponins makes them polar and functions as secondary antioxidants. Saponins inhibit lipid peroxidation by forming hydroperoxides and enhancing the activity of antioxidant enzymes like superoxide dismutase (SOD) and catalase (Abdilah et al., 2022).

Tannins were identified by the addition of $FeCl_3$, which produced a dark blue color indicating the presence of gallic tannins. Tannins are polar compounds due to their hydroxyl (OH) groups, functioning as antioxidants. These OH groups can scavenge various types of free radicals, including superoxide (O_2-), peroxyl (ROO-), hydrogen peroxide (H_2O_2), nitrite oxygen (NO-), and peroxynitrite (ONOO-). This phytochemical screening shows that the aqueous extract of butterfly pea flowers has potential as a source of natural antioxidant compounds, with flavonoids, saponins, and tannins being the main contributors to its antioxidant activity (Yurisna et al., 2022)(Handayani & Kumalasari, 2022).

This research focuses on analyzing the total flavonoid content in butterfly pea flower extract (*Clitoria ternatea* L.). First, the maximum wavelength of quercetin was determined, which served as a reference for measuring flavonoid content. The wavelength optimization results were obtained at 427 nm with an absorbance value of 0.6430, indicating the characteristic maximum absorption of flavonoids (Sunarti & Octavini, 2023).

The study also optimized the incubation time to ensure the correct reaction time between the sample and reagents. The measurements indicated that absorbance reached stability between 30-40 minutes; hence, the incubation time used was 30-50 minutes (Ayu Martini et al., 2020).

Table 3. Quercetin standard result

No	Concentration (ppm)	Absorbance
1	10	0.2645
2	20	0.3374
3	30	0.4041
4	40	0.4821
5	50	0.5029

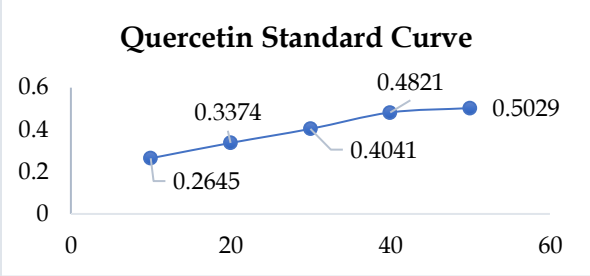

Figure 1. Quercetin standard curve result

Quercetin solutions were prepared at various concentrations (10, 20, 30, 40, and 50 ppm) to determine the linear equation relating concentration to absorbance. The resulting equation was y = 0.2117 + 0.00621x with a linearity value of 0.9875, indicating a very strong correlation between concentration and absorbance (Taufik & Ainiyah, 2021).

The total flavonoid content of the butterfly pea flower extract was determined using a colorimetric method, where the sample was dissolved in methanol and reacted with aluminum chloride and sodium acetate to form an acidic complex that could be measured by a spectrophotometer. The results showed that higher flavonoid content led to a deeper yellow color (Kushargina et al., 2022).

Table 4. Total flavonoid content result

Sample (mg)	Absorbance	Content $\frac{mg}{100\ mg}$	Average Content $\frac{mg}{100\ mg}$
10.7	0.4894	4.18	4.06
10.5	0.4733	4.01	
10.2	0.4646	3,99	

The average total flavonoid content in the butterfly pea flower extract was 4.06 mg per 100 mg of extract (0,40%). This value is higher than that reported in a previous study by Anita and Gandis (2020), which found a content of 4.65% / 100 mg butterfly pea extract. The difference may be attributed to different drying methods; in this study, drying was performed using an oven at 50°C, while the earlier study used direct sunlight. The difference in flavonoid content between oven drying and sunlight drying is due to differing conditions. Oven drying at 50°C maintains a consistent temperature, protecting flavonoids from heat and UV degradation while reducing drying time. In contrast,

sunlight drying involves fluctuating temperatures, UV exposure, and longer drying times, which can increase flavonoid degradation. (Afiah et al., 2023).

The significance of flavonoids in the butterfly pea flower extract lies in their antioxidant properties, with the ability to inhibit lipid peroxidation and reduce free radicals, thus contributing to health benefits. These compounds are capable of donating hydrogen atoms or electrons to neutralize free radicals, thereby inhibiting lipid peroxidation and reducing oxidative stress. Studies have identified key flavonoids in butterfly pea flowers, including anthocyanins (e.g., delphinidin and malvidin derivatives), quercetin, kaempferol, and myricetin (Alam et al., 2019). Anthocyanins, in particular, are prominent in butterfly pea flowers and contribute significantly to their antioxidant potential due to their multiple hydroxyl groups, which are effective in scavenging reactive oxygen species (ROS). Variations in flavonoid content may also be influenced by various factors, including environmental conditions, temperature, and drying methods (Alam et al., 2019).

The antioxidant activity testing began with determining the maximum wavelength. Based on the absorbance spectrum of the blank solution, which was prepared by mixing 1.0 mL of 0.4 mM DPPH solution in methanol up to 5.0 mL and measured within the wavelength range of 400-600 nm, the maximum wavelength was found to be 515 nm. Theoretically, the maximum wavelength of DPPH in methanol is between 515-517 nm. This difference could be due to variations in measurement instruments. Since the DPPH solution is freshly prepared for each sample, the maximum wavelength is recalibrated before each measurement (Alam et al., 2019).

The determination of the stable absorbance time was conducted to ensure the color stability of the DPPH solution in methanol (pro analysis). The test results showed a stable time at 45-50 minutes, with an absorbance of 0.2680, indicating that the DPPH solution in methanol still exhibited stable absorbance (Kushargina et al., 2022).

Antioxidant Activity Test of Vitamin C (Positive Control)

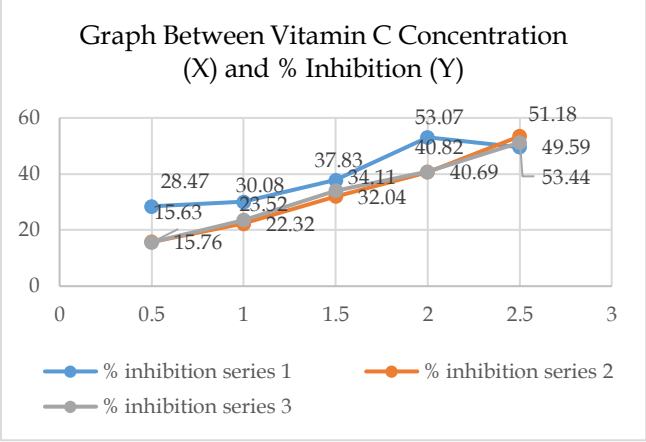

Figure 2. Graph of vitamin C concentration (X) versus % tyrosinase inhibition (Y)

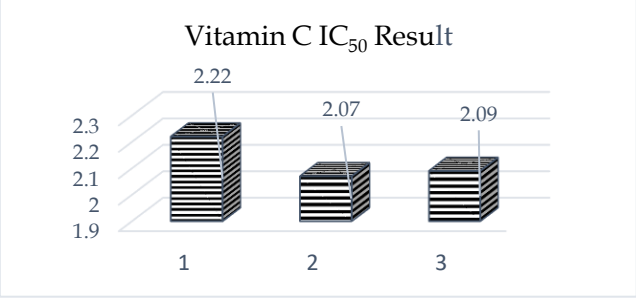

Figure 3. Vitamin C IC_{50} result

In the antioxidant activity test using the DPPH method, vitamin C was used as a positive control and produced an IC_{50} value of 2.13 ppm at concentrations of 0.5, 1, 1.5, 2, and 2.5 ppm. This indicates that at these concentrations, vitamin C can inhibit 50% of free radicals. Since the IC50 value of vitamin C is less than 50 ppm (specifically, 2.13 ppm), it demonstrates that vitamin C possesses very strong antioxidant activity. The smaller the IC_{50} value of a compound, the greater its ability to neutralize free radicals. Vitamin C was chosen as a positive control due to its water solubility and its well-known potent antioxidant properties. The IC_{50} value of vitamin C serves as a benchmark to assess the strength of the butterfly pea flower extract in neutralizing free radicals, as indicated by the IC_{50} value of the sample (Taufik & Ainiyah, 2021).

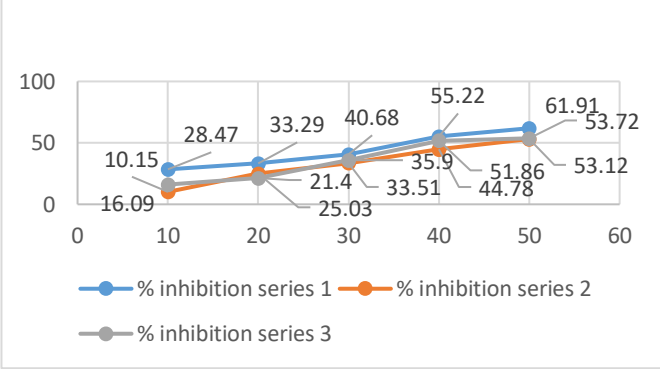

Figure 4. Graph of the Between Butterfly Pea Flower Extract Concentration (X) and % Inhibition (Y)

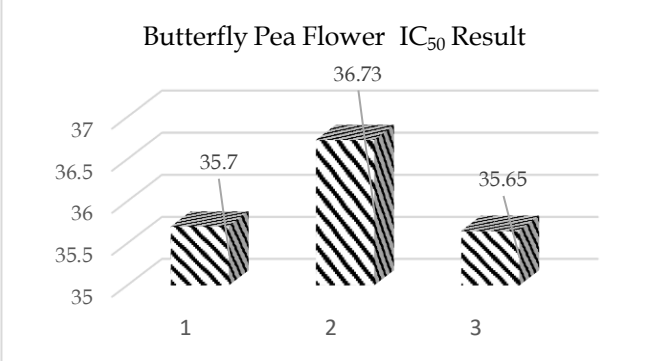

Figure 5. Butterfly pea flower IC_{50} result

The results of the antioxidant activity test showed that vitamin C had an average IC_{50} value of 2.42 ppm at concentrations of 0.5, 1, 1.5, 2, and 2.5 ppm. Meanwhile, the butterfly pea flower extract had an average IC_{50} value of 36.03 ppm at concentrations of 10, 20, 30, 40, and 50 ppm. Although the IC_{50} value of vitamin C is lower and stronger than that of the butterfly pea extract, both are classified as having very strong antioxidant activity, as their IC_{50} values are less than 50 ppm. An IC_{50} value of 36,03 ppm for the butterfly pea flower extract indicates that it is capable of inhibiting 50% of free radicals at that concentration. This strong antioxidant activity is attributed to the flavonoid content in butterfly pea flowers, which contain multiple hydroxyl groups.

Mechanistic Insight into Flavonoid Contribution to the antioxidant activity of flavonoids is influenced by their chemical structure, particularly, the number and position of hydroxyl groups on the aromatic rings. The presence of a C2=C3 double bond in conjunction with a 4-keto group in the C-ring, which enhances electron delocalization and radical-scavenging ability. Glycosylation or methylation, which can modify the bioactivity of flavonoids. (Sunarti & Octavini, 2023) (Ayu Martini et al., 2020).

Conclusion

The determination of the total flavonoid content from the butterfly pea flower extract (*Clitoria ternatea* L.), yielding a content of 4.06 mg (100 mg). The butterfly pea flower extract (*Clitoria ternatea* L.) prepared using the infusion method exhibits very strong antioxidant activity, with an IC_{50} value of 36.03 ppm.

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