

Research Article

Potential of Ciplukan Leaf Extract (*Physalis angulata* L.) Against *Streptococcus pneumoniae* Growth in Vitro

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ABSTRACT

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Pneumonia is a disease that infects the lungs, and antibiotics such as tetracycline show a high degree of resistance. Therefore, the use of alternative natural materials, such as ciplukan leaves (*Physalis angulata* L.), which the Dayak people commonly use to treat lung diseases, is necessary. This study aimed to prove that the extract of ciplukan leaves can inhibit *Streptococcus pneumoniae* by measuring the diameter of the clear zone and determining the Minimum Inhibitory Concentration (MIC). The extract was obtained by maceration using 96% ethanol, and then concentrations of 5%, 10%, 15%, 20%, and 25% were prepared. The positive control was levofloxacin 5 µg, and the negative control was DMSO 1%. The average inhibition zone diameters of the ciplukan leaf extract were 5% (0 mm), 10% (0.17 mm), 15% (0.45 mm), 20% (1.95 mm), 25% (2.67 mm). Antibacterial test using the disk diffusion method. From the Kruskal-Wallis and Mann-Whitney Post Hoc statistical test, a significance value of <0.05 was obtained. Therefore, there is an effect of ciplukan leaf extract on *Streptococcus pneumoniae* at concentrations of 15%, 20%, and 25%, with the MIC 15%.

Keywords: Ciplukan (*Physalis angulata* L.); *Streptococcus pneumoniae*; extract; in Vitro

INTRODUCTION

Pneumonia is a disease that affects the lungs, specifically the lung parenchymal tissue. The disease causes high mortality among children in low-to-medium per capita income countries such as Africa and southern Asia. In Indonesia in 2018, the mortality rate was 551 toddlers. The prevalence of pneumonia among under-fives in the districts/cities of Central Kalimantan Province in 2019 was highest at 355 under-fives in West Kotawaringin District and lowest at 2 under-fives in South Barito District (Dinkes Kalteng, 2019).

The most common pathogen of pneumonia is *Streptococcus pneumoniae*. According to Brown et al. and Feldman et al. (2018), *S. pneumoniae* bacteria can cause micro lesions in the heart myocardium, referred to as Invasive Pneumococcal Disease (IPD). In addition, treating *S. pneumoniae* has difficulties, such as a high level of antibiotic resistance, namely beta-lactams, tetracyclines, and macrolides. Microbes have developed antibiotic resistance as a result of the widespread and irrational usage of antibiotics. Furthermore, it may have negative long-term health impacts. Therefore, it is crucial to do research on the need for new antimicrobial substitutes made from natural substances that are less harmful and have fewer adverse effects (Subramanian, Henriques-Normark, and Normark 2019).

According to (Sari et al. 2015), the Dayak Jangkang community in West Kalimantan uses ciplukan leaf parts as medicine for influenza and lung pain. The most commonly used technique in processing is boiling because, according to the Dayak people, this technique can help make more compounds interesting than other techniques (Az-Zahra et al. 2021).

According to (Rohyani 2015), from the results of phytochemical tests on ciplukan leaves, active compounds such as steroids, alkaloids, flavonoids, terpenoids, anthraquinones, saponins, and tannins were found. The following is an example of the function of phytochemical compounds in flavonoids and alkaloids: flavonoids act as bacteriostatic and bactericidal agents by damaging membranes in the cytoplasm of bacterial cells, while alkaloids act to inhibit DNA formation in bacteria (Harlita, Anggrieni, and Rahmawati 2019). This study aimed to prove that ciplukan leaf extract (*Physalis angulata* L) can inhibit *S. pneumoniae* by measuring the diameter of the clear zone and determining the Minimum Inhibitory Concentration (MIC) value.

MATERIALS AND METHODS

Materials

The tools used in this study include blender (Miyako), black cloth (Jet Black), maceration jar (Ginka), sifter (Doll), rotary vacuum evaporator (HN 2005 V-N, South Korea), stirring rod, water bath (Memmert, Germany), Erlenmeyer (Pyrex, Indonesia), clean pot (Pyrex, Indonesia), Sudip (Muhltal, Germany), lighter (Afta,

Indonesia), bunsen, cotton swab (Onemed, Indonesia), ose, preparation glass (Onemed, Indonesia), light microscope (Olympus, Japan), petri dish (Onemed, Indonesia), analytical scales (Wann, Indonesia), measuring flask (Pyrex, Indonesia), beaker bowl (Pyrex, Indonesia), test tube (Pyrex, Indonesia), measuring cup (Pyrex, Indonesia), vernier (Taffware, Indonesia), tweezers (Onemed, Indonesia), autoclave (GEA LS-100LJ, China), drop pipette (Onemed, Indonesia). The materials used in this study include Ciplukan leaves (*Physalis angulata* L.), 96% ethanol (Onemed, Indonesia), Blood Agar Plate (BAP) media (Oxoid), aluminum foil (Reynolds, America), pure isolates of *S. pneumoniae* (ATCC 49619 PK/5), Mueller Hinton Agar (MHA) media (Oxoid), safranin solution (Indoreagen, Indonesia), crystal violet solution (Indoreagen, Indonesia), filter paper (Whatmann No. 1), 1% DMSO (Dimethyl Sulfoxide) (Merck), five µg levofloxacin antibiotics in the form of disc paper (Oxoid), sterile 0.9% NaCl (Onemed, Indonesia), BaCl (Merck), 6 mm disc paper (Oxoid). Ciplukan leaves were taken from the Jekan Raya sub-district, Palangka Raya City. The determination of ciplukan plants was carried out at the InaCC-BRIN Characterization Laboratory in Cibinong, Bogor, and West Java. This study was declared ethically feasible with the issuance of a letter of ethical clearance by the ethics committee of the faculty of medicine, Palangkaraya University, with number 30/UN24.9/LL/2024.

Method

Sample Extraction

Dried ciplukan leaves are pureed with a blender. Then, the leaves that have been finely sifted and mixed with 96% ethanol solution in a ratio of 1:3, 1 gram of dried leaves, and 3 ml of 96% ethanol solution. The delicate leaves are put into a glass jar, soaked using a 96% ethanol solution, covered using aluminum foil, and macerated for 3x24 hours while stirring occasionally, filtered using filter paper, and obtained filtrate one and dregs. Furthermore, the dregs will be re-soaked with 96% ethanol compared to 1:3 every 24 hours and covered using aluminum foil while stirring occasionally. Then, the dregs are filtered with filter paper, and dregs and filtrate 2 are obtained. Combine filtrate 1 and filtrate 2, then use a rotary evaporator to concentrate the filtrate, then use a water bath to evaporate the remaining solvent until a thick extract of ciplukan leaves is formed (Aldafi et al. 2023) (Sukmawati, Rahmawati, and Ainun 2024). Next, calculate the yield value to determine the ratio of the weight of the extract that has been obtained to the weight of the simplisia. The yield value can be calculated by the following formula:

$$\frac{\text{Weight of extract}}{\text{Weight of raw material}} \times 100\%$$

Phytochemical Screening

Flavonoid

A 2 mL solution of ethanol extract of ciplukan leaves was added to hot water and boiled for 5 minutes, then filtered. Filtrate added a little magnesium powder and concentrated HCl as much as 1 mL, then shook it. Positive test results are indicated by the formation of red, yellow, or orange colors (Wulan Kusumo, Kusuma Ningrum, and Hayu Adi Makayasa 2022).

Alkaloids

Ethanol extract from ciplukan leaves as much as 2 mg was weighed and then dissolved with 5 ml of distilled water before adding HCl until a reaction occurred. After adding HCl, Dragendroff reagent was added as much as 1 ml. The formation of a brown precipitate characterizes positive Dragendroff test results (Rizqiana and Sudarmin 2023).

Saponin

The plant extract is dissolved with distilled water to test for saponins and then shaken. After the foam appears, add HCL 1N and wait 10 minutes. If the foam remains >10 minutes, it means that the plant extract contains saponins (Rizqiana and Sudarmin 2023).

Tanin

If the ciplukan leaf extract contains tannins, it will turn dark blue or blackish green when it reacts with a 1% ferric chloride solution in a test tube. Additionally, gelatin solution identification is carried out by mixing the extract with the solution; if a white precipitate forms, tannins are present (Makatambah, Fatimawali, and Rundengan 2020).

Antraquinon

The color changes after adding 1 N NaOH to the ciplukan leaf extract solution. The formation of a yellow color indicates a positive reaction (Wulan Kusumo, Kusuma Ningrum, and Hayu Adi Makayasa 2022).

Steroid/Terpenoid

A total of 1 mm of the chloroform phase of ciplukan leaf extract was put into a drip plate, then five drops of Liebermann-Burchard reagent were added. Steroids and terpenoids form a blue or green ring layer (Nurjannah et al. 2022).

Preparation of ciplukan leaf extract concentration

The preparation of ciplukan leaf extract was carried out using the maceration technique, and then a 1% DMSO solution was mixed with each ciplukan leaf extract according to the concentration of 10 ml. The formula of the percentage of volume weight can obtain the concentration of the extract (Kurniati et al. 2020).

$$\% = \frac{b}{v} \times 100\%$$

Notes:

b= Weight of condensed extract of ciplukan (gram)

v= Volume of 1% DMSO

For example, to make ciplukan leaf extract with a concentration of 5%, 0.5 grams of extract is dissolved in 1% DMSO until the volume is 10 ml.

Culture Process of Streptococcus pneumoniae Bacteria on Blood Agar Plate (BAP) Media

Preparation of Blood Agar Plate

Petri dishes are sterilized using an autoclave at 160°C for 45 minutes, and Petri dishes are sealed using aluminum foil. Then, weigh 40 g of Blood Agar Base (BAB). Add 950 ml of distilled water, then stir until smooth. Next, sterilize the media in an autoclave at 121°C for 15 minutes. Fill the BAB and wait until it warms up, then add sheep blood, about 50 ml or 5-7%, then mix it. Pour into sterilized petri dishes (Nabila, Purnamasari, and Alhawaris 2021).

Bacterial Rejuvenation of Streptococcus pneumoniae onto BAP

Pure *S. pneumoniae* isolates were inoculated by one ose into a petri dish containing blood agar using the zig-zag streak method. Then, for 24 hours, incubation was carried out at 37°C. BAP media displays a light greenish-colored zone with a circular shape (M. Sari, Latief, and Massi 2020).

Confirmation test for Streptococcus pneumoniae bacteria (Gram stain)

The first step is to make a smear on a glass slide by taking bacteria using an ose from BAP media, then make a smear with a circle with a diameter of 15 mm. Dry the smear with the help of air (Tripathi 2023).

In the second step, give a purple crystal violet solution to the glass preparation, wait for 60 seconds, and then rinse with running water. Next, add iodine solution to the entire surface of the object glass, wait for 60 seconds, and then rinse with running water. Add alcohol to the glass object and let it stand for 5-10 seconds, then rinse with water. Finally, add safranin solution and wait for 40-60 seconds, then rinse with water and air dry (Tripathi 2023).

The third step is to examine the glass object under a microscope with an initial magnification of 40X objective lens to assess the distribution of the smear, then use 100X magnification with the help of immersion oil that has been dripped on the glass object (Tripathi 2023). Colonies of *S. pneumoniae* bacteria will appear purplish in color and elongated like a chain/lancet (M. Sari, Latief, and Massi 2020).

Reculture of Streptococcus pneumoniae

Preparation of Blood Agar Plate

Petri dishes are sterilized using an autoclave at 160°C for 45 minutes, and petri dishes are sealed using aluminum foil (Wulandari et al. 2022). Put 2 grams of Mueller Hinton Agar (MHA) media into an erlenmeyer. After dissolving it with distilled water to 500 ml, heat it in a water bath with cotton covered on top. Then, sterilize for two hours at 121°C. Then, it is cooled to a temperature of 40-45°C, poured into sterile petri dishes, and the media is ready when tested for bacterial culture and growth (Vanawati et al. 2023).

Planting Streptococcus pneumoniae Bacteria to Mueller Hinton Agar (MHA) Media

Previously, make a suspension of *S. pneumoniae* bacteria by taking it from a bacterial breeding site using a sterile ose and then suspending it into 10 ml of sterile 0.9% NaCl in a test tube until the turbidity is equal to 0.5% McFarland solution. McFarland 0.5% solution was made with a composition of Sulfuric acid (H₂SO₄) 1% by 9.95 ml and Barium chlorida (BaCl₂) 1% by 0.05 ml (Aldafi et al. 2023; Aviany and Pujiyanto 2020).

Antibacterial Test

Place the ciplukan leaf extract and positive and negative controls in clean, uncontaminated containers. When implanting *S. pneumoniae* bacteria in Mueller Hinton Agar (MHA) media, label the petri dish with the amount of solution to be tested. Next, prepare a 6 mm diameter paper disc and use sterile tweezers to soak it into the extract and negative control solution for 10 minutes. Then, take the paper disc and insert it into the labeled part of the container. Next, incubate at 37°C for up to 24 hours. Next, observe the clear zone in the Petri dish (Aldafi et al. 2023).

Measure the inhibition zone using horizontal and vertical diameters using a caliper using units in mm (Magvirah, Marwati, and Ardhani 2019). Calculation of the diameter of the inhibition zone is carried out through the following formula:

$$\text{Diameter of inhibit zone} = \frac{(\text{Dh}-\text{Dc})+(\text{Dv}-\text{Dc})}{2}$$

Notes:

Dh : Diameter of horizontal

Dv : Diameter of vertical

Dc : Diameter of cakram disk

The data taken is the average value of 4 repetitions.

Data Analysis Techniques

The results of the data that have been obtained in the research in the form of the calculation of the diameter of the clear zone are then entered into the Microsoft Excel application to facilitate the process of entering data. Furthermore, the data was entered into the IBM SPSS 26.0 application, and the normality test was carried out using the Shapiro Wilk test and the homogeneity test using the Levene test. The significance value of the Saphiro Wilk test was less than 0.05 (sig.<0.05), so the data was not normally distributed, then the data group did not come from a homogeneous population because the significance value was obtained <0.05 in the Levene test. Because the parametric test requirements were not met, a non-parametric test was carried out with the Kruskal-Wallis test. After the Kruskal-Wallis test was carried out, conduct a non-parametric post hoc test with the Mann-Whitney U post hoc test, in order to determine the minimum inhibitory concentration (MIC) (Singgih 2020).

RESULTS

Extraction Result

This study, using a 96% ethanol solvent and maceration extraction technique, obtained a simplisia weight of 414.88 g and an extract weight of 49.62 g, resulting in a yield value of 11.96%.

Phytochemical Screening Results

Qualitative phytochemical screening results show that ciplukan leaf extract contains flavonoids, alkaloids, saponins, tannins, anthraquinones, steroids, and terpenoids. Table 1 shows the qualitative phytochemical results of ciplukan leaf extract.

Table 1. Qualitative Phytochemical Test Results

Compound Groups	Observation and Reaction	Results
Flavonoid	Mg(s) + concentrated HCl = Changes to a brick red color	(+)
Alkaloid	Mayer + sample = Dragendorff precipitate + sample = Precipitate	(+)
Saponin	Foam lasts 10 minutes + 1N HCl = Foam persists	(+)
Tanin	FeCl 1% + Gelatin 1% + sample = Formed White precipitate	(+)
Antraquinon	Liquid sample + NaOH 1N = Formed yellow-red color	(+)
Steroid/Terpenoid	Lieberman-bucard + sample = Dark green color reaction formed	(+)

Bacterial Identification and Antibacterial Test Results

After the phytochemical test, bacterial identification and antibacterial tests were carried out using the disc diffusion technique for 4 repetitions (Figure 3.). The results of antibacterial identification can be seen in Figure 1. and Figure 2.



Figure 1. Identification results of *Streptococcus pneumoniae* bacteria on Blood Agar Plate



Figure 2. Identification results of *Streptococcus pneumoniae* bacteria using Gram stain and light microscopy with 100x magnification

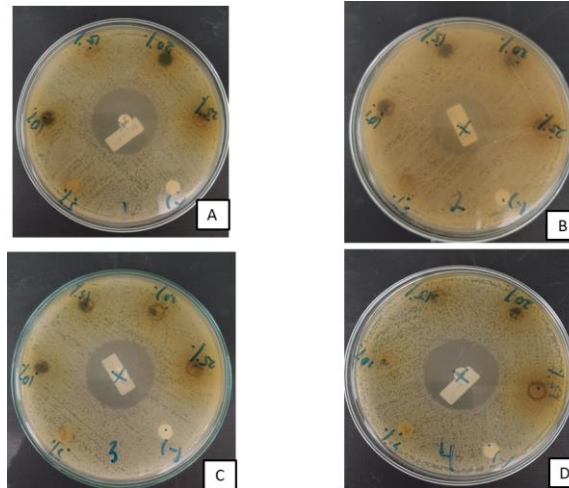


Figure 3. Antibacterial Test Results for 4 Repetitions with Disc Diffusion: (A) Repetition 1; (B) Repetition 2; (C) Repetition 3; (D) Repetition 4

The results of the antibacterial test of ciplukan leaf extract using the disc diffusion method with a repetition of 4 times obtained the average value of the diameter of the inhibition zone as follows: 5% concentration measuring 0 mm, 10% concentration measuring 0.17 mm, 15% concentration measuring 0.45 mm, 20% concentration measuring 1.95 mm, and 25% concentration measuring 2.67 mm. The mean of the positive control was 24 mm, and the negative control was 0 mm. Normality test data, homogeneity test, Kruskal-Wallis test, and mean inhibition zone diameter data along with Mann-Whitney post hoc test can be seen in Table 2 and Table 3.

Table 2. Normality (Shapiro-Wilk), Homogeneity (Levene), and Kruskal-Wallis Test Results

Group	Shapiro-Wilk Sig.	Levene Sig.	Kruskal-Wallis Sig.
Extracts (5%)	0,000*		
Extracts (10%)	0,220**		
Extracts (15%)	0,574**	0,001*	0,000*
Extracts (20%)	0,869**		
Extracts (25%)	0,085**		
Positive control (Levofloxacin)	0,986**		
Negative control (DMSO 1%)	0,000*		

Note: * Statistical $p < 0.05$

** Statistical $p > 0.05$

Table 3. Mean Data of Inhibition Zone Diameter and Mann-Whitney Post Hoc Test

Group	Mean (mm) ± SD
5% (Extracts) (n=4)	0±0 ^{c,d,e,f,B,G}
10% (Extracts) (n=4)	0,17±0,23 ^{d,e,f,A,C,G}
15% (Extracts) (n=4)	0,45±0,36 ^{a,d,e,f,g,B}
20% (Extracts) (n=4)	1,95±0,53 ^{a,b,c,f,g,E}
25% (Extracts) (n=4)	2,67±0,45 ^{a,b,c,f,g,D}
Levofloxacin (Control +) (n=4)	24±1,94 ^{a,b,c,d,e,g}
DMSO 1% (Kontrol -) (n=4)	0±0 ^{c,d,e,f,A,B}

Notes:

- a : Significantly different at 5% with $p < 0.05$
- b : Significantly different to 10% with $p < 0.05$
- c : Significantly different to 15% with $p < 0.05$
- d : Significantly different to 20% with $p < 0.05$
- e : Significantly different to 25% with $p < 0.05$
- f : Significantly different from control (+) with $p < 0.05$
- g : Significantly different from control (-) with $p < 0.05$
- A : Not significantly different from 5% with $p > 0.05$
- B : Not significantly different from 10% with $p > 0.05$
- C : Not significantly different from 15% with $p > 0.05$
- D : Not significantly different from 20% with $p > 0.05$
- E : Not significantly different from 25% with $p > 0.05$
- F : Not significantly different from control (+) with $p > 0.05$
- G : Not significantly different from control (-) with $p > 0.05$
- n : Sample

From the normality test results in Table 2, the 5% concentration obtained has a significance value ($p < 0.05$), meaning that the measurement data is not normally distributed. The results of the homogeneity test obtained a significance value ($p < 0.05$), meaning the measurement data does not have the same/homogeneous variation. Because the data did not have a normal distribution and were not homogeneous, a non-parametric statistical test was performed using the Kruskal-Wallis test, and a significance value was obtained ($p < 0.05$), meaning that there was a significant difference between the mean diameter of the inhibition zone for the 7 treatment groups. To be able to find out which groups have significant differences in means, the Mann-Whitney Post Hoc test (Table 3) was conducted.

DISCUSSION

In the research of Sukmawati et al. (2024), using the same solvent and extraction technique, the weight of ciplukan leaf simplisia was obtained at 100 g, and the weight of the extract was 10.654 g, so a yield value of 10.654% was obtained (Sukmawati, Rahmawati, and Ainun 2024). In the research of Tambunan et al. (2023), using the same solvent and extraction technique, the weight of ciplukan leaf simplisia was obtained 1000 g, and the extract weight was 284.50 g so that the yield value was 28.45%. It is concluded that the yield value is influenced by how much the amount of simplisia is used (Tambunan et al. 2023).

The results of the phytochemical test showed that ciplukan leaves positively contained alkaloid, flavonoid, saponin, tannin, anthraquinone, steroid, and terpenoid compounds. In this study, which used 96% ethanol solvent, it was found that ciplukan leaves contained flavonoids, alkaloids, saponins, tannins, anthraquinones, steroids, and terpenoids. Research by Aldafi et al. (2023), which used the same plants, solvents, and extraction techniques as this study, obtained the content of alkaloid, saponin, tannin, and flavonoid phytochemical compounds (Aldafi et al. 2023). Research by Dahlia et al. (2022), using the same plants, solvents, and extraction techniques as this study, obtained phytochemical compounds such as phenols, flavonoids, saponins, tannins, and quinones (Dahlia et al. 2022). The

difference in the content of phytochemical compounds is due to internal and external factors of the plants used. Internal factors include plant genetics and parts used by plants, while external factors can include pH, light, altitude of the place of growth, nutrient content in the soil, and humidity.

From the flavonoid test, the color changes to brick red, this is due to the reduction process of the benzopyrone nucleus in the flavonoid section (Wulan Kusumo, Kusuma Ningrum, and Hayu Adi Makayasa 2022). The alkaloid test shows a precipitate, this occurs because free electrons in the structure of hydrogen atoms in alkaloids can form ions with Dragendorff reagent. Saponin tests can form foam because saponins have polar and non-polar structures; when shaken, these structures can form micelles, giving the appearance of foam (Rizqiana and Sudarmin 2023). The tannin test forms a white precipitate due to the precipitation of FeCl_3 1% and gelatin 1%; this occurs because tannins are able to precipitate proteins (Makatambah, Fatimawali, and Rundengan 2020). The anthraquinone test forms a yellow-to-red color; this occurs because when 1 N NaOH is added, the deprotonation process of the phenol group occurs to form enolate ions; these ions can form double bonds and resonate between electrons so that they can absorb and reflect certain colors (Wulan Kusumo, Kusuma Ningrum, and Hayu Adi Makayasa 2022). Steroid and terpenoid tests produce a dark green color due to the reduction of steroid acetyl groups due to the addition of Lieberman-Buchard reagent (Nurjannah et al. 2022).

Based on the results of bacterial identification, it is found that the bacteria that grow on BAP media are true *S. pneumoniae*, which has a round shape, flat edges, white color, convex elevation, and there is a greenish zone at the bottom of the colony which indicates the process of partial hemolysis. In the Gram staining test, it was found that the test bacteria were purple, which indicates gram-positive bacteria and coccus-shaped (*streptococcus* and *diplococcus*); this result is in accordance with the characteristics of *S. pneumoniae* bacteria, which are gram-positive bacteria with the form of *streptococcus/diplococcus* (Chen et al. 2021).

In this study, the 5% concentration did not have an inhibition zone. This is supported by the research of Wijayanti et al. (2014), which uses ciplukan leaves against *Lactobacillus acidophilus* bacteria (gram +) and shows that concentrations below 6.35% also have no inhibition zone (Wijayanti, Medawati, and Mahanani 2014). Pattipeilohy et al. (2022), research using tapak dara leaves against *Staphylococcus aureus* bacteria (gram +) shows that 5% concentration also has no inhibition zone. This can occur because when the concentration of the extract is too low, it can cause the diffusion rate of the extract from the disc paper to decrease, this can make the extract unable to inhibit the growth of test bacteria so that no inhibition zone is formed (Pattipeilohy, Umar, and Pattilouw 2022).

From the results of statistical tests, the Minimum Inhibitory Concentration (MIC) value of 15% was obtained. This result was obtained from the comparison of the extract group with the negative control group (DMSO 1%), in the 5% and

10% extract groups, when compared with DMSO 1%, did not have a significant difference, meaning that the diameter of the inhibition zone formed was not much different from DMSO 1%. While the 15%, 20%, and 25% extract groups when compared with 1% DMSO had significant differences, meaning that the diameter of the inhibition zone formed was much different from 1% DMSO. MIC is determined by determining the smallest concentration that has a significant difference with 1% DMSO, namely the 15% concentration group (Aldafi et al. 2023).

This study used a negative control of 1% DMSO and a positive control of 5 µg levofloxacin. DMSO 1% was chosen because it is not toxic and does not have antibacterial properties. The positive control of levofloxacin 5 µg when tested with *S. pneumoniae* according to the Clinical and Laboratory Standards Institute (CLSI) 2023 is categorized as sensitive when it has an inhibition zone diameter ≥ 17 mm, intermediate 14-16 mm and resistant ≤ 13 mm. Compared with CLSI 2023, levofloxacin 5 µg was in the sensitive category because it had an inhibition zone diameter ≥ 17 mm. While the extract group concentrations of 5%, 10%, 15%, 20%, 25% were categorized as resistant because the diameter of the inhibition zone was ≤ 13 mm (Lewis et al. 2023).

In this study, which used a positive control of levofloxacin 5 µg to *S. pneumoniae* bacteria (gram +), the diameter of the inhibition zone was 24 mm. Research by Irfan et al. (2016), which used a positive control of levofloxacin 5 µg to *S. aureus* bacteria (gram +), obtained an inhibition zone diameter of 25.56 mm. Kusuma's (2016) research, which uses a positive control of levofloxacin 5 µg on *Pseudomonas aeruginosa* bacteria (gram -), obtained an inhibition zone diameter of 21 mm (Kusuma 2016). It can be seen that the diameter of the inhibition zone of gram (-) bacteria is smaller than that of gram bacteria; this is because gram (-) bacteria have a structure called lipopolysaccharide. This structure consists of a lipooligosaccharide core, lipid A, and O-chain structure, which can be a stimulator for bacterial immune defense (Caroff and Novikov 2020).

CONCLUSIONS

Ciplukan leaf extract (*Physalis angulata* L.) can inhibit the growth of *S. pneumoniae* bacteria starting from concentrations of 15%, 20%, and 25%. It can also be determined that the Minimum Inhibitory Concentration (MIC) value in this study is 15%.

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