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Antibacterial Effectiveness of White Champaca Flower *(Michelia alba dc)* Against Growth of *Corynebacterium diphtheriae*

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Abstract

Background: *Corynebacterium diphtheriae* is a bacterium that attacks the respiratory tract, especially the larynx, tonsils, and throat. These bacteria can release toxins that can cause diphtheria. One of the nutritious plants that people often use is the white Champaca flower, or *Michelia alba*. **Objective: T**his study aimed to examine the antibacterial effectiveness of white champaca flower (*Michelia alba dc*) against the growth of *Corynebacterium diphtheriae*. **Methods:** Experimental research method with post-test only control group design using Cup-plate technique of ethanol extract of white Champaca flower (*Micheia alba*) in various concentrations (20%, 40%, 60%, 80%, and 100%) and Erythromycin as a positive control and aquades as a negative control. **Results:** The results of this research are that the minimum inhibitory concentration against the growth of *Corynebacterium diphtheriae* bacteria at a 20% concentration is 42.16 mm, the concentration of 40% is 47.21 mm, the concentration of 60% is 51.66mm, while at the concentration of 80% the average diameter of the inhibitory zone is 54.49 mm and also at the concentration of 100% the average diameter of the inhibition zone is 54.69 mm which is the average diameter of the largest inhibition zone. The negative control with an inhibitory zone diameter of 0 mm, while the positive control with an inhibitory zone diameter of 44.21 mm. **Conclusion:** White Champaca flower extract (*Michelia alba dc*) had antibacterial effectiveness against the growth of *Corynebacterium diphtheriae* and at a concentration of 100% it is the most effective concentration in inhibiting the growth of *Corynebacterium diphtheriae* bacteria.

Keywords: Antibacteria; *Corynebacterium diphtheriae*; *Michelia alba*

Original Research Article

INTRODUCTION

Disease patterns due to infection still dominate (Putra, 2019). One of the infectious diseases is diphtheria, which is a disease caused by the pathogenic bacterium *Corynebacterium diphtheriae* (Alawiyah, 2019). In December 2019 until May 2020, the highest number of diphtheria infection cases were reported in East Java and including diseases that can cause outbreaks (Kementrian Kesehatan Republik Indonesia, 2020).

Corynebacterium diphtheriae is a gram-positive, non-spore, immobile, and acid-resistant bacterium. They usually have irregular swelling at one end that makes them look "club-shaped".

Corynebacterium diphtheriae has a length that is measured in micrometers and is between 0.5 and 1 millimeter in diameter. *Corynebacterium diphtheriae* colonies on agar blood are small, granular, gray, with uneven edges, and have small hemolysis zones (Jawets, 2019). *Corynebacterium diphtheriae* is a bacterium that causes infectious diseases, namely diphtheria (Alawiyah, 2019).

One of the plants that has been used as medicine by the community is the white Champaca flower, or Michelia alba. Various parts of the Champaca plant can be used as medicine for diseases, including fruit, flowers, leaves, bark, roots, and sap (Maesaroh & Özel, 2021). the flowers of the white Champaca or called kantil flowers are also very beneficial for the treatment of diseases, namely being able to treat Bronchitis, cough, fever, vaginal discharge, scabies, leprosy, chest pain, and joint pain (Ningsih et al., 2016). In addition, white Champaca flowers can be used for the treatment of inflammation and prostate disorders (Nurul, 2017).

In the research conducted on white Champaca plants, namely by using the methanol extract of Champaca flowers. This study used extract concentrations of 0.05%, 2%, 4%, 6%, and 8% in the antibacterial activity test. Antibacterial testing for the methanol extract of champaca flowers showed bacterial inhibition, but in the weak to moderate category. The test results showed that the methanol extract of white Champaca has the potential to inhibit bacterial growth. It is illustrated that the higher the concentration used, the greater the diameter of the inhibition zone produced (Munira et al., 2021). So, further research is needed on white Champaca flowers. White Champaca flower (*Michelia alba dc*) is included in one of the plants that can inhibit the activity of bacteria that cause infection (Safrina, 2022).

Based on the description above, the researcher is interested in conducting a study on much antibacterial effect of white Champaca flower extract on the growth of *Corynebacterium diphtheriae* bacteria entitled "Test of Antibacterial Effectiveness of White Champaca Flower (*Michelia alba dc*) Against the Growth of *Corynebacterium diphtheriae*".

MATERIALS AND METHODS

Sample Size

This study is a true experiment used a Post-Test Only Control Group using a simple random sampling method and using a homogen population. The population of this study is Corynebacterium diphtheriae. The samples used in this study were pure cultures of Corynebacterium diphtheriae. Sampling in this study was calculated using the Federer formula. In this study, there were 7 groups of 5 treatment groups and 2 control groups. K1 is a positive control using erythromycin, K2 is a negative control using aquades, K3 using 20% concentration of White Champaca Flower extract, K4 using 40% concentration of White Champaca Flower extract, K5 using 60% concentration of White Champaca Flower extract, K6 using 80% concentration of White Champaca Flower extract, and K7 using 100% concentration of White Champaca Flower extract. So, the total number of samples used was 28 because each sample had 4 repetitions. This study has obtained ethical approval from Faculty Medicine Universitas Wijaya Kusuma Surabaya with Certificate Number 43/SLE/FK/UWKS/2024. Note:

S: Sample
M: Mueller Hinton Agar (MHA) Medium
K: Research Group
P: Treatment
O: Observation

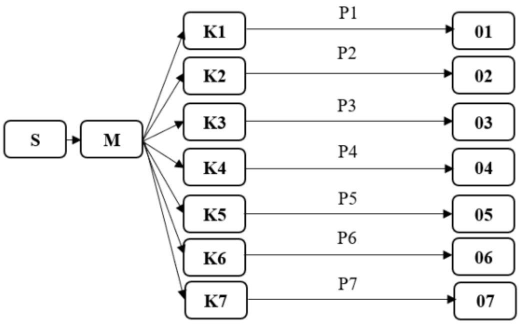

Figure 1. Research design

Materials Preparation

Sterile distilled water, suspension *Corynebacterium diphtheriae*, white champaca flower extract (*Michelia alba*) with concentrations of 20%, 40%, 60%, 80%, 100%, Mueller Hinton Agar, erythromycin antibiotic.

Extraction of White Champaca Flower

Fresh white Champaca flowers were cut into pieces and dried using an oven so as not to affect the chemical content therein, then 500 grams of powder was taken and put into a macerator. Next, the white Champaca flower powder was soaked in ethanol until it was completely submerged. Followed by a filtering process that is repeated at least once. Perform evaporation using rotavapor until the extract is perfect and a thick extract of white cempaca flower is obtained.

Preparation of White Champaca Flower Solution

In this study, white champaca flower extract concentrations were obtained at 20%, 40%, 60%, 80%, 100% through dilution with 100% white champaca flower extract and the addition of distilled water. Variations in concentrations 20%, 40%, 60%, 80%, and 100% were used to observe the antibacterial effects of white champaca flower extract on the growth of *Corynebacterium diphtheriae* in order to identify the maximum effectiveness points at different concentration levels. The 20% test solution concentration was obtained by taking 1 ml of 100% test solution plus 4 ml of distilled water. The 40% test solution concentration was obtained by taking 2 ml of 100% test solution plus 3 ml of distilled water. The test solution concentration of 60% was obtained by taking 3 ml of 100% test solution plus 2 ml of distilled water. The test solution concentration of 80% was obtained by taking 4 ml of 100% test solution plus 1 ml of distilled water. The test solution concentration of 100% is obtained by taking 5 ml of 100% test solution.

Preparation of *Muller Hinton Agar*

38 grams of MHA was dissolved in 1 L of distilled water. After that, it is heated and stirred until homogeneous. Sterilize the media using an autoclave at a temperature of 121^{o}C, a pressure of 1.5 atm for 15 minutes. After the sterilization is complete, the media is put into a 15 ml petri dish which will be used as a medium is the antibacterial test.

Procedure

white champaca flower extract (*Michelia alba*) with concentrations of 20%, 40%, 60%, 80%, and 100% was divided into 5 groups (K3, K4, K5, K6, and K7) and 2 control groups (K1 and K2) using sterile distilled water and erythromycin. Add white champaca flower extract and control solution using a micropipette into each hole in the MHA media that has been inoculated with *Corynebacterium diphtheriae.* Then, incubated all plates in an incubator for 24 hours at 37^{o}C. After that, the diameter of the inhibition zone was measured using a caliper. Next, analyze the data from the study results.

Statistical Analysis

Data analysis from the study results was carried out using SPSS (Statistical Product of Service Solution) statistical analysis. The Kolmogorov-Smirnov test was used to determine whether of the data was normally distributed. Levene's test was then used to establish data homogeneity ($p>0.05$). If the data is normally distributed, then the one-way ANOVA test is used. An absolute requirement for one-way ANOVA to be used is that the data must be normally distributed. By using one-way ANOVA, it will be known whether there is an effect of white champaca flower extract (*Michelia alba dc)* on the diameter of the inhibition zone on the growth of *Corynebacterium diphtheriae*. Least Significant Difference (LSD) test with a significance of α=0.05.

RESULTS

Table 1. Results of measuring the diameter of the growth inhibition zone for *Corynebacterium diphtheriae*

Repetition	Inhibition Zone Diameter (mm)						
	K (+)	K (-)	K3 (20%)	K4 (40%)	K5 (60%)	K6 (80%)	K7 (100%)
1	30.50	0	40.25	51.10	49.80	51.10	51.65
2	56.95	0	48.85	44.05	52.90	54.85	54.15
3	52.70	0	40.85	44.40	50.85	53.90	58.10
4	36.70	0	38.70	49.30	53.10	58.10	54.85
Total	176.85	0	168.65	188.85	206.65	217.95	218.75
Average	44.21	0	42.16	47.21	51.66	54.49	54.69
St. Deviation	12.63	0.00	4.80	3.53	1.60	2.89	2.66

The average diameter of the highest inhibition zone based on the table above was in the K6 group (the group that was given 100% concentration extract) which was 54.69 mm and the lowest average value of 0.0 mm was in the negative control group in the form of aquades. The inhibition zones of the control group and other treatments can be seen in the figure below.

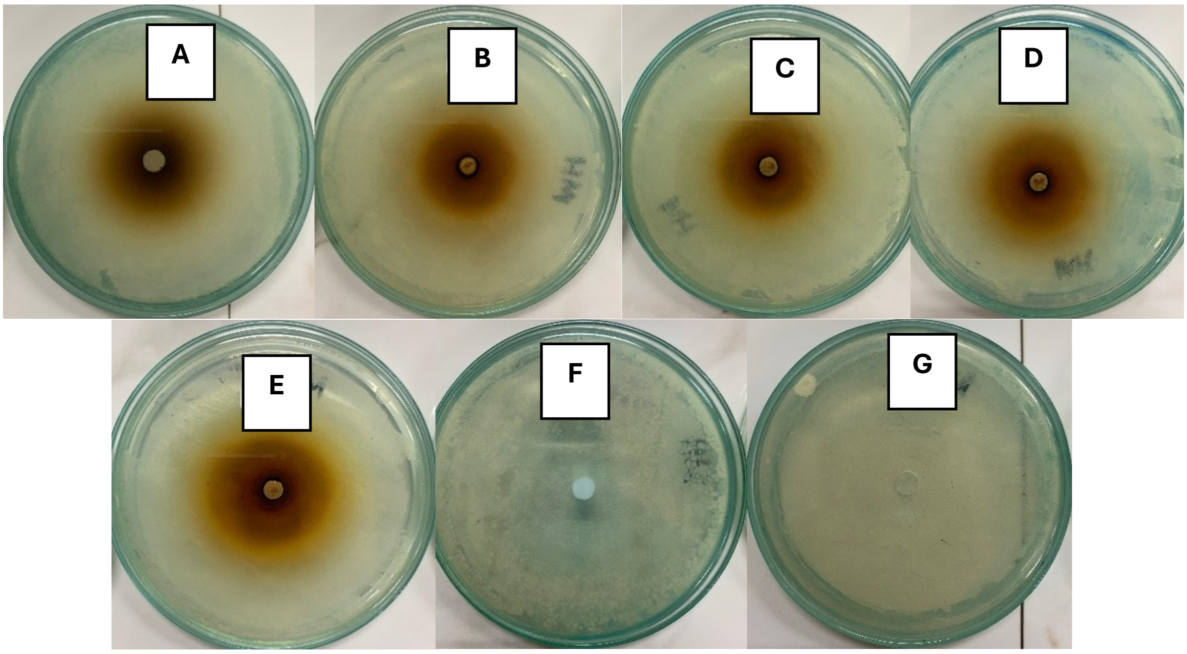

Figure 2. (A) White Champaca flower extract concentration 20%; (B) White Champaca flower extract concentration 40%; (C) White Champaca flower extract concentration 60%; (D) White Champaca flower extract with a concentration of 80%; (E) White Champaca flower extract 100% concentration. (F) Positive Control; (G) Negative control.

In the media given white Champaca flower extract (*Michelia alba dc*) produces a clear zone that is brownish in color. The brown color created in the white Champaca flower extract is due to the compounds contained in the flower interacting with ethanol. Alkaloid, flavonoid and terpenoid compounds present in flowers after extraction interact with ethanol and form complex compounds or undergo oxidation which causes the creation of brown extracts (Rauf et al., 2023). For media treated with erythromycin antibiotics in the wells, a clear zone of white color was formed. Meanwhile, the media treated with distilled water as a negative control showed the results that no visible inhibition zone was formed.

Table 2. Kolmogorov-Smirnov One-Sample Normality Test

Bacteria	Sig.
Corynebacterium diphtheriae	0.200

Based on Table 2 above, the results of measuring the diameter of the inhibition zone have a value of p = 0.200 > 0.05, meaning that the distribution is normal in the results of measuring the diameter of the inhibition zone.

Table 3. Levene Homogeneity Test

Bacteria	Sig.
Corynebacterium diphtheriae	<0.001

Based on Table 3, the results of the homogeneity test are known to have a value of p = 0.001<0.05 (5% or 0.05), which means that this data is not homogeneous in terms of variation. Although the data is not homogeneous, in the normality test, normal results were obtained so that the One-Way Anova test can still be continued.

Table 4. One-Way ANOVA Test Result

Bacteria	Sig.	Identifiying
Corynebacterium diphtheriae	<0.001	There is a difference

Based on the results of the analysis of the One-Way Anova test, the significance value was obtained of <0.001, which is smaller than p (p=0.05), meaning that there is a significant difference between groups.

Table 5. LSD Post Hoc Test Results

(I) Concentration	(J) Concentration	Sig.	Identifying
20%	40%	0.207	There is no difference
	60%	0.023	There is difference
	80%	0.005	There is difference
	100%	0.004	There is difference
	K positif	0.603	There is no difference
	K negatif	0.000	There is difference
40%	20%	0.207	There is no difference
	60%	0.264	There is no difference
	80%	0.075	There is no difference
	100%	0.068	There is no difference
	K positif	0.448	There is no difference
	K negatif	0.000	There is difference
60%	20%	0.023	There is difference
	40%	0.264	There is no difference
	80%	0.475	There is no difference
	100%	0.444	There is no difference
	K positif	0.069	There is no difference
	K negatif	0.000	There is difference
80%	20%	0.005	There is difference
	40%	0.075	There is no difference
	60%	0.475	There is no difference
	100%	0.959	There is no difference
	K positif	0.015	There is difference
	K negatif	0.000	There is difference
(I) Concentration	(J) Concentration	Sig.	Identifying
100%	20%	0.004	There is difference
	40%	0.068	There is no difference

	60%	0.444	There is no difference
	80%	0.959	There is no difference
	K positif	0.013	There is difference
	K negatif	0.000	There is difference
K positive	20%	0.603	There is no difference
	40%	0.448	There is no difference
	60%	0.069	There is no difference
	80%	0.015	There is difference
	100%	0.013	There is difference
	K negatif	0.000	There is difference
K Negative	20%	0.000	There is difference
	40%	0.000	There is difference
	60%	0.000	There is difference
	80%	0.000	There is difference
	100%	0.000	There is difference
	K positif	0.000	There is difference

The positive control group with erythromycin administration is significantly different from the concentration group of white Champaca flower extract (*Michelia alba dc*) 80%, 100% and negative control because the significance is less than 0.05. The positive control group with erythromycin administration is not significantly different from the concentration group of 20% and 40% white Champaca flower extract (*Michelia alba dc*) because the significance is more than 0.05. This can be interpreted that giving the concentration of white Champaca flower extract (*Michelia alba dc*) 100% is more effective than the concentration group of giving white Champaca flower extract (*Michelia alba dc*) 80%, positive control and negative control. And the positive control by giving erythromycin has the same effectiveness as the concentration group giving 20%, 40% and 60% white Champaca flower extract (*Michelia alba dc*).

DISCUSSION

The results of the One-Way Anova test analysis obtained a significance value of <0.001, which is smaller than p (p=0.05), which means that there is a significant difference in the effect of the treatment given to the bacteria. This shows that between the positive control and the five concentrations of white Champaca flower extract (*Michelia alba dc*) concentrations of 20%, 40%, 60%, 80% and 100% give different activity effects on inhibiting the growth of *Corynebacterium diphtheriae* bacteria. As for the negative control, it has no effect at all on Corynebacterium diphtheriae. For the antibacterial effectiveness test, the best is seen at 100% extract concentration while even the smallest concentration, 20%, can still inhibit the growth of *Corynebacterium diphtheriae* bacteria.

To see the difference between each treatment, using the LSD post hoc test and will be compared with the control group. The results of the LSD post hoc test are that the positive control group with erythromycin administration is significantly different from the concentration group of white cempaca flower extract (*Michelia alba dc*) 80%, 100% and negative control and there is no significant difference with the concentration group of white cempaca flower extract (*Michelia alba dc*) 20%, 40% and 60%. The negative control group is significantly different from the concentration group of white cempaca flower extract (*Michelia alba dc*) 20%, 40%, 60%, 80%, 100% and positive control. It can be interpreted that flowers have the ability to inhibit *Corynebacterium diphtheriae* bacteria. The concentration group giving white Champaca flower extract (*Michelia alba dc*) 100% is the most effective concentration in inhibiting the growth of *Corynebacterium diphtheriae* bacteria.

To determine the effectiveness of the inhibition of white Champaca flower extract (*Michelia alba dc*) against the growth of *Corynebacterium diphtheriae* bacteria by measuring the clear zone formed around the well after treatment. The clear zone itself is a description of the sensitivity of bacteria to antibacterial materials used in the test and is expressed by the width of the diameter of the inhibition zone formed (Rastina et al., 2015). Classification of bacterial growth inhibition activity is by looking at the clear zone formed which is divided into weak, medium, strong, and very strong. If the diameter formed is 5 mm or less, it is classified as weak, classified as moderate if the diameter ranges from 5-10 mm, the diameter ranges from 10-20 mm then classified into a strong response and if more than 20 mm then classified into a very strong response (Jamilatun et al., 2020). Based on this classification, it can be stated that in all treatments with the administration of white Champaca flower extract (*Michelia alba dc*) concentrations of 20%, 40%, 60%, 80%, 100% and the provision of positive control using erythromycin is classified in the very strong category. This indicates that the response or effect produced by all treatments has a very high level of strength or significance in the tests carried out.

The results showed that the concentration of white Champaca flower extract (*Michelia alba dc*) 100% showed the greatest average inhibition zone of 54.69mm including in the class of very strong response with a standard deviation of 2.66mm, 80% concentration in second place which formed an inhibition zone of 54.49mm with a standard deviation of 2.89mm, then followed by white Champaca flower extract (*Michelia alba dc*) 60% concentration with an inhibition zone of 51.66mm with a standard deviation of 1.60mm and white Champaca flower extract (*Michelia alba dc*) 40% concentration with an inhibition zone of 47.21mm with a standard deviation of 3.53mm. The concentration of 20% standard deviation of 4.80mm with an inhibition zone of 42.16mm is the smallest inhibition zone result compared to the positive control, which is giving treatment to bacteria using erythromycin. The inhibition formed from the positive control is 44.21mm standard deviation 12.63mm. In this case, the larger inhibition zone indicates the ability of the extract to inhibit bacterial growth more effectively than the antibiotic erythromycin because in the extract contains various antibacterial compounds that work synergistically so that the antibacterial effect can be greater. The size of the zone of inhibition and standard deviation give an idea of the antibacterial effectiveness of the extract, but do not provide direct information regarding its toxicity. To assess whether the extract is toxic, more in-depth research and safety tests are needed, one of which is in vivo or using experimental animals (Wuri et al., 2021).

In the treatment given to *Corynebacterium diphtheriae* bacteria by giving white Champaca flower extract (*Michelia alba dc*) has very strong antibacterial activity. The results of the treatment showed that the higher the concentration given, the greater the inhibition zone created around the well. This is in accordance with research conducted by Magvirah et al., (2019) found that the higher the concentration of extracts against bacterial growth, the greater the inhibition zone formed. The formation of inhibition zones that have a large diameter is in line with the statement that at higher concentrations there are more antibacterial substances. In this study using the well method, each hole is filled with a concentration of extract. This method is done to make the osmolarity more thorough and homogeneous. This method also produces a larger and stronger concentration of extract to stop bacterial growth. Using this pitting method is easier to measure the results of the inhibition zone formed because bacterial isolates will form to the bottom (Haryati et al., 2017). This method using wells has advantages in antibacterial testing, which will create higher antibacterial activity compared to using the disc method (Nurhayati et al., 2020).

The results of the study for the antibacterial inhibition zone using the positive control of erythromycin antibiotics formed an average inhibition zone diameter of 44.21mm. According to research by Mutahhar et al., (2020) which aims to determine antibacterial activity against *Corynebacterium diphtheriae* by observing its sensitivity pattern using the Epsilometer test (Etest) as a diffusion technique. From the results of this study, it was found that Erythromycin has strong antibacterial activity against *Corynebacterium diphtheriae* bacteria, namely the results showed that 90% were classified as sensitive and 10% were classified as moderate. Erythromycin is classified as a macrolide antibiotic which is very commonly used for the treatment of gram-positive bacteria. Research conducted by Benamrouche et al., (2016). using 157 isolates of Corynebacterium diphtheriae. The results obtained from the study were 157 isolates used were susceptible to erythromycin antibiotics.

Corynebacterium diphtheriae belongs to the group of Gram-positive bacteria. This group of bacteria is characterized by a layer of teichoic acid covering the peptidoglycan and in many aspects is functionally equivalent to the outer membrane of Gram-negative bacteria. In Gram-positive bacteria, the cell is created from an array of petidoglycan (PG) so that the cell wall is rigid (Ott et al., 2017). A water-soluble polymer has a function as a positive ion transport in order to get in and out of the substance. Gram-positive bacterial cell walls have more polar properties because of these soluble properties. White Champaca flower (*Michelia alba dc*) contains compounds that are polar. therefore, it can more easily enter through the peptidoglycan layer which has polar properties in the bacterial cell wall. Antibacterial molecules that enter will increase the osmotic pressure inside the cell, thus increasing the risk of lysis (Audies., 2015).

According to research conducted by Khairan et al., (2021) which shows that white Champaca flower extract contains alkaloid, steroid, terpenoid, saponin, flavonoid and phenol compounds. Flavonoid, alkaloid and phenol compounds are known as secondary metabolites that inhibit bacteria through the cell wall so that later suppress the growth of bacteria so as not to grow more (Rahmawati, 2020). Flavonoid compounds have an antibacterial mechanism to inhibit cell membrane function by forming complex materials consisting of extracellular and soluble proteins so that they can damage the bacterial cell membrane. In addition, flavonoids directly affect the microbial cell cycle by stopping the cell activity of microorganisms (Septiani et al., 2017).

Another polar compound is alkaloid. Alkaloids work by disrupting the constituent parts of peptidoglycan in bacterial cells, so that the formation of bacterial cell walls fails to form completely (Budianto et al., 2023). In addition, phenol has antibacterial activity because it interacts with bacterial cells through an absorption process that involves the formation of Hydrogen bonds (Ayuningtyas, 2018). The arrangement of phenol in the lipid bilayer can affect lipid-protein interactions and cause high membrane permeability. This in turn changes the membrane structure and accelerates the leakage of intracellular constituents, which ultimately destroys the integrity of the membrane (Wu et al., 2016)

White Champaca flowers also contain terpenoid compounds that can carry out antibacterial mechanisms. Terpenoid compounds are classified into non-polar compounds (Budianto, 2020). The mechanism of action of terpenoids has the ability to react with transmembrane proteins located on the outer membrane of the bacterial cell wall. This mechanism produces strong polymer bonds resulting in porin damage. The damage of porin, which has a function as the entrance and exit of the compound, will make the bacterial cell wall less permeable. This results in bacterial cells becoming deprived of nutrients, and will die because their growth is inhibited (Nugroho et al., 2015). Fresh Champaca flowers contain volatile compounds such as a-myrcene, (S)-limonene, (R)-fenchone, linalool, camphor, caryophyllene, and germacrene. These compounds are classified as terpenoids. α-Myrcene, (S)-Limonene, Linalool, Germacrene, Caryophyllene and Camphor can disrupt the integrity of bacterial cell membranes, cause leakage of ions and small molecules, and disrupt membrane functions that are important for bacterial survival (Wang et al., 2020)

Steroids have antibacterial properties by showing a mechanism in which steroids specifically associate with membrane lipids so that they can cause leakage from liposomes (Mulay & Shinde, 2016). Furthermore, there are saponin compounds, which are included in compounds that interfere with the activity of the bacterial wall. Finally, saponins have a content called AKP (alkaline phosphatase), which can result in the lysis of the cell wall of bacteria. This can occur because the content soars rapidly when additional saponins are added to the bacterial culture (Khan et al., 2018).

This study is also in accordance with research that has been conducted on white Champaca flower extract (*Michelia alba dc*). Munira et al., (2021) proved that the natural antibacterial ability of white Champaca flowers against pathogenic bacteria, namely Propionibacterium acnes. The inhibition formed from the methanol extract of white Champaca flowers (*Michelia alba dc*) has the largest diameter against bacterial growth with an extract concentration of 8% (9.6 mm), which is classified as a moderate response. In this study it was also found that white Champaca flower (*Michelia alba dc*) with the largest concentration of 100% is the most effective concentration in inhibiting the growth of *Corynebacterium diphtheriae* bacteria because it produces the largest inhibition zone compared to other concentrations and is classified as a very strong response.

CONCLUSION

The conclusion of the research was that white Champaca flower extract (*Michelia alba dc*) in concentrations (20%, 40%, 60%, 80%, and 100%) had antibacterial effectiveness against the growth of Corynebacterium diphtheriae and at a concentration of 100% it is the most effective concentration in inhibiting the growth of *Corynebacterium diphtheriae* bacteria because it produces the largest inhibition zone compared to other concentrations and is classified as a very strong response.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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