Study of The Antibacterial Activity of *Staphylococcus aureus* and *Eschericia coli* Combination of Butterfly Pea Flower Extract (*Clitoria ternatea* L.) and Honey

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Keywords: Antibacterial, Butterfly Pea Flower, Honey, *Staphylococcus aureus, Eschericia coli*

Background: Butterfly pea flower is a herbal plant where every part of the plant has properties. The high anthocyanin content in butterfly pea flowers shows antimicrobial properties. Apart from it, honey is also a natural ingredient that has antimicrobial performance, because the flavonoids in honey are antibacterial. Antimicrobials are materials used to eradicate microbial infections in humans, including antibiotics, antiseptics, disinfectants and preservatives. Antibacterial activity test using the disc diffusion method. There are 5 levels of concentration of the combination of butterfly pea flower extract and honey, namely F1 (100%: 0%), F2 (75%: 25%), F3 (50%: 50%), F4 (25%: 75%), F5 (0 %: 100%). The inhibition zone of 100% concentration of butterfly pea flower is 9.9 mm, the combination concentration (75%, 25%) has inhibition zone of 7.1 mm, the combination concentration (50%, 50%) shows an inhibition zone of 11.4 mm, the combination concentration (25%, 75%) has inhibition zone of 11.3 mm, and in honey with a concentration of 100%, an inhibition zone of 11.3 mm was obtained against *Staphylococcus aureus*. The inhibition zone of 100% concentration of butterfly pea flower is 7.9 mm, the combination concentration (75%, 25%) has inhibition zone of 7.3 mm, the combination concentration (50%, 50%) shows an inhibition zone of 9.7 mm, the combination concentration (25%, 75%) has inhibition zone of 8.0 mm, and in honey with a concentration of 100%, an inhibition zone of 12.4 mm was obtained against *Eschericia coli*. Based on the research, it is concluded that butterfly pea flower has antibacterial potential in the medium category and honey in the strong category. The combination of butterfly pea flowers and honey in concentrations of (50%, 50%) and (25%, 75%) against *Staphylococcus aureus* shows strong antibacterial power, but 100% concentration honey has the strongest inhibitory power against *Eschericia coli*.

1 INTRODUCTION

Butterfly pea flower is a special herbal plant because all parts from the roots to the flowers are believed to have medicinal properties (Marpaung, 2020). Butterfly pea flowers also contain high levels of natural antioxidants, namely anthocyanins (Hariadi et al., 2018), flavonoids, flavonol, glycosides, kaempferol glycosides, quercetin glycosides, myrisetin glycosides, terpenoids, tannins and steroids (Kazuma et al., 2003). Anthocyanins exhibit antimicrobial, anti- inflammatory, antioxidant, anti-allergic, anti-viral properties and many other health benefits (Khoo et al., 2017). According to Suganya (2017), the anti- inflammatory properties of butterfly pea flower extract are equivalent to the performance of aspirin.

The anti-microbial or anti-microorganism properties of butterfly pea flowers have also been studied. Butterfly flower extract can inhibit the growth of three pathogenic bacteria, one of which is the bacteria that causes acne, namely *Staphylococcus aureus* (Marpaung, 2020).

Apart from butterfly pea flowers (*Clitoria ternatea* L.), honey is also a natural ingredient that is rich in antioxidants and has antimicrobial performance. Honey contains the enzymes catalase, glucose oxidase and peroxidase as well as non- enzymatic contents such as carotenoids, amino acids, proteins, organic acids, Maillard reaction products, and more than 150 polyphenolic compounds including flavonoids, flavonols, phenolic acids, catechins and cinnamic acid derivatives (Sumarlin et al., 2014). This composition supports the antioxidant properties of honey (Sumarlin et al., 2014). Mabrouk et al. (2004) in Muhartono et al. (2013) stated that the flavonoid content in honey is high so it has good antioxidant activity, which can bind free radicals.

Arifannisa, S. J., Sartika, D., and Astuti, S.
Study of the Antibacterial Activity of Staphylococcus aureus and Escherichia coli Combination of Butterfly Pea Flower Extract (Clitoria ternatea L.) and Honey.
1st International Conference on Medical Science and Health (ICOMESH 2023), pages 80-84.

Flavonoids in honey also have antibacterial properties. Nadhilla (2014) stated that the antibacterial power of honey can be used on gram-positive bacteria such as Staphylococcus aureus and gram-negative bacteria such as Eschericia coli which has been researched by Nirwana and Silvani (2022).

Antimicrobials are compounds that can inhibit the growth of bacteria or microorganisms. This compound causes denaturation of bacterial cell membrane, thus affecting the diffusion of compounds in and out. As a result, lysis occurs on the bacteria, thereby inhibiting their growth (Putri and Febrianto, 2018).

Bacteria are one of the microbes that most often play a role in various diseases. Gram positive bacteria (*Staphylococcus aureus*) cause skin and bacterial infections, and gram- negative bacteria (*Escherichia coli*) causes diarrhea (Huda, 2017).

2 METHODS

This research was carried out at the UPTD Microbiology Laboratory, Lampung Provincial Health Center, Vahana Scientific Laboratory, Padang City and the Integrated Research and Testing Laboratory at Gadjah Mada University, Yogyakarta. The research was conducted from June to September 2023.

2.1 Sample Preparation

Butterfly pea flower (*Clitoria ternatea* L.) picked and collected from Bandar Lampung and the honey used is Trigona honey, which is obtained from Suhita Bee Farm. Butterfly pea flowers that have been picked and cleaned are dried in an oven at 50℃ for 10 hours. After drying, the simplicia obtained was ground using a grinder and sieved using a 60 mesh-sieve. The butterfly pea flower powder was then extracted using the maceration method for 3x24 hours. The solvent used is 70% ethanol. Then the solvent was evaporated using a rotary evaporator at a temperature of 60ºC and a rotation of 7 rpm.

2.2 The Antibacterial Activity Test

Antibacterial activity test using the disc diffusion method. There are 5 levels of concentration of the combination of butterfly pea flower extract and honey, namely F1 (100% : 0%), F2 (75% : 25%), F3 (50% : 50%), F4 (25% : 75%), F5 (0 % : 100%).

2.2.1 Preparation of Test Solution

The concentration level for the combination of butterfly pea flower extract and honey is 2 ml. 2 ml butterfly pea flower extract for F1 (100% : 0%),1.5 ml butterfly pea flower extract and 0.5 ml honey for F2 (75% : 25%),1 ml butterfly pea flower extract and 1 ml honey for F3 (50% : 50%),0.5 ml butterfly pea flower extract and 1.5 ml honey for F4 (25% : 75%)and 2 ml honey for F5 (0% : 100%) using a micropipette.

2.2.2 Create MHA Media

Mix 19 g MHA media powder with 500 ml distilled water in an Erlenmeyer flask. Heat on a hotplate at 80℃ while stirring with a magnetic stirrer. Sterilize the media by autoclaving at 121℃ for 15 minutes at 2 atm pressure. The medium was poured into a petri dish and allowed to solidify.

2.2.3 Inoculation of The Bacteria

The bacterial suspension of *Staphylococcus aureus* and *Eschericia coli* was inoculated using a sterile cotton swab on each medium in a petri dish by spreading it evenly, then waiting for 10 minutes.

2.2.4 Immersion of Paper disc in The Test Solution

Paper discs soaked in a series of concentrations of a combination of butterfly pea flower extract and honey, namely F1 (100% : 0%), F2 (75% : 25%), F3 (50% : 50%), F4 (25% : 75%), F5 (0% : 100%). The paper discs were also soaked in *Chloramphenicol* as a positive control for *Staphylococcus aureus* and *Eschericia coli* bacteria, then distilled water as a negative control. The process of soaking the disc paper is carried out for 15 minutes.

2.2.5 Placing Disc paper on The Media

After soaking, the disc paper is placed on MHA Media with 5 quadrants and 2 quadrants. The media was then incubated in an incubator at 37ºC for 24 hours.

2.2.6 Inhibition Zone Measurement

The inhibition zone formed measured by a caliper.

3 RESULT

2.3 The Results of Extraction

The results of collecting butterfly pea flowers in Bandar Lampung and extracting butterfly pea flowers are presented in the table 1.

Table 1: The results of making simplicia

No	Repetition	Fresh Flowers (g)	Dry Flowers (g)	Flower Powder (g)
1	Repetition 1	1000	102	100
2	Repetition 2	1000	101	100
3	Repetition 3	1000	102	100
4	Repetition 4	1000	102	100

The results of extracting butterfly pea flowers are shown in the table 2.

Table 2: The results of extracting butterfly pea flowers

No	Repetition	Flower Powder (g)	70% Ethanol (ml)	Liquid Extract (ml)	Thick extract (g)	Yield (%)
1	Repetition 1	100	1000	730	42,9	5,8
2	Repetition 2	100	1000	765	39,1	5
3	Repetition 3	100	1000	780	80,9	10
4	Repetition 4	100	1000	780	77,1	9

Based on research that has been carried out, 42.9 grams of butterfly pea flower extract from repetition 1 were obtained or a yield of 5.8%. Repetition 2 butterfly pea flower extract was 39.1 grams or a yield of 5%. In repetition 3, the result of butterfly pea flower extract was 80.9 grams or a yield of 10%, and in repetition 4, butterfly pea flower extract was 77.1 grams or a yield of 9%.

According to Maulid and Jati (2019), the yield of the thick butterfly pea flower extract produced is 10%. In repetitions 3 and 4 in this research, the yield of thick butterfly pea flower extract was not much different from previous research, however for repetitions 1 and 2 the yield of thick butterfly pea flower extract was quite different, namely 5.8% and 5%.

The extraction method used is the maceration method for 3x24 hours. The maceration method is used because this method is the easiest, most widely used method and also minimize damage to the active ingredients of butterfly pea flowers.

The choice of ethanol solvent was made because its polar nature is the same as the anthocyanin and flavonoid content in butterfly pea flowers which are also polar, so that the active ingredient content can be maximally dissolved (Rifqi, 2021).

2.4 The Results of Antibacterial Activity Test

The results of the antibacterial test for *Staphylococcus aureus* by butterfly pea flower extract and honey and their combination are presented in the table 3.

Table 3: *Staphylococcus aureus* Antibacterial Test Results

Diameter of The Inhibition Zone (mm) *Staphylococcus aureus*						
No	**Concentration**	**1**	**2**	**3**	**Average Zone of Inhibition**	**Category**
1	Control aquadest (-)	0	0	0	0	
2	Control (+)	29,1	29,1	27,4	28,5	Very Strong
3	100% Butterfly pea Flower Extract (BFE)	10,5	11	8,2	9,9	Medium
4	75%, 25% (BFE, Honey)	6,5	6,5	8,5	7,1	Medium
5	50%, 50% (BFE, Honey)	12,5	13,1	8,7	11,4	Strong
6	25%, 75% (BFE, Honey)	11,4	9,7	10,7	10,6	Strong
7	100% Honey	12,7	10,8	10,5	11,3	Strong

Table 4: *Eschericia coli* Antibacterial Test Results

Diameter of The Inhibition Zone (mm) *Eschericia coli*						
No	**Concentration**	**1**	**2**	**3**	**Average Zone of Inhibition**	**Category**
1	Control aquadest (-)	0	0	0	0	
2	Control (+)	28,3	26,2	27,0	27,2	Very Strong
3	100% Butterfly pe Flower Extract (BFE)	7,4	7,6	8,9	7,9	Medium
4	75%, 25% (BFE, Honey)	6,2	8,4	7,5	7,3	Medium
5	50%, 50% (BFE, Honey)	9,1	9,7	10,3	9,7	Medium
6	25%, 75% (BFE, Honey)	7,7	7,1	9,3	8,0	Medium
7	100% Honey	10	14,1	13,1	12,4	Strong

The results of the antibacterial test of butterfly pea flower extract and honey on *Staphylococcus aureus* bacteria showed that the inhibition zone for butterfly pea flower extract with a 100% concentration was 9.9 mm and was categorized as moderately inhibitory. Then the combination of butterfly pea flower extract and honey with a concentration of (75%, 25%) showed an inhibitory zone of 7.1 mm which could be categorized as moderately inhibitory. In the combination of butterfly pea flower extract and honey concentrations (50%, 50%), an inhibition zone of 11.4 mm was obtained, which was categorized as having strong inhibitory power. Furthermore, in the combination of butterfly pea flower extract and honey concentrations (25%, 75%), an inhibitory zone of 11.3 mm was obtained, which was categorized as having strong inhibitory power. Then in honey with a concentration of 100%, an inhibition zone of 11.3 mm was obtained. The results of the diameter of the inhibition zone in the positive control Chloramphenicol were an average of 28.5 mm which was categorized as very strong, and in the negative control distilled water no inhibition zone was formed. The results of the antibacterial test of butterfly pea flower extract and honey on *Eschericia coli* bacteria showed that the inhibition zone for butterfly pea flower extract with a 100% concentration was 7.9 mm and was categorized as moderately inhibitory. Then the combination of butterfly pea flower extract and honey with a concentration of (75%, 25%) shows an inhibitory zone of 7.3 mm which can be categorized as moderately inhibitory. In the combination of butterfly pea flower extract and honey concentrations (50%, 50%), an inhibitory zone of 9.7 mm was obtained which was categorized as having moderate inhibitory power. Furthermore, in the combination of butterfly pea flower extract and honey concentrations (25%, 75%), an inhibitory zone of 8.0 mm was obtained, which was categorized as having medium inhibitory power. Then, in honey with a concentration of 100%, an inhibition zone of 12.4 mm was obtained and its inhibitory power was categorized as strong. The diameter of the inhibition zone in the positive control Chloramphenicol was an average of 27.2 mm which was categorized as very strong, and in the negative control distilled water no inhibition zone was formed.

According to the previous research by Maulid and Jati (2019) for butterfly pea flower extract and Yunus *et al*. (2019) for honey, that the inhibition power of butterfly pea flower extract is medium and weak, and for honey the inhibition power is strong. It is because of the dilution concentration of test solution that influences the strength of the antibacterial inhibitory power of an active substance In this research, the preparation of test solution did not use the dilution concentration, it is pure of only butterfly pea flower extract and honey, so the antibacterial power is also stronger.

4 CONCLUSION

Based on the research, it is concluded that butterfly pea flower has an antibacterial potential in the medium category and honey in the strong category. The combination of butterfly pea flowers and honey in concentrations of (50%, 50%) and (25%, 75%) against *Staphylococcus aureus* shows strong antibacterial power, but 100% concentration honey has the strongest inhibitory power against *Eschericia coli*.

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