Medical Sains
Jurnal Ilmiah Kefarmasian
ISSN : 2548-2114 (e) 2541-2027 (p)
Medical Sains : Jurnal Ilmiah Kefarmasian
Vol. … No…, Month Year
https:/ojs.stfmuhammadiyahcirebon.ac.id/index.php/iojs

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

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Submitted :........................ Revised :.......................... Accepted:.....................

ABSTRACT

Butterfly Pea Flower (BPF) 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). Measurement of flavonoid and total phenolic content using UV-Vis spectroscopy with wavelengths of 431nm and 744.8nm. 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 contain antioxidants with an IC50 index of 6.621 µg/mL and have an average minimum inhibition zone of antibacterial activity at concentrations of 50%, 75% and 100% of 6.67±1.15 mm, 6.67±1.15 mm, 6.67±1.15 mm respectively with a weak category. BPF are proven to contain flavonoids, phenolic compounds and have high antioxidant activity and weak antibacterial activity. So that further researchers can develop BPF research into certain preparations.

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 impacts 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 flower is one of the plants with antioxidant activity. Phytochemical screening of Butterfly pea flower shows that Butterfly Pea Flower (BPF) has several secondary metabolic compounds such as tannins, saponins, alkaloids, triterpenoids, flavonoids and steroids. Pharmacologically, Butterfly pea flower 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, quersetin glycosides, mirisetin 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 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, oxidation of lipids and proteins (Ridwan Rais, 2015). The benefits of flavonoids include being an allergy repellent, expelling viruses in the body, avoiding thrombus, 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. Total flavonoid and phenolic levels are highly dependent on the location of the BPF plants (Safrina & Joko, 2018). This is in accordance with the results of research conducted by Rahayu et al, (2021) where the total flavonoid content from North Lombok district was 59.37 mgQE/g, and Wonosobo was 63.09 mgQE/g. Significantly different results were also obtained by Azizah (2021) in Bandung city with 4.865 gQE/100g total flavonoids. According to Andriani and Murtisiwi, (2018), the total phenolic content in the ethanol extract of telang flowers was 19.43 ± 1.621 GAE (mg/g sample), this research was conducted in the city of Kudus, Central Java, Indonesia. Similarly, a reasech in India by T.Madhavi and Sushma, (2014) obtained 67.2 mgQE/g. of total flavonoid levels and 45.6 mgGAE/mg of total phenolic levels. 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 its the functions of is an antioxidants 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 in anti-radical activity to BPF. Based on this case, it is significant to know the levels of total flavonoids and phenolics in BPF.

Antioxidants are substances that in low concentrations can neutralise 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 neutralise 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 haemolysis (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 from Pyrex, a rotary evaporator from Hidolph, a viscometer, an oven from Binder, an incubator from Memlert In55, UV-Vis Spectrophotometry from Shimadzu, a vortex mixer from VM. 300P, an autoclave from GEA. LS35HD, a furnace from Vulcan, a pH meter from Benchtop, and an autoclave from GEA. Quercetin, gallic acid, DPPH, ethanol p.a., ethano 96%, sulfuric acid, AlCl3, potassium acetate, Na2CO3, folin-ciocalceuc, and distilled water are among the substances utilized.

Research Procedure

BPF samples were obtained from Umbulharjo, Yogyakarta Province. Extraction was conducted by using maceration method with 96% ethanol solvent (1: 10) (Andhiarto et al,

2021). BPF extracts were standardised with specific and non-specific standardization. Specific extract standardization include plant determination, Organoleptic, 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 (Materia Medika Indonesia (MMI), 2023). Non-specific extract standardization include determination of extract yield with standard Ministry of Health RI (2000), total water content with standard Ministry of Health RI (2000), total ash content with standard Indonesian Herbal Formulary (IHF), (2008) and Acid insoluble ash content with standard IHF,(2008).

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 added with 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
      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 (λmaks)
      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 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 IC50 of extract

Determination of IC_{50} was calculated with the results of a linear regression curve between % inhibition with a series of sample concentrations, namely extracts and quercetin comparison solution. Determination of antioxidant activity was carried out by calculating the inhibitory concentration (IC_{50}) using equation 1: (Andriani & Murtisiwi, 2020).

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

b. Antibacterial activity

1) Sterilization

First, the necessary components are properly cleaned, dried, and sterilized. All ingredients should be covered with aluminum foil and sterilized in an autoclave for 15 minutes at 121ºC. Rubber tools can be sanitized 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 sanitized for 15 minutes under a UV lamp. The LAF is sterilized both prior to and following work on it (Hainil et al., 2021).

2) Nutrient Agar Media Preparation

Agar media was prepared by dissolving 4.2 grams of nutrient agar into 150 mL of distilled water in an erlenmeyer. Then the mixture was heated and sterilized 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 as much as 30 mL each into Petri dishes. 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 Mc Farland Standard

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

5) Preparation of Bacterial Suspension

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

6) Making Concentration of ethanol extract of *(C. ternatea)*

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 Chloramphenicol disc and the negative control used 10 ml 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).

Data Analysis

Data analysis of UV-Vis spectrophotometry results using Microsoft Excel.

RESULTS AND DISCUSSION (11pt)

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 set at 78^{0}C, 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 1.**

Table 1. 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. The 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 North Lombok district was 59.37 mgQE/g 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/mg. 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/mg. 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 (FHI, 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 = 8.578x + 22.885 with a value of R^2 = 0.8839. Based on the linear regression equation, the IC_{50} value of 6.261 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 IC50 41.36 ± 1.191 μg/mL. These studies have in common that BPF have antioxidant activity with a strong category.

Figure 1 shows the diameter of the inhibition zone of BPF extract, which was obtained by measuring the antibacterial activity of BPF extract against *S. aureus* bacteria at a concentration of 50% 6.67±1.15 mm in the weak category, at a concentration of 75% 6.67±1.15 mm in the weak category and 6.67±1.15 mm at 100% concentration in the weak category.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 number of 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 (Hendra et al, 2011). 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.

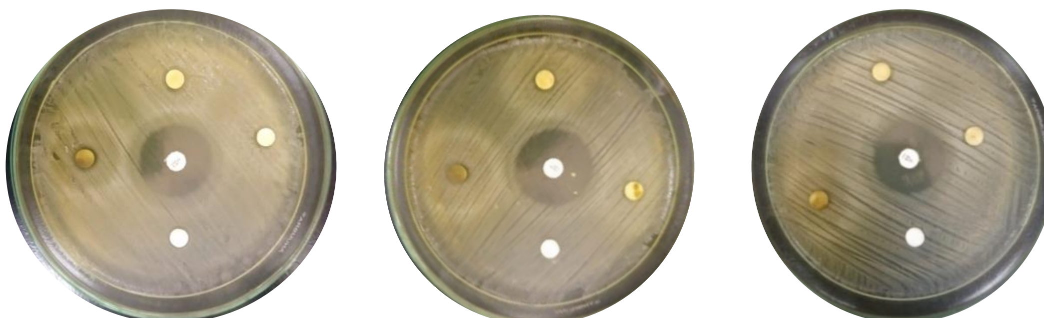

Figure 1. Antibacterial testing of BPF extracts with concentration variants of 50%, 75% and 100% using the disc paper method

CONCLUSION

BPF contained total flavonoids 19.44 ± 0.060 mgQE/g, total phenolics 36.37 ± 0.47 mgGAE/g, antioxidant activity 3,160 μg/mL with strong category and the average minimum inhibition zone of antibacterial activity at 50%, 75% and 100% concentrations were 6.67±1.15 mm, 6.67±1.15 mm, 6.67±1.15 mm respectively with weak category. The total flavonoids, phenolics, antioxidants and antibacterial activity of BPF are highly dependent on the place and location of growth, differences in altitude, temperature and environment. in further research, BPF extract may be turned into specific formulations with additional study.

ACKNOWLEDGMENT

Our thanks go to the Directoral of Research, Technology and Community Service, Directorate of the Ministry of Education, Culture, Research and Technology as the main funder for this research through contract number: 0557/E5.5/Al.04/2023.

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