ANTIBACTERIAL ACTIVITY OF Kumis Kucing LEAF EXTRACT (*Orthosiphon Stamineus*) AGAINST *Propionibacterium acnes* BACTERIA

Fiytary Noor Madani*, Putri Vidiasari D[1], and Melviani[1]

[1]*Department of Pharmacy, Health Faculty, Sari Mulia University, Indonesia*
Indonesia
fiytarynm@gmail.com

Abstract

Acne is a skin disease that often affects teenagers to adults and a skin disease that almost happens to everyone. The main bacteria that causes acne is *Propionibacterium acnes*. Previous research stated that the extract of the Kumis Kucing leaf contains secondary metabolites have antibacterial inhibition is flavonoids, alkaloids, saponins, steroids and terpenoids. This study to determine the antibacterial activity extract of Kumis Kucing leaves against *Propionibacterium acnes* bacteria through MIC and MBC testing. The methods used in this study is a true experimental method with post test only with control group design. *Propionibacterium acnes* bacteria as research subjects were given 5 treatment groups is extracts of Kumis Kucing (*Orthosiphon Stamineus*) leaves with concentrations of 50%, 75%, and 100%, as well as positive control clindamycin and negative control DMSO 10%. The method in this study uses the disc diffusion method and liquid dilution. Antibacterial testing on the disc diffusion method obtained extracts of Kumis Kucing (*Orthosiphon stamineus*) leaves is the inhibition zone of 7.46 mm while the dilution method contained MIC at a concentration of 50% (0,5 g/10 ml), while the MBC was not obtained. The results of the statistical test Kruskal Wallis 0,018 and Mann Whitney 0,025 showed a p value <0,05, indicating that there was a significant difference between all treatments and between two variations in the concentration of Kumis Kucing (*Orthosiphon stamineus*) leaf extract against *Propionibacterium acnes*. The Conclusion is extract of Kumis Kucing (*Orthosiphon stamineus*) leaves against *Propionibacterium acnes* has inhibitory at a concentration of 50% but has not bactericidal.

Keywords: Antibacterial, Kumis Kucing Leaf (*Orthosiphon stamineus*), *Propionibacterium acnes*

1. Introduction

Acne is a skin disease that often affects teenagers to adults and a skin disease that almost happens to everyone (Fitri et al, 2018). Acne creates an unattractive impression in appearance and can lower a person's self-confidence. Acne is caused by open skin pores that are clogged with oil and dead skin cells and is also caused by an excess of androgen hormones that trigger the formation of sebum.

The prevalence of acne sufferers in Indonesia ranges from 80-85% in adolescents with the highest incidence at the age of 15-18 years, 12% in women aged > 25 years and 3% at the age of 35-44 years (William et al, 2016). Factors that cause acne include genetics, hormones, dietary factors, lifestyle, environment, lack of sleep, stress, cosmetics and other chemicals. Acne can be caused by overactivity of the oil glands and exacerbated by bacterial infections such as Propionibacterium acnes (Asbullah et al., 2021).

The problem of acne on the face of course also caused by personal hygiene and environmental cleanliness. But the thing that is often done by everyone to reduce and prevent the formation of acne is to wash the face area at least 3 times a day. In addition, the most important thing is the selection of facial cleansing soap to remove dirt on the surface of the skin and usually some facial cleansing products often add an active compound or a combination of several active compounds in an effort to kill acne-causing bacteria (Marliana et al, 2018)

The main bacteria that causes acne is *Propionibacterium acnes. Propionibacterium acnes* is a gram-positive, focultative anaerobic bacterium that has the ability to grow with or without oxygen. *Propionibacterium acnes* plays a role in the process of inflammatory lesions in acne which results in inflammation, where its growth increases due to increased production (Dewi et al, 2019).

The prevalence of antibiotic-resistant *Propionibacterium acnes* varies in different countries. High prevalence occurs in various European countries with erythromycin/clindamycin resistance ranging from 45%-91% and tetracycline resistance from 5% to 26.4%. The prevalence of antibiotic-resistant *Propionibacterium acnes* in Asia is very different, for example in Japan, the rate of resistance to erythromycin or clindamicin is only 4% and tetracycline or doxycycline is only 2%. Meanwhile, in Korea, the latest research found only one of 33 strains (3.2%) isolated to be resistant to clindamycin, this is because antibiotic-resistant *Propionibacterium acnes* has not developed well enough in Korea, while the results of research in Indonesia showed resistance of *Propionibacterium acnes* to tetracycline antibiotics was 12,9%, erythromycin 45,2% and clindamycin 61,3% while doxycycline and minocycline did not get resistance (Madelina, 2019).

The benefits of Kumis Kucing leaves (*Orthosiphon Stamineus*) show that the ethyl acetate extract of Kumis Kucing leaves is able to inhibit pathogenic bacteria, namely *Peudomonas aeruginosa, Aeromonas hydrophilla, Staphylococcus aureus* and colon cancer cells (Nair, et al, 2014). The methanol extract of Kumis Kucing leaves produces high levels of antioxidants and is not toxic (Yam, et al, 2013). The ethanol extract of the Kumis Kucing leaf contains secondary metabolites that have antibacterial inhibition, namely flavonoids, alkaloids, saponins, steroids and terpenoids (Alwahid, et al., 2015). Many properties contained in this Kumis Kucing plant such as antioxidants, hepatoprotective, anti-inflammatory, cytotoxic, antihypertensive, and vasodilating (Sivakumar & Jeganathan, 2018). The Kumis Kucing leaf also acts as an antibacterial which is expected to be able to inhibit the *Propionobacterium acnes* bacteria.

Based on this, a study will be conducted on extracts of Kumis Kucing (*Orthosiphon Stamineus*) leaves as antibacterial activity on *Propionibacterium acnes* bacteria.

2. Materials and Methods

The method used in this study is a true experimental method with post test only with control group design. *Propionibacterium acnes* bacteria as research subjects were given 5 treatment groups, namely extracts of Kumis Kucing (*Orthosiphon Stamineus*) leaves with

concentrations of 50%, 75%, and 100%, as well as positive control of clindamycin and 10% negative control of DMSO.

The tools used in this study were erlenmeyer, measuring cup, test tube, test tube rack, dropper pipette, hot plate, analytical balance, watch glass, petri dish, stirring rod, magnetic stirrer, rotary evaporator, tweezers, incubator, ose needle, autoclave, Biological Safety Cabinet (BSC), glass container for maceration, funnel, aluminum foil, filter paper, refrigerator, label, caliper and Bunsen.

The materials used in this study were Kumis Kucing (*Orthosiphon Stamineus*) as the sample material, *Propionibacterium acnes* bacteria, clindamycin as a positive control, 10% DMSO solution, NA (Nutrient Agar), NB (Nutrient Broth), sterile distilled water, ethanol 96%.

Sample Preparation

The collection of raw materials from the Kumis Kucing (*Orthosiphon Stamineus*) plant which was taken directly in the Tabalong Regency area and took the leaves as a sample. Then do wet sorting, washing, chopping, drying, dry sorting and packing.

Extraction

The extraction method used in this research is the maceration method. Simplicia leaves of Kumis Kucing (*Orthosiphon Stamineus*) were put in a glass container with 96% ethanol solvent for 3 days while stirring. Then filtered with a funnel and concentrated using a rotary evaporator until the solvent evaporates at a temperature of 50ºC and a rotary speed of 60 rpm because the filtrate compound is relatively safe and avoids damage due to excessive heating, so that in the end a thick extract is obtained and the results are weighed. The extract was diluted according to the concentration to be used for research.

Phytochemical Screening

Alkaloid Test

Take 1 mL of the extract into a test tube and add 2 mL of HCl, then add 2-3 drops of Mayer's reagent. The presence of alkaloid compounds is indicated by a white precipitate (Wijaya et al, 2014).

Flavonoid Test

Take as much as 1 mL of the extract put into a test tube, then add 2 drops of concentrated HCl and shake vigorously. After that, Mg powder was added and shaken vigorously. The extract contains flavonoids if there is foam and the solution will experience a color change from the initial light green color to orange (Mailuhu et al., 2017).

Saponin Test

Take 1 mL of the extract put into a test tube and add hot water, then add a few drops of concentrated HCl. A positive test is indicated by the formation of permanent foam ± 15 minutes (Illing et al., 2017).

Tannin Test

Take 1 mL of extract add a few drops of 1% FeCl3 solution. The presence of tannins in the sample is indicated by the appearance of a green-black color (Yunus et al., 2018).

Steroid-Terpenoid Test

A total of 1 mL of the extract solution was added with Liebermann Burchard reagent. A positive test for steroids produces a green or blue color and terpenoids produce a red or violet color (Illing et al., 2017).

Equipment Sterilization

Sterilization is carried out on tools that have been washed and dried. Bunsen burner, to sterilize equipment such as loops, needles, and spatulas by burning the tip of the equipment over

a Bunsen flame until it glows. Autoclave for sterilizing petri dishes, dropper pipettes, stirring rods, test tubes, erlenmeyer, measuring cups, beakers. The tools are wrapped in aluminum foil. The wrapped tools were put in an autoclave and sterilized for 15 minutes at a temperature of 121ºC and a pressure of 1 atm (Rambiko et al., 2016).

Media Creation

Preparation of Nutrient Broth Media (NB)

NB media was made by weighing 3.25 grams of NB. Then put it in a beaker and add 250 ml of distilled water. NB and aquadest in a beaker were heated using a hot plate and stirred with a magnetic stirrer for ±10 minutes until the NB dissolved. The homogenized media was sterilized in (Rambikko, Fatimawali, & Widdhi, 2016) in an autoclave for 15 minutes at 121°C. After that, the media is waited for a bit to cool at around 40-45°C (Indarto, et al., 2019).

Preparation of Nutrient Agar (NA) Media

NA media was made by weighing 7.25 grams of NA. Then put it in a beaker and add 250 ml of distilled water. NA and aquadest in a beaker were heated using a hot plate and stirred with a magnetic stirrer for ±10 minutes until the NA dissolved. The homogenized media was sterilized in an autoclave for 15 minutes at 121°C. After that, wait for the media to cool slightly at around 40-45°C. The cooled NA medium was then poured into a 20 mL petri dish. The NA media that has been poured into a petri dish is allowed to solidify (Indarto, et al., 2019).

Muller Hinton Agar (MHA)

Weigh 38 grams of MHA media then add 1000 ml of distilled water then heat it on a hot plate and stir using a magnetic stirrer. After that, the finished media was put into an autoclave for 15 minutes at 121oC to sterilize the media. Then the sterile media was poured into a 15 ml petri dish and carried out in the BSC (Maheasy & Atun, 2017).

Bacterial Rejuvenation

The test bacteria were taken with a sterile ose needle, then implanted on the agar medium by scraping. It was then incubated in an incubator at 37°C for 24 hours.

Bacterial Inoculation

After the rejuvenation of bacteria do bacterial inoculation. The bacteria used in this research is *Propionibacterium acnes*. Before being used in the antibacterial test, the bacteria to be used must be regenerated first from the old media to the new media. The bacterial culture to be tested was planted in one loop in 10 ml of Nutrient Broth (NB) media, then incubated for 18 hours. After that, 0.1 ml of the culture was taken and then added 0.9% NaCl until the turbidity was the same as the Mc Farland standard.

Antibacterial Activity Test by Disc Diffusion

This method is done by soaking the paper disc to be used for ± 15 – 20 minutes into the extract of the leaves of the Kumis Kucing (*Orthosiphon Stamineus*), the negative control is 10% DMSO solution and the positive control is clindamycin solution (1 mg/5 ml), after that Dry briefly then place the paper disc on MHA media which has been spread by bacteria as much as 20 micropipettes and then incubated for 37°C for 20 hours then measure the clear zone around the disc. The disc paper used is 6 mm in diameter.

Antibacterial Activity Test with Liquid Dilution

Antibacterial activity test using dilution can assess the minimum inhibitory concentration (MIC) and minimum Bactericidal concentration (MBC) against *Propionibacterium acnes* bacteria. MIC can be assessed by using NB which is put in a test tube as much as 1 ml then added the concentration of the extract made, namely 50%, 75%, 100%, positive control, negative control each as much as 0.8 ml and bacterial suspension as much as 0.2 ml and then

incubated for 20 hours at 37°C, then the turbidity was observed for each concentration. Then to see the MBC by spreading the MIC results onto solid media, namely NA, incubated for 20 hours at 37°C, if there is an inhibition zone, it is measured using a caliper with units (mm) and the results obtained are averaged.

3. Results and Discussion

Results

As much as 1 kg of Kumis Kucing (*Orthosiphon Stamineus*) leaves were dried at room temperature and 100 grams of dried simplicia were produced. Then simplicia was extracted with 96% ethanol and obtained a thick extract using a rotary evaporator as much as 5.36 grams. Extract yield of 5.36%

Phytochemical screening results from thick extract of Kumis Kucing (*Orthosiphon Stamineus*) leaves have secondary metabolites, namely flavonoids, alkaloids, saponins, tannins and steroids.

Table 4.1 Phytochemical Screening Results

Phytochemical	Test	Color Result
Flavonoids	+	Foam is formed and changes color from green to orange
Alkaloids	+	White precipitate is formed
Saponins	+	Forms permanent foam 15 minutes
Tannins	+	Green-black color
Steroids	+	Green color

Table 4.2 Observation of Inhibitory Zone on Disc Diffusion

Treatment	Replication			Average
	I	II	III	
Cat's Whiskers leaf extract	7,065 mm	8,285 mm	7,03 mm	7,46 mm
Control (+)	20,69 mm	17,99 mm	21,99 mm	20,23 mm
Control (-)	0	0	0	0

Based on the results of the antibacterial activity test using the disc diffusion method, the inhibition zone of the Kumis Kucing (*Orthosiphon Stamineus*) leaf extract on replication I, II, and III was 7.065 mm, respectively; 8.285 mm; 7.03mm. Positive control is clindamycin, found the inhibition zone on replication I, II, and III was 20.69 mm, respectively; 17.99 mm; 21.99mm. The negative control is DMSO 10% in replications I, II, and III, was found to have no inhibition zone.

Table 4.3 Observation Results of Minimum Inhibitory Concentration (MIC) in Liquid Dilution

Treatment	Replication			*P value*	*P value*
	I	II	III	*Kruskal wallis*	*Man whitney*
Concentration 50% (0,5g/10 ml) (KHM)	-	-	-	0,018	0,025
Concentration 75% (0,75g/10ml)	-	-	-	0,018	0,025
Concentration 100%	-	-	-	0,018	0,025

(1g/10ml)			
Control (+)	-	-	-
Control (-)	+	+	+

Information:
Control (+): Clindamycin — Sign (+): There is bacterial growth (cloudy)
Control (-): DMSO 10% — Sign (-): No bacterial growth (Clear)

Based on the research results of testing the antibacterial activity of the Kumis Kucing (*Orthosiphon Stamineus*) leaf extract with the liquid dilution method, the Kumis Kucing (*Orthosiphon Stamineus*) leaf extract was obtained at concentrations of 50%, 75%, 100% and in positive control there was no bacterial growth indicated by the solution becoming clear while in the negative control there was bacterial growth marked by the solution becoming cloudy. It states that the Minimum Inhibitory Concentration (MIC) is at a concentration of 50%. The *p value* of *Kruskal Wallis* of 0.018 stated that there was a significant or significant difference between all treatments against the bacterium *Propionibacterium acnes* while the *p value of Man Whitney* stated that there was a significant difference between the two variations in the concentration of the extract of the leaves of the Kumis Kucing (*Orthosiphon Stamineus*) against the bacteria *Propionibacterium acnes.*

Table 4.4 Observation Results of Minimum Bactericidal Concentration (MBC) on Liquid Dilution

treatment	Replication		
	I	II	III
Concentration 50%	+	+	+
Concentration 75%	+	+	+
Concentration 100%	+	+	+
Control (+)	-	-	-
Control (-)	+	+	+

Information:
Control (+): Clindamycin — Sign (+): There is bacterial growth
Control (-): DMSO 10% — Sign (-): No bacterial growth

Based on the research results of testing the antibacterial activity of Kumis Kucing (*Orthosiphon Stamineus*) leaf extract with the liquid dilution method, it was found that the Kumis Kucing leaf extract (*Orthosiphon Stamineus*) at concentrations of 50%, 75%, 100% and in negative control there was bacterial growth marked by the solid media becoming cloudy while in the positive control there was no bacterial growth indicated by the solid media becoming clear. It states that the leaf extract of the Kumis Kucing (*Orthosiphon Stamineus*) does not have a Minimum Bactericidal Concentration (MBC).

Discussion

The sample used in this study was 1 kg of Kumis Kucing (*Orthosiphon Stamineus*) leaves. Furthermore, the simplicia was weighed where the results obtained were 100 grams. Simplicia leaves of Kumis Kucing (*Orthosiphon Stamineus*) were extracted by maceration method. This method is very simple and can be used to extract heat-resistant and non-heat-resistant substances (Dirjen POM, 2014). The solvent used in the study was 96% ethanol as a liquid filter

because ethanol has the ability to filter polar compounds and non-polar compounds and is not toxic when compared to other organic solvents, and is not easy to grow microbes (Siti, 2021).

After filtering and obtaining the liquid extract, it was thickened using a rotary evaporator at a temperature of 50ºC with a rotary speed of 60 rpm. After the liquid extract evaporates, a thick extract will be obtained. From 5 liters of liquid extract, 5.36 grams of thick extract were obtained. The thick extract obtained was then calculated the yield. Yield by comparing the obtained extract divided by the simplicia before extraction multiplied by 100%, where yield is one of the extract parameters. (Wijaya et al., 2018). The yield obtained in this study was 5.36%.

Phytochemical screening aims to provide an overview of the compounds contained in plants. One way to determine the content of compounds contained in plants is by using color reagents. Where the factors that play a role in phytochemical screening are solvents and extraction methods (Siti, 2021). The results of the phytochemical screening of the thick extract of the Kumis Kucing leaf (*Orthosiphon stamineus*) showed that there were secondary metabolites, namely alkaloids, flavonoids, saponins, tannins and steroids. Alkaloids are indicated by the presence of a white precipitate. The presence of flavonoid compounds when foam is formed and the solution will experience a color change from the initial green color to orange color. Saponin compounds were indicated by the formation of permanent foam for 15 minutes. Tannin compounds are indicated by the appearance of a green-black color, while steroid compounds are indicated by a green color.

Antibacterial activity is closely related to secondary metabolites where in this study the secondary metabolites contained were alkaloids, flavonoids, saponins, tannins and steroids. Flavonoids as antibacterial is by causing damage to the permeability of bacterial cell walls, microsomes and lysosomes as an interaction between flavonoids and bacterial DNA. Tannins can shrink the cell wall or cell membrane so that it interferes with the permeability of the cell which causes the cell to be unable to carry out life activities so that its growth is inhibited. Saponins act as antibacterials by lowering the surface tension, resulting in increased permeability or cell leakage and resulting in the release of intracellular compounds. Steroid compounds inhibit bacterial growth by inhibiting protein synthesis because they cause changes in the components that make up the bacterial cell itself (Marfu'ah et al, 2019). Alkaloids work as antibacterial by interfering with the peptidoglycan constituent components in bacterial cells so that the cell wall layer is not fully formed and causes cell death (Anuzar et al, 2017).

Antibacterial activity testing was carried out using extracts with concentrations of 50%, 75%, 100%, clindamycin is positive control and 10% DMSO is negative control. This test was carried out on *Propionibacterium acnes*, which is a gram-positive anaerobic bacterium. The negative control used was 10% DMSO solution. The substance used as a negative control is the solvent used as a diluent of the compound to be tested. In this study, the solvent used to dissolve the sample was DMSO solution. The purpose is as a comparison that the solvent used as a diluent does not affect the antibacterial test results of the sample to be tested (Utomo, 2018). The positive control used was clindamycin. Clindamycin antibiotics are bacteriostatic and work to inhibit the growth or reproduction of bacteria by inhibiting protein synthesis. The mechanism of action of clindamycin includes cutting the elongation of the peptide chain, blocking the A site on the ribosome, misreading the genetic code or preventing the attachment of oligosaccharide chains to glycoproteins.

The antibacterial activity testing method in this study used the disc diffusion method to determine the antibacterial activity and the liquid dilution method to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Based on the

results of research on the antibacterial activity of Kumis Kucing (*Orthosiphon stamineus*) leaf extract against *Propionibacterium acnes* bacteria with the disc diffusion method to see the zone of inhibition was carried out using extracts without concentration as well as a comparison of positive control clindamycin and 10% DMSO negative control with 3 repetitions for initial screening. . The results obtained showed that there was an inhibitory zone around the disc, where the extract without concentration after being averaged had an inhibition zone of 7.46 mm, a positive control of 20.23 mm and a negative control that had no inhibition zone which can be seen in table 4.2.

While the results of research on the antibacterial activity of Kumis Kucing (*Orthosiphon stamineus*) leaf extract against *Propionibacterium acnes* bacteria using the liquid dilution method was carried out using an extract concentration of 50%. 75%, 100%, clindamycin is positive control and 10% DMSO is negative control. The results of the Minimum Inhibitory Concentration (MIC) after incubation for 18-24 hours can be seen turbidity where the results of the smallest concentration indicating MIC is found at a concentration of 50% (0.5g/10ml). The results of non-parametric statistical tests in this study were *Kruskal Wallise* of 0.018 and *Mann Whitney* of 0.025 indicating a significant value is p <0.05. The *p value* of *Kruskal Wallis* of 0.018 stated that there was a significant or significant difference between all treatments against the bacterium *Propionibacterium acnes* while the p value of *Man Whitney* stated that there was a significant difference between the two variations in the concentration of the extract of the leaves of the Kumis Kucing (*Orthosiphon Stamineus*) against the bacteria *Propionibacterium acnes*. Furthermore, after the MIC was obtained, the research continued to the killing power test by means of the concentration of MIC contained in NA solid media and then incubated for 18-24 hours to see the growth of colonies in the media, where the results showed that there was not bacteridal activity in *Propionibacterium acnes* bacteria.

Propionibacterium acnes is a gram-positive anaerobic bacterium (Zahrah et al, 2018). *Propionibacterium acnes* plays an important role in causing inflammation in acne vulgaris by producing chemotactic factors and lipase enzymes that will convert triglycerides into free fatty acids (Indarto et al, 2019). Clindamycin is an antibiotic that works to inhibit bacterial growth by inhibiting protein synthesis. Clindamycin is a type of antibiotic that is indicated also to treat diseases caused by gram-positive anaerobic bacterial infections such as *Propionibacterium acnes* (Narulita, 2017). Based on research (Hindritiati, 2017) *Propionibacterium acnes* resistance to antibiotics is the highest to clindamycin, followed by erythromycin, minocycline, tetracycline and the lowest is doxycycline, while in the study (Nurwulan, 2006) got resistance to tetracycline by 12.9%, erythromycin 45.2% and clindamycin 61.3%. And there is no resistance to doxycycline and minocycline.

A similar study was conducted by (Marfu'ah et al, 2019) namely the ethanolic extract of bidara leaves on *Propionobacterium acnes* bacteria was able to form inhibition zones at concentrations of 70%, 80% and 90%. Research conducted by (Dewi et al, 2019), the diameter of the inhibition zone of the methanol extract of betel leaf (Piper betle L.) at a concentration of 6.25%; 12.5%; 25% and 50% for *Propionibacterium acnes* of 9.05 mm; 11.50mm; 12.18mm; 13.53 mm and the Minimum Inhibitory Concentration (MIC) of betel leaf (Piper betle L.) methanol extract on *Propionibacterium acnes* was 3.25%. The results of the research conducted (Indar to et al, 2019) is a leaf extract of binahong against *Propionibacterium acnes* at a concentration of 100% extract obtained an inhibition zone of 9 mm. In a study conducted (Riskawati, 2018) the results of testing the antibacterial activity of gotu kola leaf extract at a

concentration of 10% against the growth of *Propionibacterium acnes* bacteria obtained an inhibition zone of 12 mm.

4. Conclusion

Based on the results of the study, it can be concluded that the leaf extract of the Kumis Kucing (*Orthosiphon stamineus*) contains secondary metabolites, namely alkaloids, flavonoids, tannins, saponins and terpenoids which have antibacterial activity. The results of the antibacterial activity test of the extract of the leaves of the Kumis Kucing (*Orthosiphon stamineus*) in the disc diffusion method had an inhibition zone of 7.46 mm while the dilution method had the Minimum Inhibitory Concentration (MIC) at the smallest concentration, namely 50% concentration and did not have a Minimum Bactericidal Concentration (MBC). . The results of non-parametric statistical tests in this study were *Kruskal Wallise* of 0.018 and *Mann Whitney* of 0.025 indicating a significant value is $p < 0.05$.

Acknowledgements

I would like to express my gratitude to my parents, family, and friends, who have always been supportive and helpful. Putri Vidiasari D, S.Si, M.Pd, as supervisor and apt. Melviani, M.Pharm.Sci, as second supervisor, have always provided valuable advice and guidance in the preparation and improvement of the thesis.

References

Almatar, Manaf, Rahmat, & Zaidah. (2014). Identifying the Developmental Stages and Optimizing the Sample Preparation for Anatomical Study of Orthosiphon stamineus. Journal of Applied Pharmaceutical Science.

http://www.japsonline.com. doi: 10.7324/JAPS.2014.40314

Alwahid, A., Yusoff, W., Md. Nor, N. S., & Ibrahim, N. (2015). Cytotoxicity and Phytochemical Analyses of Orthosiphon Stamineus Leaves and Flower Extracts. American Institute of Physics, 3.

Anugrah, G. F., Yasa, I. N., & Sujana. (2019). Analisis Perbedaan Dasar Perhitungan Tarif Harga Air Utama.

Anuzar, C., Hazar, S., & Suwendar. (2017). Aktivitas Antibakteri Ekstrak Etanol Daun Cabe Rawit (Capsicum frustescens L.) terhadap Pertumbuhan Bakteri Penyebab Jerawat Propionibacterium Acnes secara In-vitro. Jurnal Farmasi, 457-464.

Asbullah, Wulandari, P., & Febrianita, Y. (2021). Faktor-Faktor yang mempengaruhi terhadap timbulnya Acnes Vulgaris (Jerawat) pada Remaja di SMAN 1 Pelangiran Kabupaten Indragiri Hilir Tahun 2018. Jurnal Keperawatn Abdurrab, 79-88.

Dalimartha, S. (2006). Atlas Tanaman Obat Indonesia Jilid 4. Jakarta: Puspa Swara.

Depkes RI. (2000). Parameter Standar Umum Ekstrak Tumbuhan Obat. Jakarta: Departemen Kesehatan Republik Indonesia.

Dewi, R., Febriani, A., & Wenas, D. M. (2019). Uji Aktivitas Antimikroba Ekstrak Metanol Daun Sirih (Piper betle L.) terhadap Pertumbuhan Bakteri Propionibacterium acnes dan Khamir Malasezia furfur. Jurnal Ilmu Kefarmasian: Sainstech Farma, 32-38.

Dirjen , P. (2014). Farmakope Indonesia Edisi V. Jakarta: Departemen Kesehatan Republik Indonesia.

Fitri, N., Suwendar, & Hazar, S. (2018). Uji Aktivitas Antibakteri Ekstrak Etanol Herba Seledri (Apium Graveolens L.) terhadap Bakteri Propionibacterium Acnes secara In-Vitro. Jurnal Farmasi, 62-67.

Illing, L., Wulan, S., & Erfiana. (2017). Fitokimia Ekstrak Buah Dengen. Jurnal Dinamika, Vol. 8 (1).

Indarto, Narulita, W., Anggoro, B., & Novitasari, A. (2019). Aktivitas Antibakteri Ekstrak Daun Binahong terhadap Propionibacterium Acnes. Biosfer: Jurnal Tadris Biologi Vol. 10 No.1, 67-78.

Jawetz, Melnick, & Adelberg's. (2004). Mikrobiologi Kedokteran. Jakarta: Buku Kedokteran EGC.

Mahdiyah, D., Farida, H., Riwanto, L., Mustofa, M., Wahjono, H., Laksamana, N., & Reki, W. (2020). Screening Of Indonesian Peat Soil Bacteria Producing Antimicrobial Compounds. Saudi Jurnal og Biological Sciences. doi: 10.1016/j.sjbs.2020.05.033.

Mahmudah, Lestari, F., & Atun, S. (2017). Uji Aktivitas Antibakteri Dari Ekstrak Etanol Temukunci (Boesenbergia Pandurata) Terhadap Bakteri Streptococcus Mutans. 37-39.

Mailuhu, M., Max, R., & HarryS. J. (2017). Skrining Fitokimia dan Aktivitas Antioksidan Ekstrak Metanol Kulit Batang Soyogik (Saurauia bracteosa DC. Chem prog, Vol. 1 (10).

Marfu'ah, N., Ramadhan, C., & Hasanah, A. (2019). Uji Efektivitas Ekstrak Etanol Daun Bidara (Ziziphus spina christi L.) terhadap Pertumbuhan Propionibacterim acnes. Pharmasipha, Vol. 3 No. 1.

Marliana, Sartini, & Karim, A. (2018). Efektivitas Beberapa Produk Pembersih Wajah Antiacne terhadap Bakteri Penyebab Jerawat Propionibacterium acnes. Jurnal Biologi Lingkungan, Imdustri Kesehatan, 31-41.

Nair, A., Kiruthika, D., Dheeba, B., & Tilton, F. (2014). Cytotoxic Potensials of Extracts Against Pathogenic Bacteria and Colon Cancer Cells. Asian Journal of Science and Technology, 221-225.

Pratiwi, S. T. (2008). Mikrobiologi Farmasi. Yogyakarta: Erlangga.

Rambikko, S., Fatimawali, & Widdhi, B. (2016). Uji Sensitivitas Bakteri Penyebab Infeksi Nosokimial Saluran Kemih Akibat Penggunaan Kateter Terhadap Antibiotik Ampicillin, Amoxicillin dan Ciprofloxacin di RSUP Prof. Dr. R. D Kandou Manado. PHARMACON Jurnal Ilmiah Farmasi, Vol. 5 (1).

Rollando. (2019). Senyawa Antibakteri dari Fungi Endofit. Seribu Bintang.

Sa'adah, H., Supomo, & Musaenah. (2020). Aktivitas Antibakteri Ekstrak Air Kulit Bawang Merah (Allium cepa L) Terhadap Bakteri Propionibacterium acnes. Jurnal Riset Kefarmasian Indonesia.

Saryono. (2011). Metodologi Penelitian Kesehatan: Penuntun Praktis Bagi Pemula. Mitra Cendikia Press.

Sivakumar, C., & Jeganathan, K. (2018). Phytochemical Profiling of Cat Whisker's (Orthosiphon Stamineus) Tea Leaves Extract. Journal of Pharmacognosy and Phytochemistry, 1396.

Surahmida, & Umarudin. (2019). Studi Fitokimia Ekstrak Daun Kemangi dan Daun Kumis Kucing Menggunakan Pelarut Metanol. Indonesian Chemistry and Application Journal, Vol 3. No. 1.

Susanty, & Bachdim, F. (2016). Perbandingan Metode Ekstraksi Maserasi dan Refluks terhadap Kadar Fenolik dari Ekstrak Tongkol Jagung (Zea mays L.). Konversi Vol. 5 No. 2, 87-93.

Vania, V., Yessie, K., Christel, N., & Reky, R. (2020). Uji Aktivitas Antibakteri Ekstrak Etanol Kulit Buah Pepaya (Carica papaya L.) terhadap Bakteri Penyebab Jerawat Propionibacterium acnes. Jurnal Biofarmasetikal Tropis, 112-121.

Wahyuni, R., Guswandi, & Rivai, H. (2014). Pengaruh Cara Pengeringan Dengan Oven, Kring Angin dan Cahaya Matahari Langsung Terhadap Mutu Simplisia Herba Sambiloto. Jurnal Farmasi Higea.

Widiyanto. (2013). Statistika Terapan: Konsep dan Aplikasi SPSS dalam Penelitian Bidang Pendidikan, Psikologi dan Ilmu Sosial Budaya Lainnya. Jakarta: PT. Elex Media Kompoutindo.

Wijaya, D., Jessy, E., Paendoga, & Jemmy, A. (2014). Skrining Fitokimia dan Uji Aktivitas Antioksidan Dari Daun Nasi (Phrynium capitatum) Dengan Metode DPPH (1,1-difenil-2-pikrilhidrazil). Jurnal , Vol. 3(1).

William, D., Timothy, G., Dirk, M., & Isaac, N. (2016). Andrew's Disease of the skin. Philadelphia, 225-227.

Yunus, I., Widdhi, B., & Edwin, D. (2018). Skrining Fitokimia dan Uji Toksisitas Ekstrak Etanol Daun Langsat (Lansium domesticun Corr) Terhadap Larva Artemia Salina Leasch Dengan Metode Brine Shrimp Lethality Test (BSLT). PHARMACON Jurnal Ilmiah Farmasi, Vol.7 (3).

Zahrah, H., Mustika, A., & Debora, K. (2018). Aktivitas Antibakteri dan Perubahan Morfologi dari Propionibacterium Acnes setelah Pemberian Ekstrak Curcuma Xanthorrhiza. Jurnal Biosains Pasca Sarjana, 1-10. doi: http://dx.doi.org/10.20473.

Zaluchu, S. E. (2020). Strategi Penelitian Kualitatif dan Kuantitatif di Dalam Penelitian Agama. Evangelikal: Jurnal Teologi Injili dan Pembinaan Warga.