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ANALYSIS OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY ETANOL EXTRACT OF BUTTERFLY PEA FLOWER *(Clitoria ternatea)* IN YOGYAKARTA

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ABSTRACT

Butterfly Pea Flower is one of the plants that has antioxidant activity. However, based on previous studies, the antioxidant content is highly dependent on the location and place of the BPF itself. Therefore, this study aims to measure the concentration of total flavonoids, phenolics, antioxidant and antibacterial activity in BPF so that this research can be further developed into certain products. Extraction using maceration method with 96% ethanol solvent (1:10). DPPH method for measuring antioxidant activity and the disc paper diffusion method for testing antibacterial activity. BPF contain flavonoids and total phenolics of 19.44 ± 0.060 mg Quercetin Equivalent (QE)/g and 36.37 ± 0.47 mg Gallic Acid Equivalent (GAE)/g, respectively. BPF also contains antioxidants with an IC50 index of 49.47 µg/mL and BPF extract has no antibacterial activity using the paper disc diffusion method. This antioxidant-rich BPF extract has a lot of promise for development into a cosmetic preparation with more research.

Keywords: Butterfly Pea Flower, Antioxidant, Antibacterial, Flavonoid, Phenolic.

INTRODUCTION

Oxidative stress is a state of imbalance between the production of free radicals or Reactive Oxygen Species (ROS) and antioxidants as a protective mechanism against free radicals. Because of this imbalance, it can have an impact on bio-molecular damage with potential consequences on the body (Anbualakan et al., 2023). Untreated oxidative stress that lasts for a long time will trigger cell damage developing into degenerative diseases (Haryoto & Frista, 2019). Some examples of degenerative diseases are cardiovascular disease, cataracts, hypertension, diabetes, cancer and premature aging (Haryoto & Frista, 2019). To maintain the balance of free radicals, extra antioxidant compounds that can be obtained from outside the body are needed. The flower crown is one area of the plant with a high antioxidant content. Numerous studies have indicated that *Soka (Ixora coccinea)* flowers (Salmataj et al., 2018), Chrysanthemum (Han et al., 2019), and *Telang (Clitoria ternatea)* flowers (Bujak et al., 2022) are among the flowers with high antioxidant content .

Butterfly pea flowers (BPF) are plants that have antioxidant activity. Phytochemical screening of BPF shows that it has several secondary metabolic compounds such as tannins, saponins, alkaloids, triterpenoids, flavonoids and steroids. Pharmacologically, the BPF also has antimicrobial, antiparasitic, anti-inflammatory, antioxidant, antidepressant and antidiabetic activities (Al-Snafi, 2016). BPF studies revealed that these flowers contain phenolic chemicals, flavonoids, anthocyanins, flavonol glycosides, kaempferol glycosides, quercetin glycosides, myrisetin glycosides (Hiromoto et al., 2013).

Flavonoids are found in almost all parts of the BPF including fruit, roots, leaves and outer bark of the stem. Flavonoids are natural compounds that have the potential as antioxidants to counteract free radicals which play significant role in the emergence of degenerative diseases through mechanisms of destruction of the body's immune system, and

oxidation of lipids and proteins (Ridwan Rais, 2015). The benefits of flavonoids include being an allergy repellent, expelling viruses in the body, avoiding thrombus, and functioning as anti-diarrhea and immunity (Widiasari, 2019). Apart from the flavonoids, BPF is also rich in phenolic compounds.

Prior studies have never compared the antioxidant activity of *Telang* flowers according to their growth region. The quantity of phenols and the presence of flavonoids are characteristics of antioxidant activity. Antioxidant activity is highly dependent on the location of the BPF plants (Safrina & Joko, 2018). This is following the results of research conducted by Andriani & Murtisiwi, (2020) where the antioxidant activity content from Seleman was IC_{50} 41,36 ± 1,191 µg/mL, and West Sumatra was IC_{50} value of 27.1 µg/mL (Putra et al. 2021) Significantly different results were also researched by Hidayati et.al (2019) in Central Java reported an IC_{50} value of 25.7 µg/mL. Based on these findings, this study aims to measure the concentration of flavonoids and total phenolics in BPF in Yogyakarta as an early detection of antioxidant activity.

Phenolic compounds are widely used natural compounds. One of its functions is as an antioxidant for the prevention and treatment of degenerative diseases (Apsari & Susanti, 2011). Phenolic compounds have a positive correlation with antioxidant activity (Huda-Faujan et al., 2009), hence polyphenols are probably the compounds that have the most potential to contribute to anti-radical activity to BPF. Based on this case, it is important to know the levels of total flavonoids and phenolics in BPF.

Antioxidants are substances that in low concentrations can neutralize free radicals by donating electrons so that free radicals become stable (Fahleny et al, 2015). Free radicals in high concentrations in the absence of an effective antioxidant mechanism can cause extensive damage to cell structures. The mechanism of action of antioxidants to neutralize free radicals by becoming electron donors to free radicals so that the electrons in free radicals become paired so as to stop damage in the body (Widhowati et al, 2022). The mechanism of antibacterial action of saponins is by increasing the permeability of the cell membrane so that the membrane becomes unstable and causes cell hemolysis (Widhowati et al, 2022). Uncontrolled bacterial growth can cause various skin problems, such as S. aureus bacteria that can cause skin infections (Hanina et al., 2022), and *Propionibacterium acnes*, and *Staphylococcus epidermidis* bacteria that cause acne (Hanina et al., 2022).

RESEARCH METHODS

Equipment and Materials

Glass tools (Pyrex), rotary evaporator (Hidolph), viscometer (Rheosys), oven (Binder), incubator (Memmlert In55), UV-Vis Spectrophotometry (Shimadzu), vortex mixer (VM. 300P), autoclave (GEA. LS35HD) and furnace (Vulcan). Quercetin (Sigma-Aldrich), gallic acid/$C_7H_6O_5$ p.a (Sigma-Aldrich), 2,2-Diphenyl-1-picrylhydrazy (DPPH) (Sigma-Aldrich), ethanol/C_2H_5OH p.a (Sigma-Aldrich), C_2H_5OH 96%, $AlCl_3$ (Sigma-Aldrich), potassium acetate/CH_3COOK (Sigma-Aldrich), Na2CO3 (Sigma-Aldrich), folin-ciocalceuc (Sigma-Aldrich), and distilled water are among the substances utilized

Research Procedure

BPF samples were obtained from Umbulharjo, Yogyakarta Extraction was conducted by using maceration method with 96% ethanol solvent (1: 10) (Andhiarto et al, 2021). BPF extracts were standardized with specific and non-specific standardization. Specific extract standardization include plant determination, organoleptic, and total flavonoids using UV-Vis spectrophotometry with a wavelength of 431 nm (Andhiarto et al., 2015) and total phenolic using UV-Vis spectrophotometry with a wavelength of 744.8 nm (Andhiarto et al., 2015). Plant determination was carried out at the Department of Health UPT Laboratory Herbal Materia Medica Batu, Malang City, East Java, Indonesia (Yulianti, 2023). Non-specific extract standardization includes the determination of extract yield with standard (Depkes RI, 1995), total water content with standard (Depkes RI, 1995), total ash

content with standard (Kemenkes RI, 2017) and Acid insoluble ash content with standard Kemenkes RI, (2017).

The antibacterial and antioxidant properties of BPF extracts were investigated. The disc paper method was used for testing antibacterial activity, while the DPPH method was used for determining antioxidant activity. To be more specific, the test was conducted as follows:

a. Antioxidant activity
   1) Preparation of DPPH solution
      DPPH solution was made with a concentration of 1 mM through 9.8 mg of DPPH dissolved with ethanol p.a to 25 mL. DPPH solution of 0.15 mM was made by taking 15 mL of 1 mM DPPH solution and adding ethanol p.a to 100 mL (Febrianti et al, 2022). DPPH solution coated with aluminum foil was placed in the refrigerator (Andriani & Murtisiwi, 2020).
   2) Preparation of quercetin standard solution
      The preparation of 100 ppm quercetin standard solution was carried out by taking 10 mg of quercetin, dissolved in 100 mL of ethanol p.a and homogenized. Through the standard solution, continued making series with concentrations of 0.5 ppm, 1 ppm, 1.5 ppm, 2 ppm and 2.5 ppm (Dominta et al., 2019).
   3) Preparation of sample solution
      The preparation of the test solution was carried out by dissolving 10 mg of extract in 100 mL of ethanol p.a, from the solution a series of extract concentrations of 10ppm, 20ppm, 30ppm, 40ppm and 50ppm were made by taking 1mL, 2 mL, 3mL, 4 mL and 5 mL of extract solution and put in a 10 mL volumetric flask add ethanol p.a until it reaches the mark.
   4) Determination of maximum wavelength (λmax)
      A total of 1.0 mL of 0.15 mM DPPH solution was added with 1.0 mL of ethanol p.a, and then measured the absorption with UV-Vis spectrophotometry at visible wavelengths of 400-800nm. After obtaining a graph of the relationship between wavelength and absorbance. The highest absorbance is the maximum wavelength (Kusbandari et al, 2018).
   5) Preparation of control solution
      Control was made by mixing 1.0 mL of ethanol p.a and 1.0 mL of 0.15 mM reagent (Dominta et al., 2019). Furthermore, it was read with the maximum wavelength that had been obtained previously.
   6) Determination of operating time
      Preparation of a solution for operating time is made by mixing 1.0 mL of test solution and 1.0 mL of 0.15 mM DPPH solution, then observing the absorbance for 30 minutes at a maximum wavelength of 517 nm (Dominta et al., 2019;Kusbandari et al, 2018).
   7) Absorbance Measurement
      Test solution and comparison solution were taken as 1 mL of solution in each concentration, added 1 mL of 0.15 mM DPPH solution and homogenised and allowed to stand for operating time. Furthermore, the absorbance or absorbance is read with the maximum wavelength of DPPH on a UV-Vis spectrophotometer. The blank used is ethanol p.a which will read the absorbance (Dominta et al., 2019).
   8) Determination of IC_{50} of extract
      The determination of IC_{50} was calculated with the results of a linear regression curve between inhibition and a series of sample concentrations, namely extracts and quercetin comparison solutions. The determination of antioxidant activity was carried out by calculating the inhibitory concentration (IC_{50}) using Equation 1: (Andriani & Murtisiwi, 2020).

$$\%\text{inhibition} = \frac{\text{Abs.Control} - \text{Abs.Sampel}}{\text{Abs.Control}} \text{ X } 100\% \quad (1)$$

b. Antibacterial activity

1) Sterilization

First, the necessary components are properly cleaned, dried, and sterilised. Glass tools should be covered with aluminum foil and sterilised in an autoclave for 15 minutes at 121ºC. Rubber tools can be sterilised by submerging them in 70% alcohol. A Bunsen burner is used to disinfect these needles. 70% alcohol was sprayed on Laminar Air Flow (LAF) after it had been sterilised for 15 minutes under a UV lamp. The LAF is sterilised both prior to and following work on it (Misna & Diana, 2016).

2) Nutrient Agar Media Preparation

Agar media was prepared by dissolving 4.2 gr of nutrient agar into 150 mL of distilled water in an Erlenmeyer. Then the mixture was heated and sterilised in an autoclave at 121ºC for 15 minutes. After being sterilized, the media was then cooled to a temperature of 45^0C, and then poured into Petri dishes in as many as 30 mL. Nutrient agar (NA) media that had been poured into petri dishes were allowed to harden (Retnaningsih et al, 2019).

3) Bacterial culture

On agar media in petri dishes, bacteria are cultivated. The zig-zag approach was used to distribute sterile round rows of bacteria, which were subsequently cultured for 24 hours at 30^0C.

4) Preparation of the Mc-Farland Standard

Put 0.05 mL of 1.75% BaCl2 .2H_2O solution mixed with 9.95 mL of H_2SO_4 solution into a test tube, then shake until a cloudy solution is formed. This solution is used as a standard for the turbidity of test bacteria (Pertiwi et al, 2022).

5) Preparation of Bacterial Suspension

A sterile cotton bath was used to collect 24-hour-old bacterial cultures, and the test bacterial colonies were suspended in 10 millilitres of sterile 0.9% NaCl in a sterile test tube. subsequently whirled into homogeneity. Comparing turbidity to Mc Farland (Muljono and Manampiring, 2016).

6) Preparation of the extract

In this study, three concentration variants were used, namely 50%, 75% and 100%. Each was done three times. The concentration of the extract was made in 2 ml of distilled water. The positive control used was a chloramphenicol disc and the negative control used 10 ml of sterile distilled water.

7) Antibacterial activity test

Testing antibacterial activity using the diffusion method using paper discs, this test uses *Staphylococus sureus* bacteria with chloramphenicol as a positive control and sterile aquadest as a negative control. BPF extract was made in 3 concentrations, namely 50%, 75% and 100%. The diffusion paper was dabbed with 200µl of each extract solution and allowed to stand for 5 minutes. The diffusion paper, positive control and negative control were placed on the media that had been induced by bacteria and then incubated for 24 hours. Then the antibacterial activity was determined by measuring the diameter of the clear zone formed using a caliper (Misna & Diana, 2016).

RESULTS AND DISCUSSION

900 grams of simplisia macerated with 70% ethanol (1:10). 8.5 liters of macerate were created during the extraction by maceration process; however, technical issues during the evaporation process limited the amount of macerate that could be effectively evaporated to 7 liters. After that, it was gradually evaporated using a rotary evaporator at 78^0C, yielding a 130-gram thick extract.

Following the completion of standardization, both non-specific and specific extracts were obtained. The results of extract standardization are shown in **Table I**.

Table I. Results of tests for the BPF extract's particular and non-specific properties.

Parameter Test	Result
Organoleptic	Thick texture, dark purple and a distinctive aromatic smell.
Total flavonoids	19.44±0.060 mgQE/g
Total phenolic	36.37±0.47 mgGAE/g
Yield	14.4%
Total water content	15.24 ± 1.3%
Total ash content	3.58 ± 0.55%
Acid insoluble ash content	1.45±0.2 %

The chemical content of flavonoid and total phenolic simplisia is strongly influenced by the location of the altitude where a plant grows. A similar result was shown by previous research (Safrina & Joko, 2018), mentioning there are significant differences in the comparison of the altitude of the plants' growing area on the levels of its chemical content. The total flavonoid content of the North Lombok district was 59.37 mgQE and Wonosobo was 63.09 mgEQ/g (Rahayu et al, 2021). Significantly different results were also obtained by (Fikayuniar et al, 2023) in the city of Bandung with a total flavonoid result of 4.865 gQE/100g. Similar research was also conducted in India by T.Madhavi & Sushma, (2014), obtaining total flavonoid levels of 67.2 mgEQ. The significant difference of the result in this study is strongly influenced by the location and differences in plant growth altitude. According to research conducted by Andriani & Murtisiwi, (2018), the total phenolic content in the ethanol extract of BPF was 19.43 ± 1.621 GAE (mg/g sample). The research was conducted in the city of Kudus, Central Java, Indonesia. Similar research was also conducted in India by T.Madhavi & Sushma, (2014) who obtained total phenolic content of BPF of 45.6 mgGAE. The significant difference in results in this study is strongly influenced by the location and differences in plant growth altitude (Safrina & Joko, 2018). The yield was calculated using the formula (%b/b) resulting in 14.4% yield. Less than 30% of the water content is needed for a BPF extract Kemenkes RI, (2017). This is to stop the fungus in the extract from growing too quickly. Repetition 1, 2, and 3 yielded total water content findings of 15.00, 16.67%, and 14.04%, respectively. The test for water content was conducted three times. Consequently, the BPF extract's average total water content was 15.24 ± 1.3%. These outcomes satisfy quality requirements. The test for ash content was run three times, with total ash content values of 2.94%, 3.85%, and 3.96% in repetitions 1, 2, and 3, respectively. The BPF extract obtained with this test had an average total ash level of 3.58 ± 0.55%. The outcomes meet the MMI (1989) ash content standards, which are <8%. The average acid-insoluble ash content of BPF extract obtained through this test was 1.45±0.2 %%, which is in accordance with the requirements of ash content according to MMI (1989) (<2%).

Antioxidant activity testing of BPF extract preparation using DPPH method measured absorbance at a maximum wavelength of 515.5 nm. Determination of IC_{50} was calculated by making a linear regression curve where %inhibition as the Y-axis and sample concentration series as the X-axis so as to obtain a linear regression equation y = 1.3183x + 15.232 with a value of $R^2 = 0.9724$. Based on the linear regression equation, the IC_{50} value of 49.47 µg/mL was obtained with a strong index. Research on the antioxidant activity of BPF has been widely carried out with different results, such as research conducted by Bujak et al., (2022) that BPF have antioxidant activity with an IC_{50} index of 47.5 ± 1.01 µg/mL with a strong category. The same research was also conducted by Grzebieniarz et al., (2023) with IC_{50} 45.91 ± 2.79 µg/mL. The results of research from Andriani & Murtisiwi, (2020) with IC_{50} 41.36 ± 1.191 µg/mL. These studies have in common that BPF has antioxidant activity with a strong category.

The diameter of the BPF extract's inhibition zone, as determined by testing the extract's antibacterial activity against S. aureus bacteria at 50%, 75%, and 100% concentrations, is displayed in **Figure 1. Figure 1** illustrates the high antibacterial activity of the positive control, chloramphenicol disk, with an inhibition zone diameter of 23.33 ± 1.15 mm, the disc paper's diameter and the inhibition zone's diameter are what determine the inhibition zone's true diameter. In contrast, the concentrations of BPF extract at 50%, 75%, and 100% did not display an inhibition zone. expressed in words BPF extract, however, possesses no antimicrobial properties. The antibacterial activity of BPF extract based on several studies showed significantly different results. According to research by Febrianti et al., (2022) in the city of Surakarta, Central Java BPF have an inhibition zone of 13 ± 1 mm (medium). According to research by Widhowati et al., (2022) in the city of Surabaya, East Java, BPF extract with a concentration of 80% showed an average of 6.36 mm (weak), at a concentration of 90% showed an average of 11.62 mm (medium). Based on the results of this study it can be seen that the location of BPF growth greatly affects its antibacterial activity.

The inhibition zone results from this study differ from those from other studies for a some reasons. The extract's storage conditions and testing methods are two such aspects. The stability of the active chemicals in an extract can be impacted by factors such as temperature, light, and humidity (Wang et al., 2018); in this instance, the extract is stored in a locker before to antibacterial testing. In addition to storage, the testing method matters because every method has a unique sensitivity (Budiasih, 2022). This test employs the diffusion method, which has a rather poor sensitivity. This approach works well for screening and preliminary testing. It is recommended that the Dilution Method or the Well Diffusion Method be used in future studies.

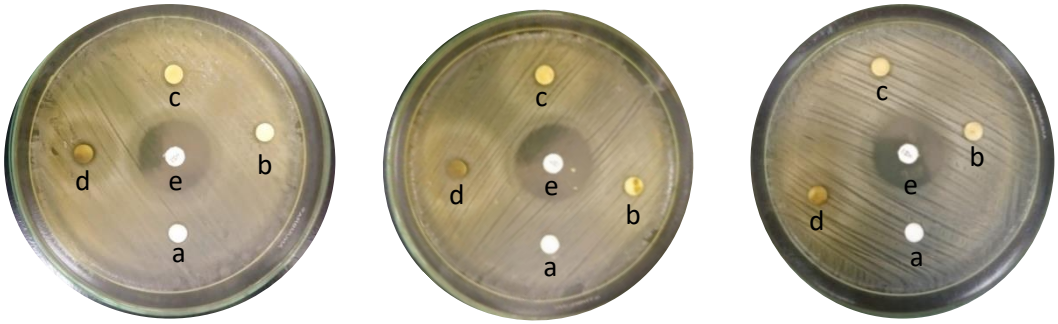

Figure 1. Antibacterial testing of BPF extracts with concentration variants of (a) negative control (b) BPF extract (50%), (c) BPF extract (75%), (d) BPF extract (100%) and (e) positive control (chloramphenicol disk)

CONCLUSION

Butterfly pea flower (BPF) has a strong category and a high IC50 value of 49.47 µg/mL for antioxidant activity. An antibacterial activity test it has been determined that BPF extract lacks antibacterial activity by using the paper disc diffusion method. The environment in which plants grow has a significant impact on the antioxidant and antibacterial properties of BPF extract. This antioxidant-rich BPF extract has a lot of promise for development into a cosmetic preparation with more research..

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