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Antibacterial Activity of Methanol Extract of Butterfly Pea Leaves (*Clitoria ternatea* L.) by Maceration and Ultrasonic-assisted Extraction Methods Against *Propionibacterium acnes* ATCC-6919 and *Staphylococcus aureus* ATCC-29213

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Abstract:- Acne is a type of skin disease, caused by blackheads that can trigger some bacteria to cause acne. Butterfly pea leaf (*Clitoria ternatea* L.) is a plant that contains tannin and phenol that show antibacterial characteristics. The compounds can inhibit pathogenic bacteria such as *Propionibacterium acnes* and *Staphylococcus aureus*. The production of tannin and phenol can be maximized by applying the right extraction methods. Maceration and ultrasound-assisted extraction (UAE) are commonly used in extracting plant compounds. The objectives of the study were to determine the best extraction method that can produce higher tannins and phenols from butterfly pea leaves, the diameter of the inhibition zone, and the minimum inhibitory concentration (MIC) against *P. acnes* and *S. aureus* The variation of concentrations applied for the inhibition zone is methanol extract of butterfly pea leaves 40, 60, and 80 %, while MIC concentrations were of 5, 10, 20, and 40 % with five replications. The results show that the UAE method produced higher tannin and phenol content than the maceration method, namely 84.70 mg TAE/g extract and 640 mg GAE/g extract, respectively. The diameter of the inhibition zone of methanol extract of butterfly pea leaves by sonication method against *P. acnes* and *S. aureus* were higher than maceration at 20.60 mm and 22.90 mm, respectively, while the MIC of the butterfly pea leaves by sonication method against *P. acnes* and *S. aureus* were better than maceration at 10 % concentration.

Keywords:- *Clitoria Ternatea L., Antibacterial Activity, Butterfly Pea Leaves Extract, Minimum Inhibitory Concentration, Ultrasonic-Assisted Extraction*

I. INTRODUCTION

Acne is a type of skin disease that occurs on the skin surface of the face, neck, chest, and back. Excessive oil production in the skin can clog the skin pores, causing fat deposits that mix with sweat, dust, and dirt and may cause the appearance of blackheads. When the bacteria that cause acne are nourished, it will trigger acne. *Propionibacterium acnes* is the common bacterium in acne with a percentage of 78.8 percent [1]. *P. acnes* is a Gram-positive rod-shaped bacterium that can live in several parts of the human body such as the skin, oral cavity, colon, conjunctiva, and external ear canal [2].

Staphylococcus aureus is a Gram-positive bacterium that generally grows on the skin mucous and human mucous membranes and is harmless, but if there is a wound or puncture, the bacterium will infect the affected area, triggering mild infections such as skin infections and otitis media to severe infections such as pneumonia, bacteremia, and endocarditis [3]. *P. acnes* and *S. aureus* are the pathogenesis of acne which can cause skin infections in the form of acne due to the breakdown of triglycerides into free fatty acids as a trigger for inflammation [4]. Therefore, antibacterials are needed to prevent infection in the acne disease caused by *P. acnes* and *S. aureus.*

Antibacterials are substances that function to inhibit the growth and kill the bacteria that cause skin infections [5]. In general, the most recognized antibacterial as a treatment for a disease is antibiotics, but antibiotics can cause resistance and side effects [6]. Antibiotic resistance occurs due to gene mutations in the bacterial genome [7]. The side effects of long-term use of antibiotics cause skin irritation, organ damage, and immune hypersensitivity [2]. Therefore, alternatives of antibacterial are needed in the form of natural ingredients such as butterfly pea plant extracts to reduce the side effects.

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Butterfly pea plants are tropical plants that are widely found in Indonesia and are easy to find because the plants are tolerant of rainy or dry weather, so they are easily found in tropical countries [8]. Butterfly pea leaves are used to treat boils and swelling which are processed by brewing butterfly pea leaves using hot water which has been given coconut sugar, then attached to swollen parts or boils [9]. Butterfly pea leaves contain secondary metabolite compounds such as alkaloids, flavonoids, saponins, quinones, phenols, triterpenoids, and steroids [10].

Therefore, it is interesting to compare the extraction methods between ultrasonic-assisted extraction (UAE) or sonication and maceration. UAE is a method used to extract compounds in a material using ultrasonic waves [11]. The advantage of the UAE is that the active compounds produced are higher than the maceration method in a relatively short time and can prevent the loss of active compounds [12]. The novelty of this study is to compare extraction methods, namely UAE and maceration to produce higher tannin and phenol compounds from the plant. This research also tries to reveal the best antibacterial activity of methanol extract of butterfly pea leaves against *P. acnes* and *S. aureus*. The antibacterial activities of the methanolic extracts of butterfly pea leaves against *P. acnes* and *S. aureus* are done based on the inhibition zone and the minimum inhibitory concentration (MIC).

II. MATERIAL AND METHODS

A. Materials

Butterfly pea (*Clitoria ternatea* L.) leaves are obtained from Bantul, Yogyakarta Special Province, Indonesia. The chemicals utilized such as chloroform, 96 %, absolute ethanol, HCl, methanol 70 %, FeCl 10 %, H_2SO_4, Dragendorff reagent, Mayer reagent, Wagner reagent, distilled water, tannic acid, gallic acid, Folin reagent 10 %, Na_2CO_3, NaOH 1 %, Nutrient Agar (NA), Nutrient Broth (NB), alcohol 70 %, Gram A stain (Hucker's Crystal Violet), Gram B stain (Morgan Lugol's Iodine), Gram C stain (acetone alcohol), phenol red, NaOH 0.1 N, glucose powder, sucrose powder, lactose powder, hydrogen peroxide (H_2O_2) and Clindamycin powder 1 % (Novella). *P. acnes* ATCC-6919 and *S. aureus* ATCC-29213 pure cultures are obtained from PT AGAVI, Indonesia.

The present research applied a factorial randomized complete block design with variations of extraction using UAE and maceration. The concentration of methanolic extracts used is 40, 60, and 80 % for the zone of inhibition assay and 5, 10, 20, and 40 % for the MIC assay. All treatments are done in five replications.

B. Research Procedures

➢ *Clitoria Ternatea Leaves Extraction*

20 kilograms of butterfly pea leaves were obtained from local farmers in Bantul, Yogyakarta Special Province, Indonesia. The plant samples in the form of butterfly pea leaves were determined at the Plant Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia. The sample's preparations consist of a sampling process, dry sorting, washing, wet sorting, and drying. The plant leaves are chosen with the criteria of fresh light green leaves, leaves size of 2-5 cm, and no spots on the surface of the leaves. The dried leaves were crushed using a blender. The leaves powder is then sieved using mesh #60. The leaf powder that had been refined was put into a jar.

➢ *Leaves Maceration Processes*

Butterfly pea leaves powder was macerated with 70 % methanol solvent in a ratio of 1:10. The mixtures were put into a shaker incubator at 27 °C and 130 rpm for 24 hours, then continued with the re-maceration process. Re-maceration was carried out for 24 hours at 37 °C and 120 rpm. The samples from maceration and re-maceration were filtered with filter paper and evaporated within a rotary evaporator at 60 °C. The result of the evaporation process is a thick extract of butterfly pea leaves [13]. The yield of the condensed extract was calculated using the following formula.

$$\%rendement = \frac{amount\ of\ condensed\ extract\ (g)}{amount\ of\ dry\ weight\ (g)} \times 100\%$$

➢ *Leaves Ultrasonic-assisted Extraction Processes*

The leaf powder was extracted using the sonicator with 70 % methanol in a 1:10 ratio. The powder was extracted as much as 25 grams of samples each with 250 mL of methanol and sonicated for 25 minutes with a sonication frequency of 35 kHz and at the temperature of 30 °C. The samples were then filtered using filter paper and evaporated within a rotary evaporator at 60 °C [14]. The yield of the condensed extract was calculated using the following formula.

$$\%rendement = \frac{amount\ of\ condensed\ extract\ (g)}{amount\ of\ dry\ weight\ (g)} \times 100\%$$

➢ *Phytochemical Analysis*

- *Quantitative Tannin*

The determination of the total tannin content (TTC) of the methanol extract of the plant leaves was done by dissolving 6 mg of the leaves extract into 1 mL of 70 % methanol to obtain a 6000-ppm extract solution. One mL of 6000 ppm extract solution was taken and added with 9 mL of 70 % methanol to obtain 600 ppm extract solution. The preparation of the test solution was carried out by taking as much as 1 mL 6000 ppm extract solution into a test tube, then adding 0.5 mL 10 % Folin reagent and adding 3.5 mL 20 % Na_2CO_3. The samples were incubated at room temperature in a dark room for 30 minutes. Absorbance was determined with a UV-Vis spectrophotometer at a wavelength of 1000 nm [15].

The absorbance results of each replication were calculated using a linear regression equation to obtain the tannic acid equivalence (TAE). The TAE results were averaged, and the tannin total content (TTC) was calculated by the formula.

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$$\text{Tannin Total-Content} = \frac{\bar{x} \times Fp \times V}{m} \text{ (mg TAE/g extract)}$$

Where:
$\bar{X}$: Tannic acid equivalence result (mg/mL)
F_p: Dilution factor
V: Volume of test solution (mL)
m: Sample weight (mg)

- *Quantitative Phenol*

The determination of the total phenol content (TPC) of the methanolic extract of the plant leaves was done by dissolving 1 mg of the leaves extract into 1 mL of 70 % methanol to obtain a 10000-ppm extract solution. One mL of 10000-ppm extract solution was added with 9 mL of 70 % methanol to obtain 1,000 ppm extract solution. The preparation of the test solution was done by taking 1 mL of 1000 ppm extract solution into a test tube, then adding 0.4 mL 10 % Folin reagent and 3.6 mL 1 % NaOH. The samples were incubated at room temperature in a dark room for 30 minutes. The absorbance was determined with a UV-Vis spectrophotometer at a wavelength of 1000 nm [16].

The absorbance results of each replication were calculated using a linear regression equation to obtain gallic acid equivalence (GAE). The GAE results were averaged, and the phenol total content (PTC) was calculated by the formula.

$$\text{Phenol Total-Content} = \frac{\bar{x} \times Fp \times V}{m} \text{ (mg GAE/g extract)}$$

Where:
$\bar{X}$: Gallic acid equivalence result (mg/mL)
F_p: Dilution factor
V: Volume of test solution (mL)
m: Sample weight (mg)

➢ *Antibacterial Activity Assays*

- *Well Diffusion Method*

Antibacterial assay samples were the methanolic extracts of the plant leaves in three different concentrations i.e. 40, 60, and 80 %, as well as a positive control sample in the form of Clindamycin powder with a concentration of 1 %. *P. acnes* and *S. aureus* cultures were inoculated on Nutrient Broth (NB) medium, then *P. acnes* was incubated in an incubator, while *S. aureus* was incubated using a shaker incubator for 12 hours. One mL of *P. acnes* culture was taken using a micropipette and poured into a Petri dish and then nutrient agar (NA) medium was poured at a lukewarm temperature using the pour plate method. *S. aureus* culture was taken at 0.1 mL using a micropipette, and then put into a solid NA medium in a petri dish using the spread plate method. Wells were made with perforator number #2 as many as the number of the treatments and negative controls. Every treatment was then put into each well in as much as 0.1 mL.

The petri dishes were incubated at 37 °C for 18 hours. The zone of inhibition was measured with a caliper [17]. The zone of inhibition diameter was calculated using the formula:

$$Inhibition\ Zone = \frac{(Dv - Dc) + (Dh - Dc)}{2} \text{ (mm)}$$

Where:
D_v: Vertical Diameter
D_h: Horizontal Diameter
D_c: Well Diameter

- *MIC Determination Assay*

The MIC assay was carried out utilizing test tubes containing assay media marked with variations of the leaves extract concentrations i.e., 5, 10, 20, and 40 %, positive control and negative control. The viscous extract was made in 100 % concentration stock solution and then diluted into the assay media within concentrations of 5, 10, 20, and 40 %. The test tubes containing the assay media were incubated at 37 °C for 24 hours.

The MIC assay was conducted with the inoculation of each test solution into a Petri dish containing NA medium. Inoculation of *P. acnes* was done by pour plate, while *S. aureus* was done by spread plate. All the petri dishes that were inoculated with the test solution were incubated at 37 °C for 24 hours. Observations were made on each petri dish to see whether there was colony growth or not in each treatment [18].

III. RESULTS AND DISCUSSIONS

A. *Clitoria ternatea L.* Extraction

The extraction results of maceration and UAE methods are concentrated using a rotary evaporator to evaporate the solvent so that a thick extract is obtained. The thick extract obtained will be followed by a weighing process to determine the percentage of yield obtained.

The extraction of butterfly pea leaves uses a solvent in the form of 70 % absolute methanol. Methanol was chosen as a solvent because it can attract polar compounds and their derivatives such as phenolic acids and tannins, and the active compounds extracted are greater than other solvents such as ethanol [19]. The ratio used was 1:10 (b/v) between the sample and the solvent. The ratio was chosen because the yield produced was greater, the more volume of the solvent, the greater the active substance extracted so the yield produced was higher (Table 1) [8].

Table 1: Extraction Result of Butterfly Pea Leaf

Treatment	Weight of Extract (g)	Viscous Extract Weight (g) ± SD	Extract Yield (%) ± SD
Maceration	125	22,63 ± 0,92	18,11 ± 1,22
Sonication	125	30,51 ± 0,94	24,40 ± 1,25

Notes: Maceration for 24 Hours and Re-Maceration

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The yield of the sonication method is 1.3 times higher than that of the maceration method. This is due to the cavitation event. The cavitation event is the process of forming microbubbles due to ultrasonic waves and the microbubbles were unstable and easily broken. When the microbubbles break, it will result in large energy and generate heat effects so that the contact between the solvent and the plant samples becomes maximized and mass transfer increases so that the yield of the extract produced is much higher [11].

Solvent is an important factor in producing high yields, in the extraction of butterfly pea leaves using 70 % absolute methanol. Methanol can attract polar and non-polar compounds in the plant samples so that the resulting yield is high. In addition, butterfly pea leaves have compounds such as flavonoids, saponins, tannins, and terpenoids that can be extracted by methanol so the resulting yield will be high [20].

B. Quantitative Tannin

The TTC in the maceration method is 1.4 times lower than the research result of Jamil and Pae'e (2018) which states that the TTC is 78.75 mg TAE/g extract. The factor that causes the difference in TTC is the time used in the maceration process. The maceration is carried out within 2 x 24 hours so that more active substances can be extracted (Table 2).

Table 2: Total Tannin Content of Butterfly Pea Leaf Extract

Extraction	Absorbance ($\bar{X}$)	TTC (mg TAE/g extract)
Maceration	0,6104	54,84
Sonication	0,7806	84,70

However, this result follows the statement of Fajri *et al.* (2021), which states that if the maceration time is too short, the compound cannot be dissolved in the solvent [22]. The results show that the total tannin content of butterfly pea leaf methanol extract using the sonication method is 1.5 times higher than the maceration method (Table 2). The active compounds obtained in the extraction using the sonication method are higher [12]. This follows the research result of Khoddami *et al.* (2013) which states that sonication using ultrasonic waves can produce higher active compounds because the contact between the solvent and the sample occurs continuously [23].

C. Quantitative Phenol

The total phenol content (TPC) in the maceration method is 1.3 times higher than the research conducted by Jamil and Pae'E (2018) which states that the total tannin content is 245.14 mg GAE/g extract (Table 3).

Table 3: Total Phenol Content of Butterfly Pea Leaf Extract

Extraction	Absorbance ($\bar{X}$)	TPC (mg GAE/g extract)
Maceration	0,4020	331,08
Sonication	0,6348	640

The factor that causes the difference in the TPC is the solvent, in the previous study used absolute ethanol solvent, while in this study used absolute methanol. This result follows the result of Wiraningtyas *et al.* (2019) that methanol has fewer C atoms than ethanol, so the number of compounds bound by methanol is lower than ethanol [24].

The TPC of butterfly pea leaf methanol extract using the sonication method is 1.9 times higher than the maceration method (Table 3). The active compounds obtained from the sonication method are higher than maceration [12]. This follows the result of Khoddami *et al.* (2013) which states that the continuous contact between solvent and sample will allow ultrasonic waves to produce higher active compounds.

D. Well Diffusion Method Assay

The results of the methanolic extract of butterfly pea leaves were followed by an antibacterial activity assay based on the inhibition zone against *P. acnes* and *S. aureus* (Table 4). *P. acnes* is anaerobic, so it uses the pour plate inoculation method, while *S. aureus* is aerobic, so it uses the streak plate inoculation method.

Table 4: Zone of Inhibition of Butterfly Pea Leaf Extract against *P. acnes*

Treatment	Inhibition Zone Diameter ± SD (mm)	
	Maceration	Sonication
Methanol Butterfly Pea Leave Extract 40%	$12,00 \pm 0^{b}$	$17,80 \pm 0,02^{e}$
Methanol Butterfly Pea Leave Extract 60%	$13,80 \pm 0^{c}$	$19,80 \pm 0,02^{f}$
Methanol Butterfly Pea Leave Extract 80%	$15,00 \pm 0^{d}$	$20,60 \pm 0,02^{g}$
Clindamycin 1%	$39,30 \pm 0,22^{h}$	$39,30 \pm 0,22^{h}$
Methanol 70% Absolute	0 ± 0^{a}	0 ± 0^{a}

It was shown that in all treatments using maceration and sonication methods revealed the formation of inhibition zones. The higher the concentration of the methanol extract of butterfly pea leaves, the greater the diameter of the inhibition zone formed. The results of the largest inhibition zone using the maceration method are in the treatment of 80 % extract with an average inhibition zone diameter of 15 ± 0 mm, while the sonication method is in the treatment of 80 % extract with an average inhibition zone diameter of 20.6 ± 0.22 mm. These results are following the result of Brooks *et*

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al. (2005) that the greater the concentration of the extract, the higher the active substance component which causes the size of the inhibition zone formed to be greater [6].

The inhibition zone is divided into 4 categories, namely strong, very strong, medium, and weak. The inhibition category of the positive control (clindamycin 1 %), 80 % butterfly pea leaf extract with sonication method is classified as very strong because it has an inhibition zone diameter ≥ 20 mm (Figure 1). In contrast, the 40, 60, and 80 % butterfly pea leaf extracts in maceration and sonication methods are classified as a strong category because it has an inhibition zone diameter of 10-20 mm. These results follow the result of Davis and Stout (1971) which states that a diameter of ≤5 mm indicates a weak category, a diameter of 5-10 mm indicates a medium category, a diameter of 10-20 mm indicates a strong category while a diameter of ≥20 mm indicates very strong category. The results of the zone of inhibition of methanolic extract of butterfly pea leaves against *P. acnes* can be seen in Figure 1

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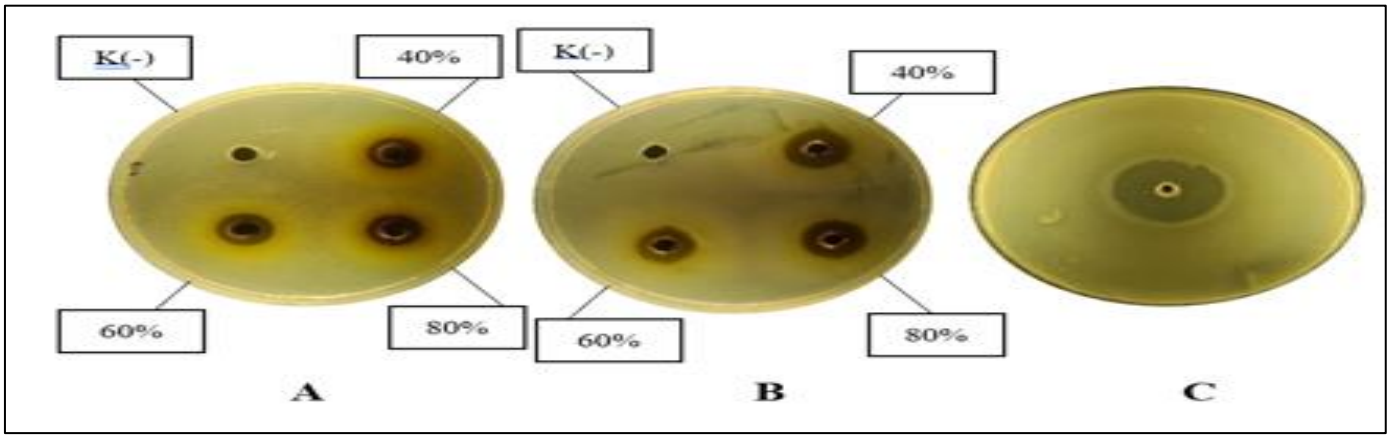

Fig 1: Inhibition Zone Assay Results of Butterfly Pea Leaf Methanolic Extract against *P. acnes.* Note: A. Inhibition Zone Result of Maceration Method, B. Zone of Inhibition Results of Sonication Method, K(+). Positive Control (Clindamycin 1 %), C.70 % Absolute Methanol

The diameter of the inhibition zone using the sonication method showed a higher number due to the cavitation process in the cell wall which caused the active compounds to be extracted more optimally than the maceration method. This result follows Liu's (2010) that there is a double effect, namely the disruption of the cell wall due to ultrasonic waves and the heating process resulting in kinetic energy followed by the appearance of cavitation bubbles so that the cell wall will experience mechanical effects and increased transfer of active compounds.

Table 5: Zone of Inhibition of Butterfly Pea Leaf Extract against *S. aureus*

Treatment	Inhibition Zone Diameter ± SD (mm)	
	Maceration	Sonication
Methanol Butterfly Pea Leave Extract 40%	$13,80 \pm 0,06^{b}$	$16,70 \pm 0,02^{e}$
Methanol Butterfly Pea Leave Extract 60%	$15,00 \pm 0^{c}$	$18,70 \pm 0,02^{f}$
Methanol Butterfly Pea Leave Extract 80%	$16,50 \pm 0^{d}$	$22,90 \pm 0,02^{g}$
Clindamycin 1%	$37,50 \pm 0,09^{h}$	$37,50 \pm 0,09^{h}$
Methanol 70% Absolute	0 ± 0^{a}	0 ± 0^{a}

All the treatments using maceration and sonication methods showed the formation of inhibition zones against *S. aureus* (Table 5). The higher the concentration of methanol extract of butterfly pea leaves, the greater the diameter of the inhibition zone formed. The results of the largest inhibition zone using the maceration method are in the treatment of 80 % methanolic extract with an average inhibition zone area of 16.5 mm, while the sonication method is in the treatment of 80 % methanolic extract with an average inhibition zone diameter of 22.9 ± 0.14 mm. These results are following the result of Brooks *et al.* (2005) that the greater the concentration of the extract, the higher the active substance component which causes the size of the inhibition zone formed to be greater.

The results of the antibacterial activity against *S. aureus* show that the methanol extract of butterfly pea leaves using the maceration method has the potential to become an antibacterial agent, these results follow the research result of Ramdani *et al.* (2021) which states that the antibacterial assay of 40, 60 and 80 % methanolic extracts of butterfly pea leaves are 8.6, 9.3, and 10.1 mm, respectively. The diameter of the inhibition zones using the sonication method shows higher numbers due to the cavitation process in the cell wall which causes the active compounds to be extracted more optimally than using the maceration method (Table 5). This result follows Liu's (2010) that there is a double effect, namely the disruption of the cell wall due to ultrasonic waves and the heating process resulting in kinetic energy followed by the appearance of cavitation bubbles so that the cell wall will experience mechanical effects and increased transfer of active compounds. The results of the zone of inhibition of methanol extract of butterfly pea leaves against *S. aureus* can be seen in Figure 2.

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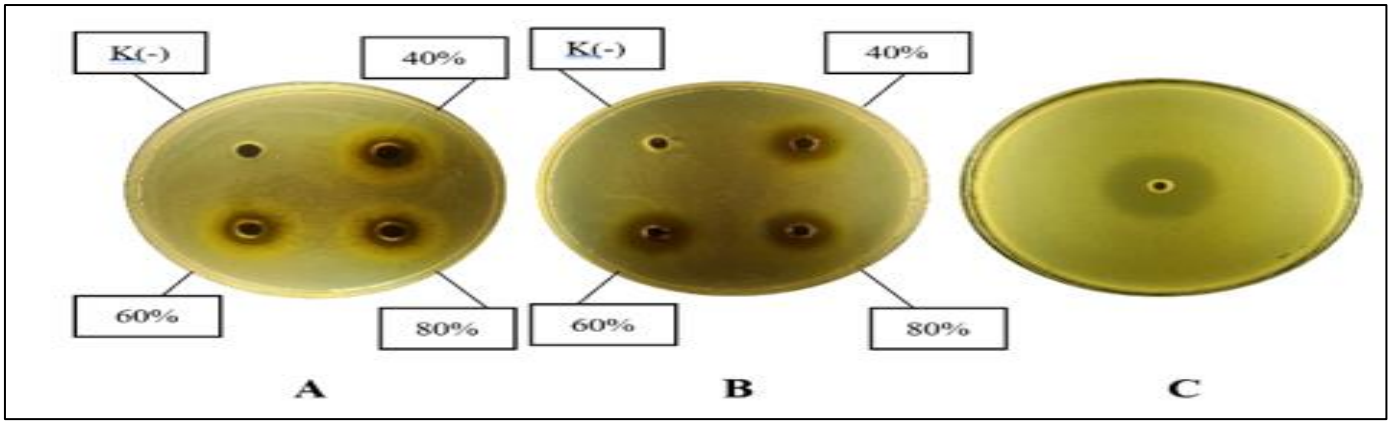

Fig 2: Inhibition Zone Test Results of Butterfly Pea Leaf Methanol Extract against *S. aureus.* Note: A. Inhibition Zone Result of Maceration Method, B. Zone of Inhibition Results of Sonication Method, K(+). Positive Control (Clindamycin 1 %), C. 70 % Absolute Methanol

E. MIC Determination Assay

The MIC assay results against *P. acnes* can be seen in Table 6.

Table 6: Minimum Inhibitory Concentration of Butterfly Pea Leaf Extract against *P. acnes*

Extraction	Concentration	Colony Number
Maceration	Extract 5%	4
	Extract 10%	0
	Extract 20%	0
	Extract 40%	0
	K(+)	0
	K(-)	TNTC
Sonication	Extract 5%	1.6
	Extract 10%	0
	Extract 20%	0
	Extract 40%	0
	K(+)	0
	K(-)	TNTC

Notes: K(+): Clindamycin 1 % ; K (-): 70 % Absolute Methanol; TNTC: Too Numerous To Count

The plant extracts with concentrations of 10, 20, and 40 % and positive control (clindamycin 1 %) have no bacterial growth. The 5 % concentrations of butterfly pea leaf methanol extract and the negative control have bacterial growth. The MIC assay results against *P. acnes* can be seen in Figure 3.

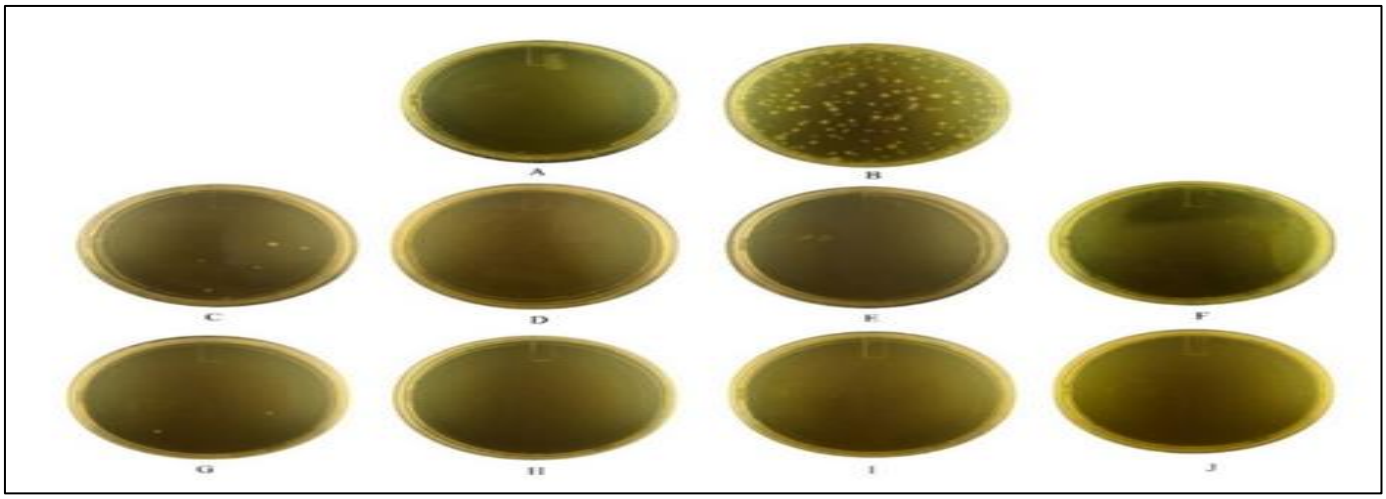

Fig 3: Minimum Inhibitory Concentration Results of Butterfly Pea Leaf Methanol Extract against P. Acnes. There is the Growth of Bacterial Colonies (B,C,G) and no Growth of Colonies (A,D,E,F,H,I,J). Note: A. Positive Control (Clindamycin 1 %), B. Negative Control (70 % Methanol), C-F. Extract by Maceration Methods (5%, 10% ,20%, 40% Respectively), G-J. Extract by Sonication Methods (5%, 10% ,20%, 40% Respectively)

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➢ *The MIC Test Results on S. aureus can be Seen in Table 7.*

Table 7. Minimum Inhibitory Concentration of Butterfly Pea Leaf Extract against S. Aureus

Extraction	Concentration	Colony Number
Maceration	Extract 5%	1
	Extract 10%	0
	Extract 20%	0
	Extract 40%	0
	K(+)	0
	K(-)	TNTC
Sonication	Extract 5%	0,4
	Extract 10%	0
	Extract 20%	0
	Extract 40%	0
	K(+)	0
	K(-)	TNTC

Notes: K(+): Clindamycin 1 %; K (-): 70 % Absolute Methanol; TNTC: Too Numerous To Count

The results prove that the MIC of butterfly pea leaf methanol extract can inhibit the growth of *S. aureus* and *P. acnes* is 10 %. This result follows the statement of Tortora *et al*. (2010) and Fadia *et al*. (2020) that the extract can inhibit a bacterium if there is no colony growth in the MIC assay. The factor that causes bacterial inhibition is the active compounds possessed by butterfly pea leaves, namely tannins, and phenols, because the two compounds have the same cellular target namely the Gram-positive cell wall. The MIC assay results against *S. aureus* can be seen in Figure 4.

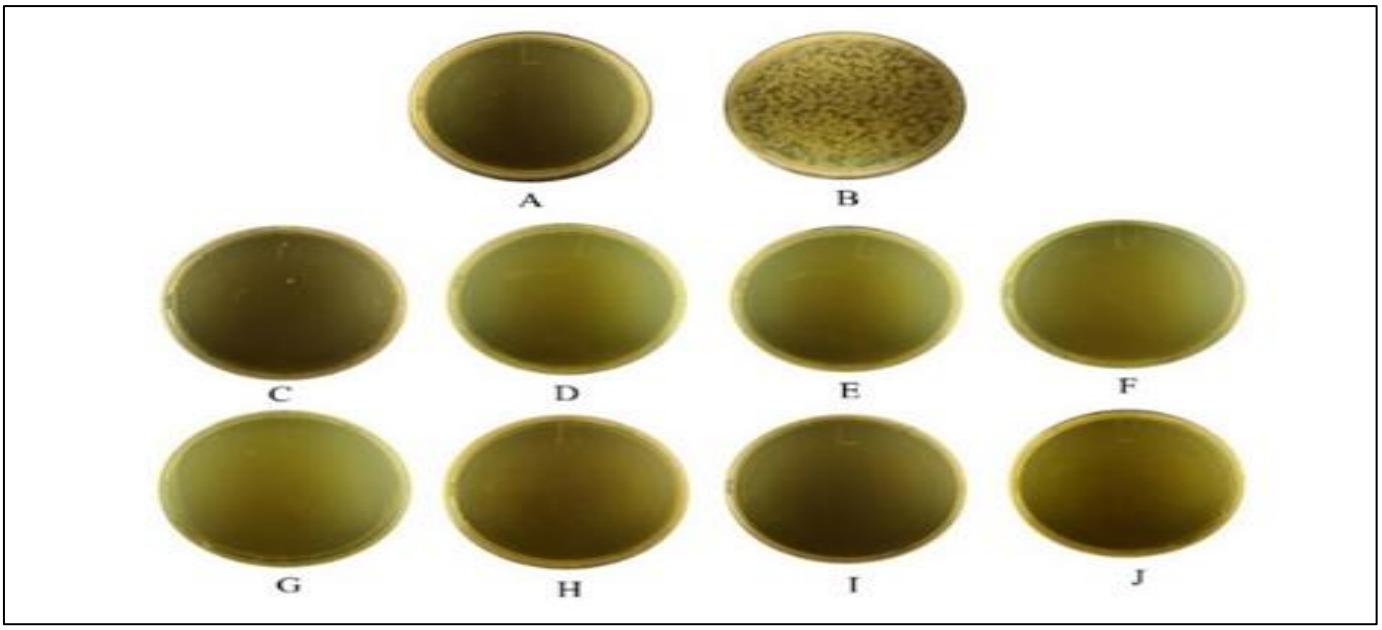

Fig 4: Minimum Inhibitory Concentration Results of Butterfly Pea Leaf Methanol Extract against *S. aureus.* There is the Growth of Bacterial Colonies (B,C,G) and no Growth of Colonies (A,D,E,F,H,I,J). Note: A. Positive Control (Clindamycin 1 %), B. Negative Control (70 % Methanol), C-F. Extract by Maceration Methods (5%, 10% ,20%, 40% Respectively), G-J. Extract by Sonication Methods (5%, 10% ,20%, 40% Respectively)

The mechanism of action of tannin on Gram-positive bacteria causes the cell wall to lysis because tannins have a target on the polypeptide so cell wall formation becomes less perfect, and the bacteria will die [29]. The mechanism of action of phenol is by denaturing bacterial cell proteins so that the metabolic activity of bacterial cells stops due to all metabolic activities of bacterial cells catalyzed by enzymes which are proteins [30]. Factors that determine success in sonication are frequency, temperature, time, and solvent [31].

IV. CONCLUSIONS AND RECOMMENDATIONS

Methanolic extract of butterfly pea leaves using the UAE method produced higher active compounds than the maceration method with a 1.5-fold higher TTC of 84,70 mg TAE/g extract, while the TPC was 1.9-fold higher at 640 mg GAE/g extract. The diameter of the inhibition zone and the MIC of methanol extract of butterfly pea leaves by the UAE method against *P. acnes* were better than the maceration method, namely 20.60 mm and 10 %, respectively, while against *S. aureus* 22.90 mm and 10 %.

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The researchers recommend that it is necessary to fractionate the active compounds contained in the methanol extract of butterfly pea leaves and conduct an antibacterial activity assay. It is also necessary to optimize the frequency, temperature, and time of the sonication method to obtain the highest active compounds.

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