

Antibacterial Activity and Preliminary Phytochemical Screening of Forest Chili Leaf (*Piper caninum* Bl.) Ethanol Extract against *Staphylococcus epidermidis*

Flavia Fionessa Falenda Shanzet¹, Ida Bagus Gede Darmayasa¹, Ni Luh Suriani^{1*}, Anak Agung Istri Sri Wiadnyani², Ting Seng Ho³, Rusdianasari⁴, Mariani⁵

¹Biology Study Program, Faculty of Mathematics and Natural Sciences, Udayana University Bali 80361 – Indonesia

²Departement of Food Science and Technology Faculty of Agricultural Technology Udayana University Bali 80361 – Indonesia

³Back2nature Regenerative Farm, Kuala Pilah, Malaysia

⁴Politeknik Negeri Sriwijaya, 30139, Bukit besar Palembang, Indonesia

⁵Agrotechnology Study Program, Faculty of Agriculture, Nahdlatul Wathan University, Mataram

*Corresponding author: niluhsuriani@unud.ac.id

Article History: Received: 22 Oktober 23, Final Revision: 12 December 23, Accepted: 21 December 23, Online Publication: 31 Januari 2024

ABSTRACT

Forest chili leaves (*Piper caninum* Bl.) has a potential to become an alternative to conventional antibiotics. This study aims to determine the Minimum Inhibitory Concentration (MIC) of forest chili leaf extract, assess the Lethal Concentration (LC₅₀), and analyze the phytochemical composition of the extract. This study also conducted a Completely Randomized Design (CRD) with four repetitions. The agar well diffusion method is used in this study with various concentrations such as T1 ethanol 96% (negative control), T2 (5% concentration), T3 (10% concentration), T4 (15% concentration), T5 (20% concentration), T6 (25% concentration), and T7 Ciprofloxacin 1% (positive control). Data were subjected to ANOVA at a significance level of 5% and further analyzed using Duncan's test. The results showed that all concentrations of forest chili leaf extract (*P. caninum* Bl.) exerted a statistically significant effect ($P < 0.05$) against *Staphylococcus epidermidis* bacteria. The research found that the most effective concentration to inhibits *S. epidermidis* is at 25%, which generates an inhibitory zone of 25.38 ± 0.52 mm. The estimated MIC value for forest chili leaf extract against *S. epidermidis* was found to be 0.2%, producing an inhibitory zone of 6.38 ± 0.32 mm. Additionally, the calculated LC₅₀ value was 0.26%. Phytochemical screening revealed the presence of various bioactive compound groups in the forest chili leaf extract, including alkaloids, flavonoids, phenols, tannins, and steroids.

Keywords: Forest chili leaf extract, acne, antibacterial activity, *Staphylococcus epidermidis*

INTRODUCTION

Bacterial infections are one of the most common causes for diseases in various countries, especially Indonesia. An example of a disease caused by a bacterial infection is acne (Radji, 2011). Acne is a common disease that occurs on the surface of the skin on the face, chest, neck and back. This happens when the skin's oil (sebaceous) glands are too active which causes the skin pores to be covered with excess fat deposits (Sawarkar et al., 2010). One of the bacteria that triggers inflammation is *Staphylococcus epidermidis*. *S. epidermidis* are bacteria that cause swelling (abscesses) such as pimples, which can also cause skin, kidney and urinary tract infections (Radji, 2011).

Treatment of acne at a skin clinic generally uses drugs that contain antibiotics, such as ointments and certain patients require additional action performed directly by a specialist. Antibiotics play an important role in treating acne because they can suppress the activity of pathogenic bacteria and provide anti-inflammatory effects (Zaenglein et al., 2012; Thiboutot et al., 2009). However, long-term use of antibiotics can trigger resistance and result in organ damage and immunohypersensitivity (Djajadisastra et al., 2007). The use of plants as an alternative made from natural ingredients to treat infections has been widely developed because they are relatively safer to be consumed in the long term compared to drugs made from chemicals such as antibiotics. Natural raw materials also have relatively cheaper prices and are easy to obtain (Putri, 2010).

Forest chili (*Piper caninum* Bl.) is a plant in

Indonesia that can be used as natural antibiotic because it contains antibacterial compounds, namely alkaloids and flavonoids as well as antioxidant compounds such as polyphenols and steroids (Salleh et al., 2015). This plant is geographically distributed with as many as 700 species in America and about 340 species spread across Indonesia and Malaysia (Parmar et al., 1997). Since ancient times, Piperaceae plants have been used for medicinal purposes such as treating wounds, urological, skin, liver and digestive diseases, also as natural antioxidants and antimicrobial agents to preserve food (Salehi et al., 2019). Forest chili leaves are generally consumed directly to treat hoarseness (de Guzman and Siemonsma, 1999) and are also used to help women recover after giving birth by washing the female area (Chan and Wong, 2014).

Research conducted by Salleh et al. (2011) proved that an aqueous extract of forest chili leaves had better inhibition against Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* with an inhibition zone range of 7 to 12 mm with a Minimum Inhibitory Concentration (MIC) value of 250 to 500 µg/mL. Suriani et al. (2015) also reported that forest chili leaf extract can also inhibit the growth of the fungus *Pyricularia oryzae* that causes blast disease in rice plants. Furthermore, Suriani et al. (2020) found an antifungal activity of forest chili leaf extract against *Curvularia verruculosa* in rice plants. Based on the description above, the authors are interested in researching and testing the inhibitory power of forest chili (*P. caninum* Bl.) leaf extract against *S. epidermidis*.

MATERIALS AND METHODS

Time and place of study

This research was conducted from February to April 2023. All observations were carried out at the Microbiology Laboratory, Biology Study Program, Faculty of Mathematics and Natural Sciences, Udayana University.

Tools and materials

The materials used in this research were forest chili leaves, aquadest, ethanol 96%, nutrient agar (NA), mannitol salt agar (MSA), nutrient broth (NB), sulfuric acid (H₂SO₄), and barium chloride (BaCl₂). The tools used were glass

petri dishes, test tubes and the rack, glass bottle, ruler, blender, digital scale with 0,01 gr precision, lighter, cork-borer, cotton swab, aluminium foil, spatula, spiritus burner lamp, inoculation loop, microtips, micropipette, vortex mixer, Eppendorf tubes with 2 mL capacity, porcelain spot plate, autoclave, rotary evaporator *Buchi R-100*, and laminar air flow.

Methods

Research design

A Completely Randomized Design (CRD) was used in this study, which consisted of 7 treatments, which consisted of the variations of the concentration of forest chili leaf extract 5% (w/v), 10% (w/v), 15% (w/v), 20% (w/v), 25% (w/v), positive control (*Ciprofloxacin* 1%), and negative control (ethanol 96%). Each experiment was repeated 4 times, so there was a total of 28 experimental units.

Plant Material and Extraction

The forest chili leaves used in this research were collected from Senganan Village, Penebel District, Tabanan Regency, Bali, Indonesia in January 2023. This study picked the third mature leaf from top to bottom based on Harborne (1987). Final collected leaves amount to 1 kg then washed thoroughly and air-dried without being exposed to direct sunlight until the weight was constant. Dried forest chili leaves were processed into a simplicia powder using blender. The extraction process was continued to a multilevel maceration stage. 100 g forest chili leaf powder was macerated using 1000 mL ethanol 96% with sample and solvent ratio of 1:10 (w/v) for 48 hours. The residue from first maceration stage was reused for second maceration which was carried out for 24 hours. The filtrates obtained from both maceration stages were combined to yield a combined filtrate. The combined filtrate was then concentrated using a rotary evaporator *Buchi R-100* at 60°C to obtain a crude extract.

Preliminary Phytochemical Screening

The major phytochemical classes such as alkaloids (Mayer's, Wagner's, and Dragendorff's tests), phenols and tannins (ferric chloride test), flavonoids (Wilstater's test), saponins (foam test), steroids and terpenoids (Liebermann-Burchard's test) were investigated according to the commonly described phytochemical methods (Harbone, 1973; Poumale et al., 2013)

Bacterial Suspension

S. epidermidis were inoculated in to NA using sterile loops and incubated for 24 hours at 37°C. Colonies that grew on the medium were taken with inoculation loops and then inoculated into a test

tube containing NB. The bacteria density tested was equivalent to a 0.5 McFarland standard solution, which is 10^8 cells/mL.

Antibacterial activity

This test was determined using the agar well diffusion method. NA medium was poured into petri dishes and subsequently allowed to solidify. *S. epidermidis* bacterial suspension was taken using sterile cotton swab. Cotton swab rubbed on solid NA media surface. Each well was filled with approximately 15 until 20 μ L of forest chili extract with various concentrations: 5% (w/v), 10% (w/v), 15% (w/v), 20% (w/v), 25% (w/v), positive control (*Ciprofloxacin* 1%), and negative control (ethanol 96%) then incubated for 24 hours at 37°C. Inhibitory zone diameter formed around wells was measured using ruler. Calculations were made by measuring diameter length vertically, horizontally, and diagonally. The research was continued with MIC test using the same method.

Lethal Concentration (LC_{50}) Test

The LC_{50} test was carried out using pour plate method. Eppendorf filled with 100 μ L bacterial suspension and 400 μ L a mixture of extract concentrations in the previous MIC test and ethanol 96% (calculated using the dilution formula). Eppendorf tubes were homogenized using vortex mixer then left for 15 minutes at room temperature. Whole solution in Eppendorf and NA were poured into petri dish. Petri dished were incubated for 24 hours at 37°C. Bacterial colonies number is counted with the assumption that one colony comes from one cell. This stage was repeated thrice to obtain representative results. The data obtained was then processed into a graph in the form of a curve showing the relationship between the concentration of forest chili leaf extract and the density of the test bacterial cells, and the regression equation is also determined.

Data analysis

Quantitative data were analyzed using one-way analysis of variance (ANOVA) at a significance level of 5%. If there is a significant difference ($p < 0.05$) between treatments, it is necessary to proceed with Duncan's test (Duncan Multiple Range Test) to find out the difference in each treatment using the IBM SPSS Statistics version 26.0.

RESULTS AND DISCUSSION

Antibacterial Activity

Inhibitory tests of forest chili leaf extract against *S. epidermidis* are presented in Table 1. The widest inhibition was found at a concentration of 25% (w/v) with a diameter of 25.38 ± 0.52 mm and the smallest inhibition zone was found at a concentration of 5 % (w/v) with a diameter of 13.31 ± 0.24 mm. No inhibition zone was formed in the

negative control treatment using 96% ethanol, indicating no bacterial activity. The positive control of Ciprofloxacin 1% showed antibacterial activity with an inhibition zone diameter of 33.38 ± 0.97 mm. Antibacterial activity is shown by the formation of an inhibition zone which can be seen in Figure 1.

Table 1. Inhibitory zone data of forest chili leaf extract against *S. epidermidis*

Concentration Treatment (%)	Inhibitory Zone Diameter (mm)
Negative control	$0,00 \pm 0,00^a$
5	$13,31 \pm 0,24^b$
10	$14,63 \pm 0,32^c$
15	$16,88 \pm 0,85^d$
20	$19,19 \pm 0,77^e$
25	$25,38 \pm 0,52^f$
Positive control	$33,38 \pm 0,97^g$

Note: The values in the table above $\pm$ standard deviation is the average of 4 repetitions. Different letter notation indicates a significant difference ($P < 0.05$) based on Duncan's multiple range test, after analysis of variance (ANOVA)

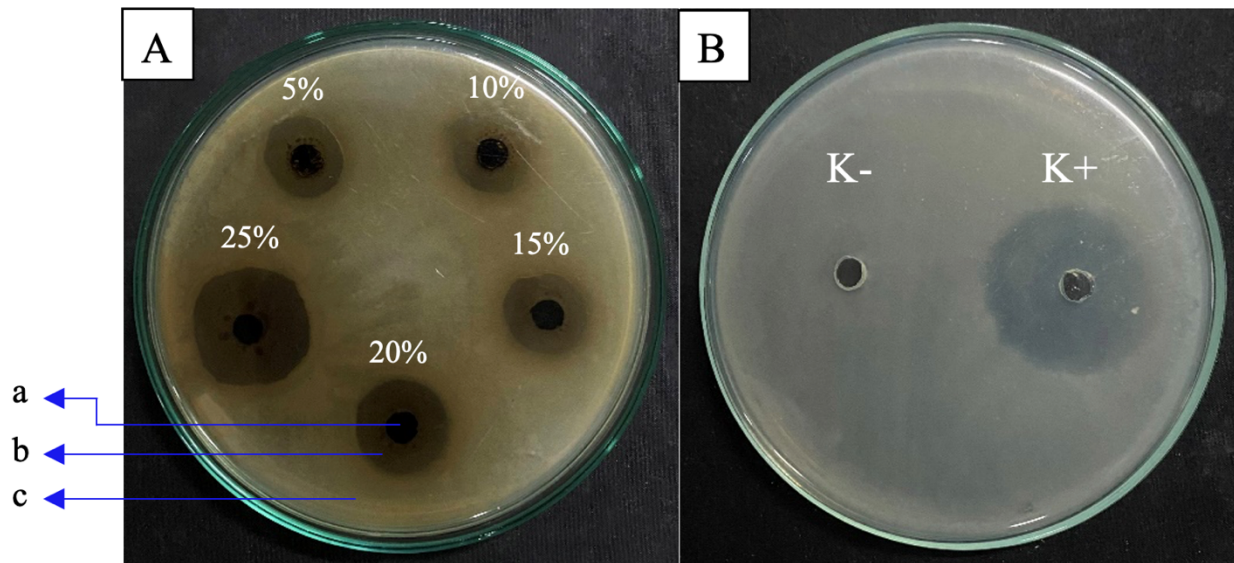


Figure 1. The inhibition zones of forest chili leaf extract against *S. epidermidis* bacteria; A. Testing several concentrations of forest chili leaf extract; B. Positive and negative control testing; (a) forest chili leaf extract; (b) clear zone; (c) bacterial culture

Minimum Inhibitory Concentration (MIC)

MIC test results of forest chili leaf extract against *S. epidermidis* are presented in Table 2. The data in the table shows that the forest chili leaf extract did not form a clear zone around the wells at 0.1% while at 0.2% to 0.5% still showed

antibacterial activity with a large diameter varying from 6.38 mm to 8.88 mm (Figure 2). Therefore, it is suspected that the MIC value of the extract lies at a 0.2%.

Table 2. Minimum Inhibitory Concentration (MIC) of forest chili leaf extract against *S. epidermidis*

Concentration Treatment (%)	Inhibitory Zone Diameter (mm)
0,1	0,00 ± 0,00
0,2	6,38 ± 0,32
0,3	7,19 ± 0,24
0,4	8,31 ± 0,24

Note: The values in the table above ± standard deviation is the average of 4 repetitions.

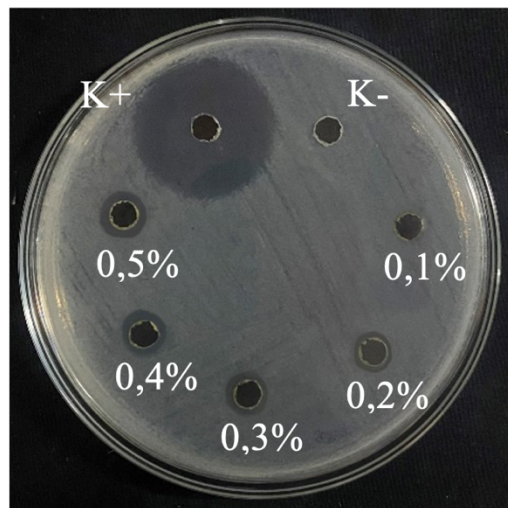


Figure 2. MIC of forest chili leaf extract against *S. epidermidis*

Lethal Concentration (LC50)

The LC50 value of forest chili leaf extract on *S. epidermidis* bacteria growth was obtained at

Phytochemical Screening

Phytochemical screening result of forest chili leaves crude extract obtained positive results for the secondary metabolite such as alkaloids,

0.26% with a linear equation $y = -211.71x + 109.1$. This value is determined through the regression equation and the regression line is obtained. A graph showing the relationship between the concentration of forest chili leaf extract and the density of the test bacterial cells is shown in Figure 3.

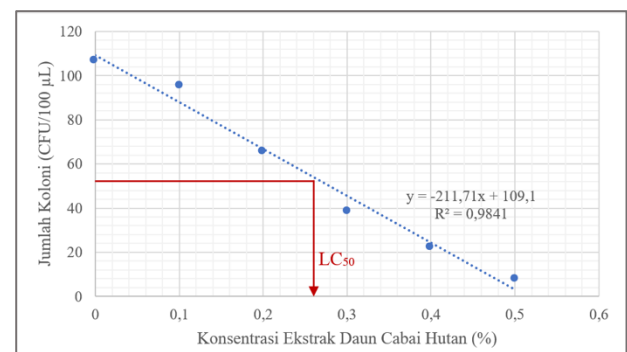


Figure 3. LC50 value graph of *P. caninum* leaf extract

flavonoids, phenols, tannins, and steroids. The result is shown in Table 3.

Table 3. Phytochemical screening result of *P. caninum* leaf extract

Phytochemical Constituent	Test	Result
Alkaloids	Mayer's test	+
	Wagner's test	+
	Dragendorff's test	+
Flavonoids	Wilstater's test	+
Saponins	Foam test	–
Phenols	Ferric chloride test	+
Tannins	Ferric chloride test	+
Steroids/Terpenoids	Liebermann-Burchard's test	Steroid (+)

Note: present: +; absent: –

DISCUSSION

Growth Parameters

Test results showed that the forest chili leaf extract had a significantly different effect ($p < 0.05$) on the growth of *S. epidermidis* bacteria. Inhibition zone diameter formed determines inhibition strength category of the extract. According to Surjowardojo et al. (2015), inhibition strength is divided into four categories, namely the weak (< 6 mm), moderate (6 – 10 mm), strong category (11 – 20 mm), and very strong (> 20 mm). The ability of inhibition which is included in the very strong category was found in the positive control (Ciprofloxacin 1%) and the concentration of 25% (w/v). Meanwhile, concentrations of 20% (w/v), 15% (w/v), 10% (w/v), and 5% (w/v) belong to the strong category. This inhibition ability proves that all concentrations of the extract given are able to inhibit the growth of *S. epidermidis* bacteria. However, each concentration certainly has different abilities according to the number of active substances contained in the extract (Kholifah, 2014).

Magvirah et al. (2019) explained that the higher concentration of plant extracts used, the higher content of secondary metabolites. The higher the concentration, the wider the inhibition zone is formed. This is due to more antibacterial compounds contained therein. The previous explanation is consistent with this study as evidenced by the greatest inhibition zone diameter found at the highest concentration, which is 25% and the smallest inhibition zone found at the lowest concentration, namely 5% (Table 1). *P. caninum* leaf extract concentration which produces an average diameter of the inhibition zone of 13 mm

to 25 mm has great potential as a natural antibiotic because the strength of its inhibition is classified into the strong to very strong category. The range of data on the diameter of the inhibition zone which has increased is in line with research conducted by Alydrus and Khofifah (2022) regarding betel leaves ethanol extract (*P. betle* L) on *S. aureus* bacteria growth which tested extract concentrations of 20%, 40%, 60 %, and 80% produced an average inhibition zone diameter of 17.6 mm respectively; 20mm; 21.6mm; 23.3mm.

One of the factors that influence the secondary metabolite content of forest chili leaves is the environmental conditions in which these plants live. Putra et al. (2014) examined the inhibition of betel leaves ethanol extract (*P. betle* L.) against *Propionibacterium acnes* at different heights. In the lowlands with an altitude of 20 meters above sea level, the concentration of the extract is 10 mg/mL; 7.5 mg/mL; 5mg/mL; 2.5 mg/mL; and 1.25 mg/mL resulted in an average diameter of the inhibition zone of 16.5 mm; 14.7mm; 11.5mm; 0mm; and 0 mm. Whereas in the highlands with an altitude of 800 meters above sea level, the concentration of the extract is 10 mg/mL; 7.5 mg/mL; 5mg/mL; 2.5 mg/mL; and 1.25 mg/mL showed an average diameter of 17.3 mm; 16mm; 14.5mm; 10.7mm; and 0 mm respectively. Therefore, it can be seen that the antibacterial activity of *P. betle* leaves in the highlands can produce stronger inhibition than in the lowlands. So, it can be said that the height where it grows affects the active compounds contained in a plant. In addition to geographical factors, the diameter of the diffusion well, the

volume of concentration of the extract used, the thickness of the agar media, the pH of the media, the temperature and the length of incubation also affect the inhibition zone that is formed (Horváth et al., 2016).

Ciprofloxacin was used as a positive control because *S. epidermidis* is known to have a high sensitivity to this antibiotic. This was confirmed by Susilowati (2007) that *S. epidermidis* had the highest sensitivity to Imipenem antibiotics at 92.31% then Ciprofloxacin at 61.54%; Gentamicin of 53.85%; Cefotaxim 38.46%; and Oxacillin 0%. Ciprofloxacin is also known to belong to the fluoroquinolone class of antibiotics which has a broad spectrum for treating various infections (Raini, 2016). The negative control test used 96% ethanol solvent and found no inhibition zones around the diffusion wells. This indicated that the inhibition zone formed was purely due to secondary metabolite compounds of forest chili leaf extract without any influence from 96% ethanol as diluent.

The MIC test results of the extract were suspected to be present at a concentration of 0.2% with an average diameter of the inhibition zone of 6.38 ± 0.32 mm (Table 2), but there is a possibility that the MIC value could be lower because the MIC was not tested at a concentration range of 0.19% to 0.11%. Based on the results of the MIC test, it can be seen that forest chili leaf extract has a strong effective inhibition against *S. epidermidis*. Jusanti's research (2020) found the MIC value of cubeb fruit extract (*Piper cubeba* L.) against *S. epidermidis* and *S. aureus* bacteria, namely at a concentration of 2.5 mg/mL. Meanwhile, the research by Salleh et al. (2011) reported that the MIC value of chili leaf extract (*P. caninum* Bl.) showed varying results against Gram-positive and negative bacteria and even fungi. The MIC value of the extract for Gram-positive bacteria, namely *B. subtilis* and *S. aureus* were each obtained at a concentration of 250 µg/mL. The results were different for Gram-negative bacteria, the MIC value was found at a concentration of 250 µg/mL for *Pseudomonas aeruginosa*; concentration of 125 µg/mL for *Pseudomonas putida*; and a concentration of 62.5 µg/mL for *Escherichia coli*. MIC values of the extract for *Candida albicans* and

Aspergillus niger were found at similar concentrations, namely >1000 µg/mL.

LC50 value of forest chili leaf extract in this study was 0.26027% or 2603 ppm. Rachmawati et al. (2021) conducted research on the toxicity of jamblang leaf extract (*Syzygium cumini* (L.) Skeels) on the growth of *S. epidermidis* bacteria using the Brine Shrimp Lethality Test (BSLT) method. The LC50 value of jamblang leaf extract was found, namely at a concentration of 485.5 ppm. If the LC50 value is below 1000 ppm or 0.1%, then the plant extract can be declared toxic (Rachmawati et al., 2021). Based on this, the forest chili leaf extract can be said to be non-toxic.

Phytochemical screening of forest chili leaf extract showed that positive contained alkaloids, flavonoids, phenols, tannins, and steroids. These five groups of compounds have different mechanisms of action. However, forest chili leaf extract obtained negative results on the saponin examination which was indicated by the absence of foam or foam that formed after shaking. The test results were in line with other Piperaceae plant species, namely betel leaf (*P. betle* L.) and pepper elder leaf (*Peperomia pellucida* (L.) Kunth) which were studied by Rukmini et al. (2020) got negative results on the saponin test.

Phytochemical screening for alkaloids showed positive results for three reagents used. A positive reaction with Mayer's test is indicated by the formation of a white precipitate. Meanwhile, the Wagner test produces a brown precipitate and the Dragendorff test forms an orange red precipitate. Alkaloids work as antibacterial by interfering with the work of the peptidoglycan constituent of bacterial cells. Later the cell wall layer cannot be completely formed and eventually the cell dies (Sampulawa and Nirmala, 2021).

Flavonoid compounds were tested for their presence by adding Mg and HCl. As a result, the forest chili leaf extract, which initially had a greenish yellow color, turned red over time, meaning that the extract contained positive flavonoids (Harborne, 1987). Heating is done because generally most of the class of flavonoid compounds dissolves in hot water. While the addition of Mg and HCl metal aims to reduce the benzopiron nucleus in the structure of flavonoid

compounds to produce red or orange flavilium salts (Robinson, 1995). According to Rijayanti et al. (2014), flavonoids work in inhibiting bacterial cell metabolism, including the function of cell membranes, nucleic acid synthesis, and energy metabolism.

Testing for the presence of phenolic compounds was carried out by adding FeCl₃ solution. A positive reaction is indicated by a color change to black-green (Pratt and Hudson, 1990). Phenol has antibacterial properties by disrupting the peptidoglycan layer on the cell wall of gram-positive bacteria, for example *S. epidermidis*, destroying the cell wall, inactivating enzymes and genetic material (Volk and Wheeler, 1988; Mhaske et al., 2012, Brooks et al., 2005). In addition, phenolic compounds are also able to denature proteins by binding them so that hydrogen bonds are created and eventually the protein structure is damaged (Cowan, 1999).

The tannin test procedure uses the same reagent as the phenol test, namely FeCl₃. The nature of the tannin group generally tends to dissolve in water, so it tends to have polar properties (Harborne, 1987). The tannin compounds contained in forest chili leaves have the ability to inhibit bacteria which is almost the same as how phenol works, namely interfering with the permeability ability of bacteria. Hydrogen bonds that were previously formed due to the presence of phenolic compounds reduced the permeability of the cell wall and cytoplasmic membrane because they are both made of protein. So, then there will be an imbalance between ions and macromolecules resulting in cell disintegration (lysis) (Carolia and Noventi, 2016).

Phytochemical testing to determine the presence of steroids and terpenoids was carried out

using the Liebermann-Burchard's method whose composition is composed of anhydrous acetic acid and concentrated sulfuric acid. Based on the results of the analysis in Table 3, it is presented that the forest chili leaf extract is positive for steroid compounds and negative for terpenoid compounds. A positive reaction is indicated by a change in color to bluish green, this condition occurs because the steroid compound group is oxidized and forms a conjugated double bond (Rahayu et al., 2019). Steroid compounds stored in wax or wax on plant leaves and fruit are useful in protecting plants from microbial or insect attacks (Puspita et al., 2018). Madduluri et al. (2013) explained that the role of steroids as an antibacterial is to interfere with the work of the lipid membrane as well as the sensitivity of the bacterial cell until a leak occurs in the liposome. Membrane phospholipids that have permeable properties are able to associate with steroid compounds to lipophilic compounds so that they can trigger a decrease in the integrity of the cell membrane as well as changes in cell morphology and cause cell fragility and rupture (lysis) (Sapara et al., 2016).

The overall results of the phytochemical tests were almost the same as those of *P. caninum* leaf extract which was studied by Suriani (2016) that found alkaloids, flavonoids, polyphenols, steroids and got negative results for the saponin test. However, negative reactions were also found in the tannin test, in contrast to this study. Another study conducted by Salleh et al. (2015) explained that phytochemical screening of *P. caninum* stems succeeded in isolating eight compounds including six flavonoid compounds including flavanones, flavones, and chalcones as well as two long-chain isobutyl amide compounds.

CONCLUSION

Based on the research that has been done, it can be concluded that the Minimum Inhibitory Concentration (MIC) of forest chili extract that is able to inhibit the growth of *S. epidermidis* is a concentration of 0.2% with an average diameter of

the inhibition zone of 6.38 ± 0.32 mm. The LC₅₀ value of forest chili leaf extract against *S. epidermidis* was 0.26%. The group of compounds contained in forest chili leaf extract are alkaloids, flavonoids, phenols, tannins, and steroids.

REFERENCES

- Alydrus, N. L. dan Khofifah, N. 2022. Efektifitas Antibakteri Ekstrak Daun Sirih Hijau (*Piper Betle* L) Terhadap *Staphylococcus aureus*. *Indonesian Health Journal (INHEALTH)*. 1 (1): 56-61.
- Brooks, G. F., Janet, S. B., dan Stephen, A. M. 2005. *Jawetz, Melnick & Adelberg's Mikrobiologi Kedokteran (Medical Microbiology) Buku I*. Penerjemah: Mudihardi, E., Kuntaman., Wasito, E. B., Mertaniasih, N. M., Harsono, S., dan Alimsardjono, L. Penerbit Salemba Medika. Jakarta.
- Carolia, N. dan Noventi W. 2016. Potensi Ekstrak Daun Sirih (*Piper betle* L.) sebagai Alternatif Terapi *Acne vulgaris*. *Majority*. 5 (1): 140-145.
- Chan, E. W. C. and Wong, S. K. 2014. Phytochemistry and Pharmacology of Three Piper Species: An Update. *International Journal of Pharmacognosy*. 1 (9): 534-544.
- Cowan, M. M. 1999. Plants as Antimicrobial Agents. *Clinical Microbiology Review*. 12 (4): 564-582.
- de Guzman, C. C. dan Siemonsma, J. S. 1999. Plant Resources of South-East Asia No. 13. Spices. Backhuys Publishers. Leiden.
- Djajadisastira, J., Mu'nim, A., dan Hidayah, O. 2007. Formulasi dan Uji Kestabilan Fisik Suspensi Topikal yang Mengandung Ekstrak *Nerii folium* sebagai Antibakteri dalam Sediaan Anti Jerawat. *Majalah Ilmu Kefarmasian*. 4 (3): 100-110.
- Harborne, J. B. 1987. *Metode Fitokimia: Penuntun Cara Modern Menganalisis Tumbuhan*. Penerjemah: Padmawinata, K. dan Soediro, I. ITB. Bandung.
- Horváth, G., Bencsik, T., Ács, K., and Kocsis, B. 2016. *Antibiotic Resistance*. Academic Press. United States.
- Jusanti, K. 2020. Uji Aktivitas Antibakteri Ekstrak Buah Kemukus (*Piper cubeba* L.) terhadap Bakteri Penyebab Jerawat. Fakultas Pendidikan Matematika dan Ilmu Pengetahuan Alam Universitas Pendidikan Indonesia. (Skripsi). Tidak Dipublikasikan.
- Kholifah. 2014. Uji Aktivitas Ekstrak Etanol dan Ekstrak Air Buah Pare (*Momordica charantia* L) terhadap Daya Hambat Pertumbuhan Bakteri *Edwardsiella tarda* Penyebab Penyakit *Edwardsiellosis* pada Ikan. Fakultas Sains dan Teknologi Universitas Islam Negeri Maulana Malik Ibrahim Malang. (Skripsi). Tidak Dipublikasikan.
- Madduluri, S., Rao, K. B., and Sitaram, B. 2013. In Vitro Evaluation of Antibacterial Activity of Five Indigenous Plants Extract against Five Bacterial Pathogens of Human. *International Journal of Pharmacy and Pharmaceutical Science*. 5 (4): 679-684.
- Magvirah, T., Marwati., dan Ardhani, F. 2019. Uji Daya Hambat Bakteri *Staphylococcus aureus* Menggunakan Ekstrak Daun Tahongai (*Kleinhovia hospita* L.). *Jurnal Peternakan Lingkungan Tropis*. 2 (2): 41-50.
- Mhaske, M., Samad, B. N., Jawade, R., and Bhansali, A. 2012. Chemical Agents in Control of Dental Plaque in Dentistry: An Overview of Current Knowledge and Future Challenges. *Pelagia Research Library*. 3 (1): 268-272.
- Parmar, V. S., Jain, S. C., Bisht, K. S., Taneja, R. J, P., Jha, A., Tyagi, A. D., Prasad, A., Wengel, J., Olsen, C. E., and Boll, P. M. 1997. Phytochemistry of the Genus Piper. *Phytochemistry*. 46 (4): 597-673.
- Poumale, H. M. P., Hamm, R., Zang, Y., Shiono, Y., and Kuete, V. 2013. Coumarins and Related Compounds from the Medicinal Plants of Africa. In: *Medicinal Plant Research in Africa*. Elsevier. Oxford.
- Pratt, D. E. and Hudson, B. J. F. 1990. *Natural Antioxidants Not Exploited Commercially*. Elsevier Applied Science Publisher. Amsterdam.
- Puspita, P. J., Safithri, M., dan Sugiharti, N. P. 2018. Antibacterial Activities of Sirih Merah (*Piper crocatum*) Leaf Extracts. *Current Biochemistry*. 5 (3): 1-10.
- Putra, I. M. D. S., Yustiantara, I. P. S., dan Paramita, N. L. P. V. 2014. Perbandingan Aktivitas Antibakteri *Propionibacterium acnes* dari Ekstrak Etanol Daun Sirih (*Piper betle* L.) Dataran Rendah dan Dataran Tinggi. *Jurnal Farmasi Udayana*. 3 (1): 106-109.
- Putri, Z. F. 2010. Uji Aktivitas Antibakteri Ekstrak Etanol Daun Sirih (*Piper betle* L.) terhadap *Propionibacterium acne* dan *Staphylococcus aureus* Multiresisten. Fakultas Farmasi Universitas Muhammadiyah Surakarta. (Skripsi). Tidak dipublikasikan.
- Rachmawati, N., Maulidiyah, G., dan Aminah. 2021. Uji Daya Hambat dan Toksisitas Ekstrak Daun Jamblang [*Syzygium cumini* (L.)

- Skeels] terhadap Pertumbuhan Bakteri *Staphylococcus epidermidis*. *Jurnal Biologi Indonesia*. 17 (1): 39-46.
- Radji, M. 2011. *Buku Ajar Mikrobiologi Panduan Mahasiswa Farmasi dan Kedokteran*. EGC. Jakarta.
- Rahayu AM, A. S., Salempa, P., dan Wijaya, M. 2019. Isolasi dan Identifikasi Senyawa Metabolit Sekunder Ekstrak n-Heksana Daun Kayu Jawa (*Lannea coromandelica* Houtt Merr.). *Chemica: Jurnal Ilmiah Kimia dan Pendidikan Kimia*. 20 (2): 111-118.
- Raini, M. 2016. Antibiotik Golongan Fluorokuinolon: Manfaat dan Kerugian. *Media Litbangkes*. 26 (3): 163-174.
- Rijayanti, R. P. 2014. Uji Aktivitas Antibakteri Ekstrak Etanol Daun Mangga Bacan (*Manfigera foetida* L) terhadap *Staphylococcus auerus* secara *In Vitro*. Fakultas Kedokteran Universitas Tanjungpura. (Skripsi). Dipublikasikan.
- Robinson, T. 1995. *Kandungan Organik Tumbuhan Tinggi*. ITB Press. Bandung.
- Rukmini, A., Utomo, D. H., dan Laily, A. N. 2020. Skrining Fitokimia Familia Piperaceae. *Jurnal Biologi dan Pembelajarannya*. 7 (1): 28-32.
- Salehi, B., Zakaria, Z. A., Gyawali, R., Ibrahim, S. A., Rajkovic, J., Shinwari, Z. K., Khan, T., Sharifi-Rad, J., Ozleyen, A., Turkdonmez, E., Valussi, M., Tumer, T. B., Fidalgo, L. M., Martorell, M., and Setzer, W. N. 2019. Piper Species: A Comprehensive Review on Their Phytochemistry, Biological Activities and Applications. *Molecules*. 24 (7): 1364-1481.
- Salleh, W. M. N. H. W., Ahmad, F., and Yen, K. H. 2015. Chemical Constituents from *Piper caninum* and Antibacterial Activity. *Journal of Applied Pharmaceutical Science*. 5 (6): 20-25.
- Salleh, W. M. N. H. W., Ahmad, F., Yen, K. H., and Sirat, H. M. 2011. Chemical Compositions, antioxidant and Antimicrobial Activities of Essential Oil of *Piper caninum* Blume. *International Journal of Molecular Sciences*. 12 (11): 7720-7731.
- Sampulawa, S. dan Nirmala, W. 2021. Potensi Antibakteri Ekstrak Alga Hijau *Halimeda makroloba* Decaisne dalam Menghambat Pertumbuhan Bakteri *Escherichia coli* dan *Staphylococcus aureus*. *Jurnal Sain Veteriner (JSV)*. 39 (2): 138-144.
- Sapara, T. U., Waworuntu, O., dan Juliatri. 2016. Efektivitas Antibakteri Ekstrak Daun Pacar Air (*Impatiens balsamina* L.) terhadap Pertumbuhan *Porphyromonas gingivalis*. *Pharmacon; Jurnal Ilmiah Farmasi*. 5 (4): 10-17.
- Sawarkar, H. A., Khadabadi, S. S., Mankar, D. M., Farooqui, I. A., and Jagtap, N. S. 2010. Development and Biological Evaluation of Hebal AntiAcne Gel. *International Journal of PharmTech Research*. 2 (3): 2028-2031.
- Suriani, N. L. 2016. Identification of the Substance Bioactive Leaf Extract *Piper caninum* Potential as Botanical Pesticides. *International Journal of Pure and Applied Bioscience*. 4 (4): 26-32.
- Suriani, N. L., Suparapta, D. N., Suadan, I. M., and Temaja, I. G. R. M. 2015. Antifungal Activity of *Piper caninum* against *Pyricularia oryzae* Cav. The Cause of Rice Blast Disease on Rice. *Journal of Biology, Agriculture and Healthcare*. 5 (8): 72-78.
- Suriani, N. L., Suprpta, D. N., Nazir, N., Darmadi, A. A. K., Parwanayoni, N. M. S., Sudatri, N. W., and Yamin, B. M. 2020. Inhibitory Activity of *Piper caninum* Leaf Extract against *Curvularia* Spotting Disease on Rice Plants. *Indian Journal of Agriculture Research*. 54 (4): 411-419.
- Surjowardojo, P., Susilorini, T. E., dan Sirait, G. R. B. 2015. Daya Hambat Dekok Kulit Apel Manalagi (*Malus sylvestris* Mill.) terhadap Pertumbuhan *Staphylococcus aureus* dan *Pseudomonas* sp. Penyebab Mastitis pada Sapi Perah. *Jurnal Ternak Tropika*. 16 (2): 40-48.
- Susilowati, R. I. 2007. Isolasi, Identifikasi dan Uji Sensitivitas *Staphylococcus epidermidis* dari Pus Pasien di Rumah Sakit Umum Islam Kustati Surakarta terhadap Beberapa Antibiotik. Fakultas Farmasi Universitas Muhammadiyah Surakarta. (Skripsi). Tidak Dipublikasikan.
- Thiboutot, D., Gollnick, H., Bettoli, V., Dréno, B., Kang, S., Leyden, J.J., Shalita, A. R., Lozada, V. T., Berson, D., Finlay, A., Goh, C. L., Herane, M. I., Kaminsky, A., Kubba, R., Layton, A., Miyachi, Y., Perez, M., Martin, J. P., Ramos-E-Silva, M., See, J. A., Shear, N., and Wolf Jr, J. 2009. New Insights into the Management of Acne: An Update from the Global Alliance to Improve Outcomes in Acne group. *Journal of the American Academy of Dermatology*. 60 (5): 1-50.
- Volk, W. A. dan Wheeler, M. F. 1988. *Mikrobiologi Dasar Jilid 1 (Terjemahan)*. Penerbit Erlangga. Jakarta.
- Zaenglein, A. L., Graber, E. M., and Thiboutot, D. M. 2012. Chapter 80. *Acne Vulgaris and Acneiform Eruptions*. In: Fitzpatrick's Dermatology in General Medicine 8th Edition Volume One. McGraw-Hill Education. New York.