TESTING THE ANTIBACTERIAL EFFECTIVENESS OF ETHANOL EXTRACTS FROM CHINESE BETEL LEAF (PEPEROMIA PELLUCIDA L.) USING A COMPARISON OF MACERATION AND SOXHLET EXTRACTION METHODS AGAINST STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI BACTERIA

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ABSTRACT

Chinese betel leaf known by the Latin name Peperomia pellucida L. is one of the plants that has secondary metabolite compounds that can act as medicinal ingredients that are efficacious to inhibit the growth of bacteria that cause infection. This study aims to determine the antibacterial effectiveness of Chinese betel leaves taken in Aimas, Sorong Regency, Southwest Papua. The bacteria used in this study are Staphylococcus aureus and Escherichia coli bacteria. maceration and sohxletasi with extract concentrations of 12.5%, 25%, 50%. The results showed that Chinese siri leaves at extract concentrations with the maceration method showed effectiveness on S.aureus bacteria with inhibition zones formed at a concentration of 12.5% with a diameter of 5.83 mm, 25% with a diameter of 10 mm, and 50% with a diameter of 12.83 mm. Pada bakteri E. coli ketiga konsentrasi terbentuk zona hambat masing-masing 4,75 mm, 6 mm, 10,83 mm. In contrast to the results of the ethanol extract of Chinese siri leaves with the soxhletation method did not show any inhibition zone on S. aureus and E. coli bacteria. The conclusion in this study is that extra ethanol of Chinese siri leaves using the maceration method has effectiveness against Staphylococcus aureus and Eschericia coli bacteria.

Keywords: Antibakteri, Daun Sirih Cina, E. coli, S. aureus.

INTRODUCTION

Bacteria are microorganisms that circulate widely in the environment and can multiply in the human or animal body that can cause infectious diseases (Sugiharti, et al., 2016). Pathogenic bacteria found in the body are at risk of causing various infections such as respiratory infections, gastrointestinal infections, and skin infections, namely Staphylococcus aureus and Escherichia coli bacteria.

Staphylococcus aureus is a gram-positive bacterium that is purple and cocci-shaped and can cause infections of the skin and human respiration, this bacterium will become pathogenic if there is a quantity and decrease in body resistance (Roni et al., 2019). Escherichia coli is a gram-negative bacterium that is short rod-shaped, anaerobic and has no color. E.coli bacteria in the human body are found in the large intestine and function to maintain the digestive system in humans. E. coli bacteria can also be pathogenic, causing disorders of the digestive system such as diarrhea (Fatiqin, et al., 2019).

Treatment of infections caused by bacteria generally uses antibiotics, but inappropriate use of antibiotics will cause resistance (Rahmawati, 2018). Therefore, it is necessary to develop traditional treatments from natural ingredients that are more effective, efficient, and safe in an effort to inhibit the growth of S. aureus and E. coli bacteria. One of the natural ingredients used as an antibacterial drug is Chinese betel leaf (P. pellucida L.).

Chinese betel leaf has the Latin name peperomia pellucida L. can inhibit the growth of bacteria that cause infection. Chinese betel leaves contain active metabolite compounds that can inhibit antibacterials such as alkaloids, tannins, flavonoids, saponins, and steroids (Khairani., 2021). Alternative medicine using Chinese betel leaf is very effective so the author is interested in conducting research on the antibacterial effectiveness of ethanol extract of Chinese betel leaf (Peperomia pellucida L.) against Staphylococcus aureus and Escherichia coli bacteria.

METHODS

This research was conducted in October 2024 - January 2025 at the Natural Materials Pharmacy Laboratory and Microbiology and Virology Laboratory of Unimuda Sorong.

The samples used were Chinese betel leaves taken from Aimas, Sorong Regency. Good and fresh Chinese betel leaves are picked and cleaned of dirt, washed under running water until clean, drained and then aerated, dried using an oven at 50°C, after drying the sample is mashed using a blender. Blended samples were sieved using a sieve and ready for sample extraction.

The extraction methods used in this study are cold (maceration) and hot (Soxhlet). The maceration extraction method is a simple extraction procedure that uses a solvent with repeated shaking or stirring at room temperature. Meanwhile, the soxhletation extraction method is an extraction with a new solvent and is usually carried out using a special tool so that continuous extraction is carried out in a relatively constant amount of solvent with recooling (Putri et al., 2021).

Chinese betel leaf simplisia powder as much as 300 grams was macerated using 96% ethanol solvent as much as 2,000 ml for 3 × 24 hours while occasionally stirring. After maceration for 3 × 24 hours the maceration dregs are filtered and then remacerated again for 2 × 24 hours using the same solvent as much as 500 ml. after that it is filtered again, the remaceration filtration results are put together and evaporated using a waterbath with a temperature of 50°C to obtain pure thick extract (Mulyani, et al, 2017). And as a comparison as much as 100 grams of Chinese betel leaf simplisia powder wrapped using filter paper, filter paper that has been filled with Chinese betel leaf simplisia powder is inserted into the sohxlet lead, add 96% ethanol solvent as much as 500 ml into a round bottom flask, after assembling all the tools sohxlet extraction by heat using a temperature of 70°C with nine repetitions for 90 minutes to obtain pure filtrate results in a round bottom flask.

Phytochemical Screening Test of Chinese Betel Leaf Extract

Flavonoids

Pure extract of Chinese betel leaf as much as 1 ml was poured into a test tube, then added lead(II) acetate reagent. A slight brownish yellow color change occurs, indicating the presence of flavonoid compounds.

Alkaloids

Pure extract of Chinese betel leaf as much as 1 ml was poured into three test tubes that had been labeled, then added mayer reagent, dragen drof, and boucardat to each tube. White precipitate occurs in mayer reagent, brown precipitate occurs in dragen drof reagent, and brick red precipitate occurs in boucardat reagent.

Tannins

Pure extract of Chinese betel leaf as much as 1 ml is poured into the reaction tabunng, then add FeCl3 reagent there is a brownish green color change indicating the presence of tannins.

Saponins

Pure extract of Chinese betel leaf as much as 1 ml is poured into a test tube, then add 3 mL of aqudest to the test tube, the solution is shaken gently and observed if foam is formed, indicating the presence of saponins.

Steroids

Pure extract of Chinese betel leaf as much as 1 ml was poured into a test tube, then added Clorofom reagent, H2SO4, Acetic Acid. Green ring formation occurs indicating the presence of steroids.

Antibacterial Effectiveness Test

Before conducting antibacterial testing, the tools used were washed, dried and wrapped using aluminum foil, sterilized first. Sterilization in this study by means of moist heat using an autoclave with a temperature of 121°C for 15 minutes.

Na was weighed as much as 8 g and dissolved in 250 ml of sterile aqudest. Heat until boiling and dissolved, removed and sterilized using an autoclave at 121°C for 15 minutes. After sterilization, Na is poured into the Petri dish that has been provided as much as 10-20 ml and left to solidify (Julianti, et al. 2017).

The effectiveness test of ethanol extract of Chinese betel leaf uses three concentrations,namely 12.5%, 25%, and 50%. The positive control used in this study used chloramphenicol disks and negative control used sterile distilled water.

The agart medium that has solidified is scratched with a suspension of S. aureus and E. coli bacteria on each petri dish that has been labeled, scratched using a sterile cutton bad. Soak the discs in each concentration of ethanol extract of Chinese betel leaf both concentration of extracts of maceration and sohxletation methods and negative control for 15 minutes. Disc paper is placed on agar media that has been scratched with test bacteria and replicated 3 times, and incubated for 24 hours at 37°C (Kristanti, 2014).

RESULTS AND DISCUSSION

Extraction Result of Chinese Betel Leaf (P. pellucida L.)

In this study, maceration and soxhletation extraction used 96% ethanol solvent. Ethanol has volatile properties in the evaporation process using a waterbath to produce a thick extract from Chinese betel leaf (P. pellucida L.). Ethanol 96% is semi-polar so that it can attract compounds in Chinese betel leaves that are polar and non-polar.

Table 1. Chinese Betel Leaf Maceration Extraction Results

Sample	Weight of Simplisia	Solvent Volume	Extract Weight
Chinese Betel Leaf (*P. Pellucida* L.)	300 gr	1.500 ml	55,5 gr

Maceration is done by soaking 300 grams of Chinese betel leaf simplisia into a solvent of 1,500 ml for 3 × 24 hours and stirring for 3 × 8 hours, the results of the first macerate are filtered and remacerated again for 2 × 24 hours after that the first and second macerate results are combined for evaporation of the sample so that the results of pure thick extract are obtained as much as 55.5 grams of thick extract of Chinese betel leaf (P. Pellucida L.).

Table 2. Soxhletation Extraction Results of Chinese Betel Leaf

Sample	Weight of Simplisia	Solvent volume	Cycle Repetition / Hour	Extrat Weight
Chinese Betel Leaf				

(*P. Pellucida* L.)	100 gr	500 ml	9 kali siklus / 2 jam	14,9 gr

Extraction by means of soxhletation using a special soxhlet tool, 100 grams of Chinese betel leaf simplisia wrapped using filter paper and then placed in a sleeve and extracted with a repetition of nine cycles for two hours at a temperature of 70°C. The soxhletation results were then evaporated until a pure viscous result of 14.9 gr was obtained.

Yield Results

Rendemen Maserasi	Rendemen Sohxletasi
$\text{Rendemen (\%)} = \frac{\text{berat ekstrak}}{\text{berat sampel}} \times 100\%$	$\text{Rendemen (\%)} = \frac{\text{berat ekstrak}}{\text{berat sampel}} \times 100\%$
$\text{Rendemen} = \frac{55\ \text{gr}}{300\ \text{gr}} \times 100\%$	$\text{Rendemen} = \frac{14{,}9\ \text{gr}}{100\ \text{gr}} \times 100\%$
$= 0{,}185 \times 100\%$	$= 0{,}149 \times 100\%$
$= 18{,}5\ \%$	$= 14{,}9\%$

Results of Phytochemical Screening of Chinese Betel Leaf (P. pellucida L)

Phytochemical screening can be used as a basis for developing the biological activity of red betel leaf extracts using maceration and Soxhlet extraction methods, which include testing for alkaloids, tannins, flavonoids, and saponins. The test results can also show the amount of secondary metabolites contained in Chinese betel leaves (Sukma et al., 2018).

Table of phytochemical screening of Chinese betel leaves. Phytochemical screening was performed on two extraction samples using hot and cold methods. Screening was carried out in this study to determine the secondary metabolites contained in Chinese betel leaves (P. pellucida L.).

The following table shows the phytochemical screening of Chinese betel leaf ethanol extract, which yielded several secondary metabolites:

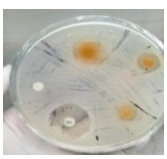
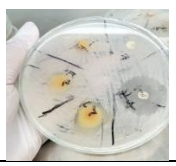
Table 3. Results of Phytochemical Screening using Maceration Samples

Test Bacteria	Extracti Concentration	Diameter Zona Hambat (mm)			
		Replication I	**Replication II**	**Replication III**	**Average**
S. aureus	K+	22,75	24,5	22,5	23,25
	12,5%	5,25	5,5	6,75	5,83
	25%	11	10,75	8,25	10
	50%	12,25	14	12,25	12,83
	K (-)	-	-	-	-
E. coli	K +	23	20,25	20,25	21,16
	12,5%	4	3	7,25	4,75
	25%	3,35	7,5	10,25	6
	50%	11	10,5	11	10,83
	K (-)	-	-	-	-

Note : K (+) = Positive Control

K (-) = Negative Control

Table 4. Results of Observation of the Inhibition Zone of S. aureus and E. coli

Pengamatan *S. aureus*		
Replikasi I	Replikasi II	Replikasi III
		
Pengamatan *E.coli*		
Replikasi I	Replikasi II	Replikasi III

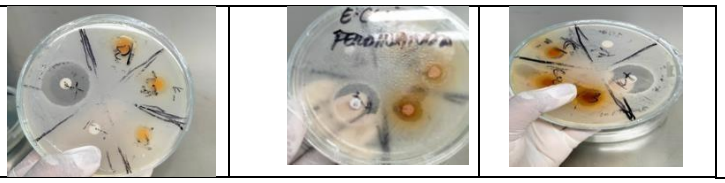
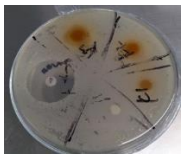
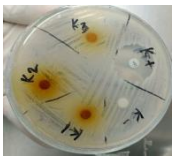
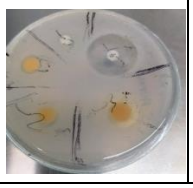
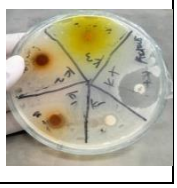


Table 5. Results of Observation of the Inhibition Zone of S. aureus and E. coli Using the Soxhlet Method

Pengamatan S. aureus		
Replikasi I	Replikasi II	Replikasi III
		
Pengamatan E.coli		
Replikasi I	Replikasi II	Replikasi III
		

Extraction methods are based on whether or not heating is involved and are divided into two types: cold extraction and hot extraction. Cold extraction, in principle, does not require heating during the extraction process to prevent the desired compounds from being damaged. On the other hand, hot extraction involves heating during the extraction process to accelerate the extraction process (Hujjatusnaini et al., 2021).

Maceration is the process of soaking a sample in an organic solvent at room temperature. The maceration method is very useful in isolating active compounds. This occurs when the cell wall is soaked, causing lysis due to the pressure difference between the inside and outside of the cell. After cell wall rupture, the active compounds present in the cytoplasm are dissolved into the organic solvent. The choice of organic solvent for maceration also has a significant impact. Based on previous research, ethanol is the most commonly used solvent in the maceration process for isolating active compounds (Hasrianti et al., 2016).

The principle of maceration is to isolate active substances from a plant organ by soaking it in a suitable solvent and storing it at room temperature away from sunlight. The organic solvent then diffuses through the cell wall into the cell, and the active compounds inside the cell dissolve in the solvent due to the difference in concentration between the solution outside the cell and inside the cell. The high- concentration solution is forced out of the cells (diffusion process) and replaced by a low-concentration solvent. The diffusion process continues until equilibrium is reached between the concentration of the solution inside the cells and the solution outside the cells. During the extraction process, the mixture is occasionally stirred. The precipitate obtained is then separated, and the filtrate is concentrated. The maceration method has several advantages, including the use of relatively simple equipment. However, its disadvantages include the need for a relatively long extraction time, the requirement for a large amount of solvent, and the inability to

apply the maceration method to materials with hard textures, such as benzoin, wax, and tirix (Hasrianti et al., 2016).

Soxhlet is a new solvent extraction method. It is usually performed using a special device so that constant extraction occurs with the presence of a counterflow cooler. Heating causes the solvent to rise, and once at the top, it is condensed by the air cooler into droplets that collect again. If the droplets exceed the limit of the side pipe opening of the Soxhlet, a continuous circulation occurs, resulting in efficient extraction (Hujjatusnaini et al., 2021). The advantages of the Soxhlet extraction method include the ability to extract a large number of compounds using a smaller amount of solvent, suitability for heat-resistant plant materials, no need for filtration, and the ability to adjust the extraction temperature according to the properties of the solvent and the material being extracted. The disadvantage of this method is that it is not suitable for thermolabile materials (Triastuti, 2022).

Phytochemical screening was conducted to obtain information on the secondary metabolites contained in Chinese betel leaf extract. Phytochemical screening was performed qualitatively, and the compounds tested were flavonoids, tannins, saponins, and alkaloids. The results of qualitative phytochemical screening of Chinese betel leaf extract can be seen in Tables 1 and 2 of the phytochemical screening test results. The results of the screening on Chinese betel leaf extract using the hot method (Soxhlet) showed several compounds such as alkaloids, tannins, and saponins, and in this extraction method, there was no color change in flavonoids. In contrast, the screening results using maceration showed positive results or the presence of secondary metabolites in Chinese betel leaves (P. pellucida L.). The alkaloid test in the Mayer test is characterized by the formation of a white precipitate. This precipitate is a potassium alkaloid complex. In the preparation of the Mayer reagent, a solution of HgCl2 with potassium iodide will react to form a red HgI2 precipitate. If excess potassium iodide is added, K2[HgI2] will form (Shevla, 1990). Alkaloids contain nitrogen atoms with free electron pairs, enabling them to form coordinate covalent bonds with metal ions (McMurry, 2004). In the alkaloid test using Mayer's reagent, it is hypothesized that the nitrogen in the alkaloid reacts with the K+ ions from K2[HgI2] to form a potassium-alkaloid complex that precipitates.

A positive result for alkaloids in the Dragendorff test is also indicated by the formation of a light brown to yellow precipitate. This precipitate is a potassium alkaloid. In the preparation of the Dragendorff reagent, bismuth nitrate is dissolved in HCl to prevent hydrolysis, as bismuth salts are easily hydrolyzed to form bismuth ions (BiO+). To keep the Bi3+ ions in solution, acid is added to shift the equilibrium to the left. Subsequently, the Bi^{3+} ions from bismuth nitrate react with KI to form a black BiI4 precipitate, which then dissolves in excess KI to form K[BiI4] (Svehla, 1990). In the alkaloid test using the Dragendorff reagent, nitrogen is used to form a coordinate covalent bond with K^{+}, which is a metal ion.

Positive results for alkaloids in the Boucardat test are indicated by the formation of a light brown to yellow precipitate (Marliana, Suryanti, and Suyono, 2005). It is estimated that the precipitate is potassium alkaloid. In the preparation of the Boucardat reagent, iodine reacts with I- ions from potassium iodide to produce brown-colored I3- ions. In the Boucardat test, K+ metal ions form covalent coordinate bonds with nitrogen in the alkaloid, forming potassium alkaloid complexes that precipitate.

In the tannin test using FeCl3 reagent, a positive result is indicated by the formation of a blackish- green complex compound in the sample, which indicates the presence of tannin compounds. In the saponin test, a positive result is indicated by the formation of stable foam for 10 minutes in the sample.

Flavonoids play a very important role and are the largest compounds in the phenol group, which are polar in nature and soluble in polar solvents such as ethanol, methanol, acetone, and butanone (Rachmawati and Suriawati, 2019). Phenols possess antibacterial and antimicrobial properties, while flavonoids have chemical properties that can inhibit disease attacks, such as antibacterial and antiviral effects on plants. The mechanism of action of flavonoids involves damaging the phospholipid layer of cell membranes and reducing bacterial permeability (Saputra and Anggraini, 2016).

Alkaloids can inhibit antibacterial agents. The mechanism of action of alkaloids is to disrupt the components that make up peptidoglycan in bacterial cells, thereby preventing the cell wall from forming properly and causing cell death (Kurniawan et al., 2015).

Tannins act as antibacterial agents because they have the ability to form complex compounds with proteins through hydrogen bonds. When hydrogen bonds form between tannins and proteins, the proteins become denatured, disrupting bacterial metabolism (Rahayu, 2019).

Saponins act as antibacterial agents by denaturing bacteria, as the active substances on the surface of saponins can be used as antibacterial agents, reducing the tension on the surface of bacterial cell walls and damaging the permeability of bacterial membranes. Bacteria are disrupted due to the damage to the cell membrane, and saponins then diffuse through the cytoplasmic membrane, disrupting membrane stability. This can cause cytoplasmic leakage and exit from the cell, leading to cell death (Sudarmi et al., 2017).

In this study, ethanol extract of Chinese betel leaf (P. pellucida L) was prepared using 96% ethanol solvent, and the Chinese betel leaf extract used was in the form of powdered crude drug. This study used three extract concentrations, namely 12.5%, 25%, and 50%, and two controls, namely positive and negative. The positive control used a chloramphenicol blank disk antibiotic, and the negative control used sterile distilled water.

Positive control used chloramphenicol discs, chloramphenicol antibiotic is a broad-spectrum bacteriostatic active against gram-positive and gram-negative bacteria. The mechanism of action of chloramphenicol is to inhibit microbial protein synthesis (Gunawan, 2020). Meanwhile, the negative control in this study uses sterile distilled water, as it has no antibacterial activity, is stable, non-toxic, and does not easily evaporate (Lombogia et al., 2016).

The solidified Na medium in the Petri dishes was then streaked with S. aureus and E. coli bacterial suspensions using cotton buds in a zig-zag pattern on each labeled Petri dish. After streaking, blank disks that had been soaked in the extract concentration and positive and negative controls were placed in the Petri dishes that had been streaked with bacteria. This was repeated three times.

The results of the study on the above maceration extract showed that the ethanol extract of Chinese betel leaf (P. pellucida L.) has antibacterial efficacy, as indicated by the presence of a clear zone in the determination of the diameter of the inhibition zone. Each concentration can form an inhibition zone on agar medium inoculated with S. aureus and E. coli bacteria, with average results as shown in Table 1. The inhibition zone test results for the three concentrations of the extract on the growth of S. aureus and E. coli indicate that the largest inhibition zone is at a concentration of 50%, with average values of S. aureus 12.83 mm and E. coli 10.83 mm. It can be said that the higher the concentration of Chinese betel leaf (P. pellucida L.) ethanol extract, the larger the inhibition zone obtained (Rahayu, 2019).

In the hot method (soxhletation), each extract concentration in the three replicates did not have an inhibitory zone on S. aureus and E. coli bacteria. Therefore, it can be stated that in the soxhletation method, the ethanol extract of Chinese betel leaves was not

effective against these two bacteria. This is because some compounds were damaged during the Soxhlet extraction process, as some of the metabolites contained in the extract cannot withstand extremely high temperatures.

The antibacterial effectiveness of Chinese betel leaf ethanol extract is classified as intermediate against the test bacteria S. aureus, but the extract can be said to have very little inhibitory ability with an inhibition zone of 12.83 mm against S. aureus compared to E. coli, which has an inhibition zone of 10.83

mm. The antibacterial efficacy of 96% ethanol extract of Chinese betel leaves using the maceration extraction method is classified as weak against both test bacteria. The antibacterial efficacy of an extract can be categorized based on the diameter of the inhibition zone produced. Weak if the diameter produced is <12 mm, intermediate (13-19 mm), and >20 mm is considered very strong (Zhang et al., 2017).

CONCLUSION

Ethanol extract of Chinese betel leaf (P. pellucida L.) using the maceration extraction method is effective against Staphylococcus aureus and Escherichia coli bacteria. In contrast, ethanol extract of Chinese betel leaf using the Soxhlet extraction method is not effective against either of these bacteria.

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