

THE EFFECTIVENESS TEST OF TURMERIC EXTRACT TOWARD BACILLUS CEREUS BACTERIA WITH THE COMPARISON OF CIPROFLOXACIN

Uji Efektivitas Ekstrak Rimpang Kunyit Terhadap Bakteri Bacillus Cereus Dengan Perbandingan Ciprofloxacin

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Abstract This research aims to find out effectiveness test of turmeric rhizome in concentration of 25%, 50%, 75% and 100% toward *Bacillus cereus* bacterial and Ciprofloxacin the same concentration as the comparison. *Bacillus cereus* Bacteria is a gram-positive rod-shaped bacteria and it is dangerous for humans. This research was conducted with an experimental method with post-test only design and sampling using purposive sampling method. The effectiveness test of turmeric rhizome extract toward *Bacillus cereus* bacteria was carried out by diffusion using disc paper by calculating the diameter of the bacterial inhibition zone against disc paper that had been moistened with turmeric rhizome extract or Ciprofloxacin solution. The results obtained in this research were carried out with the Post Hoc test, the test was found that there were significant differences from each treatment given with a 95% confidence index. In bacteria treated with 25% ciprofloxacin toward *Bacillus cereus*, the effectiveness was above 50% turmeric rhizome extract and under 75% turmeric extract. Turmeric extract of 75% 10.6 mm had bacterial effect as Ciprofloxacin 25% of 10.1 mm toward the growth of *Bacillus cereus* bacterial.

Keywords: Antibacterial, Turmeric extract, *Bacillus cereus*.

Abstrak Penelitian ini bertujuan untuk mengetahui uji efektivitas dari ekstrak rimpang kunyit pada konsentrasi 25%, 50%, 75% dan 100% terhadap bakteri *Bacillus cereus* dengan Ciprofloxacin konsentrasi yang sama sebagai pembandingnya. Bakteri *Bacillus cereus* merupakan bakteri gram positif berbentuk batang dan berbahaya bagi manusia. Penelitian ini dilakukan dengan metode eksperimental dengan desain post-test only design dan pengambilan sampel menggunakan metode Purposive sampling. Uji efektivitas ekstrak rimpang kunyit terhadap bakteri *Bacillus cereus* dilakukan dengan cara difusi menggunakan kertas cakram, yaitu dengan menghitung diameter zona hambat bakteri terhadap kertas cakram yang sudah dibasahi dengan ekstrak rimpang kunyit maupun larutan Ciprofloxacin. Hasil yang didapat pada penelitian ini yang dilakukan dengan uji Post Hoc, Test tersebut dijumpai bahwa terdapat perbedaan yang bermakna dari setiap perlakuan yang diberikan dengan indeks kepercayaan 95%. Pada bakteri yang diberi perlakuan ciprofloxacin 25% terhadap *Bacillus cereus*, efektivitas yang didapat berada diatas ekstrak rimpang kunyit 50% dan berada dibawah ekstrak rimpang kunyit 75%. Ekstrak rimpang kunyit 75% 10.6 mm memiliki efek antibakteri sama dengan Ciprofloxacin 25% yaitu 10.1 mm terhadap pertumbuhan bakteri *Bacillus cereus*.

Kata Kunci: Antibakteri, Ekstrak Rimpang Kunyit, *Bacillus cereus*.

INTRODCUTION

Traditional medicine is an alternative medicine from plant which has been used by society in various parts of the world to treat several diseases. According to a WHO (World Health Organization) survey, it is stated that 80% of the world's 100 inhabitants have used herbal medicines (Pangemanan, ., & Budiarmo, 2016). Based on Permenkes (Ministry of Health regulations) No. 007 of 2012 concerning traditional medicine has been used for generations and is useful as a treatment according to norms circulating in the community. Traditional medicine is in the form of whole ingredients which can come from nature (Yusnidar, 2017). Turmeric (*Curcuma domestica* VALET) can be used as a Family Medicinal Plant (TOGA) which is very useful as a correctional effort in family health (Rukmana, 1995). Turmeric consists of roots, rhizomes, stems, leaves, flower stalks and flower buds. The type of fibrous turmeric root (radix adventica) and the shape is like a thread connected to the turmeric rhizome (Rukmana, 1995). The efficacy of turmeric as a medicine is due to its curcuminoid compound and its essential oils which are antioxidant, antitumor, anti-cancer, antimicrobial, antiseptic and anti-inflammatory (Balitro & Hartati, 2013). In essential oils contained phenol derivatives namely hydroxyl and carbonyl functional groups are used as antibacterial (Yuliati, 2016). Curcumin activity results have the ability to regulate inflammatory cytokines, protein-kinases, enzymes, transcription factors, and growth factors (Mutiah, 2015). There are three components were found in turmeric rhizome, namely Curcumin, Desmetoksikurkumin and Bis-Desmetoksikurkumin. Turmeric rhizome contains around 3% of essential oil content and 10% of curcuminoid content (H. Widyaningrum, 2011). Curcumin is a polyphenol compound (1,7-bis (4 '-hydroxy-3'-methoxyphenyl) -, 6-heptadiene-3,5-dion). Polyphenols have a structure generally divided into flavonoids

and nonflavanoids. Flavonoids-2-alls, flavonols, and tannins as polyphenols which have greater microbial inhibitory activity than other polyphenols and some polyphenols can also inhibit virulence. Turmeric rhizomes contain active ingredients which can be used as antibacterial (Nadifah, Farida Muhajir, & Retnoningsih, 2018).

The genus of *Bacillus cereus* belongs to a gram-positive, aerobic chain. *Bacillus cereus* is usually found in food such as fried rice which has been inhabited for hours at room temperature. Thus, these bacteria cause "fried rice syndrome" (Brooks, 2013).

Toxic food can cause gastrointestinal disorders because it is usually contaminated by *Bacillus cereus*. This occurs due to the presence of enterotoxins contained in the food consumed. The presence of enterotoxins in food is usually due to the presence of endospore. This endospore is not completely turned off by high temperatures when cooking food (Yuliati, 2016).

Pathogenesis of *Bacillus cereus*, The soil organism which often contaminates rice is *Bacillus cereus*. Then in the log-phase growth (sporulation), bacterial spores grow and vegetative cells in the bacteria release toxins. The incubation period of diarrhea is about 1-24 hours. Treatment for the *Bacillus cereus* bacteria is partly resistant to penicillin containing β -lactamase. Effective alternatives to penicillin are doxycycline, erythromycin or ciprofloxacin (Brooks, 2013).

Ciprofloxacin is antibiotic group of second generation broad-spectrum florokuinolone that functions as an anti-infective or synthetic antibiotic. Ciprofloxacin works by inhibiting the growth of DNA-gyrase. Thus, it can stop the metabolism of germs. Ciprofloxacin is absorbed mainly in the small intestine and the peak concentration after 60-90 minutes later the results of its metabolites are excreted in urine and feces (Kementerian

Kesehatan RI, 2008). Ciprofloxacin Antibiotic Medication is a therapy classified as effective against bacteria with a clinical cure rate of 98% (I. Pratiwi, Aziz, & Kusumastuti, 2018). Yet, the unstable ciprofloxacin antibiotic is absorbed by the digestive tract (Y. Pratiwi & Swantari, 2017).

Based on the description above, the researcher wants to test the effectiveness of antibiotics from Turmeric rhizome extract toward the proliferation of *Bacillus cereus* with Ciprofloxacin as a comparison.

RESEARCH METHOD

Method used was experimental method with post test only design. In this research design, the interventions which had been carried out were then carried out measurements (observations) or post-tests of the results. Treatment was as independent variables and results as dependent variables.

The location of the research was conducted at the Faculty of Medicine of Universitas Prima Indonesia, part of the clinical pathology laboratory, Medan, North Sumatera and the time the research was in September.

Sampling used a purposive sampling technique. The extract used was turmeric rhizome extract. The samples tested were obtained at Gambir Market, Tembung.

Research tools were calipers, weighing devices, autoclaves, stirring rods, petri dishes, erlenmeyer tubes, measuring cups, incubators, flannel cloths, cotton, Ose needles, filter woods, measuring flasks, bunsen burners, ovens, paper disks, markers, test tubes, labels, aluminum coils, rotary evaporators, micropipets, and fial bottles.

The research materials were rhizome of turmeric (*Curcuma domestica* VALET) 2000 grams (2 kg), 70% alcohol, DMSO, *Bacillus cereus* bacteria and Ciprofloxacin antibacterial.

Tools which would be used should be sterilized. The oven is used for dry heat sterilization and sterilization tools in this

research made of glass using an oven with a temperature of 170°C for 2 hours. Whereas in wet heat, it used an autoclave with a temperature of 121°C for 15 minutes.

Fresh turmeric was cleaned using running water. Furthermore, turmeric is divided into several pieces. Then dry it in a place that is not exposed to direct sunlight. Thus, after the material has been dried and then smoothed, the material became powdery form.

The calculation of Turmeric Rhizome Flour Fluid before being drying was 2000 gr. Turmeric rhizome powder was 200 g. 70% of alcohol volume was needed:

$$V = B / (B_j \text{ 70\% alcohol}) = (2000 \text{ g}) / (0.884 \text{ g / ml}) = 2262.4 \text{ ml}$$

$$75 \text{ parts of extract fluid: } 75/100 \times 2262 \text{ ml} = 1696.5 \text{ ml}$$

$$25 \text{ parts of extract fluid: } 25/100 \times 2262 \text{ ml} = 565.5 \text{ ml}$$

Making Turmeric Rhizome Extract

1. Turmeric powder is weighed as much as 200 grams, put in a container and pour 75 parts of liquid filter (1696.5 ml).
2. Cover the container, wait 5 days and protect from the sun while stirring several times.
3. After 5 days, the pulp is rinsed with the remaining 25 parts of filter liquid until 2262.4 ml is obtained.
4. Then left for 2 days, then pour and transfer into a container.
5. Then the maceration is evaporated using a Rotary evaporator (60°C-65°C) until a thick turmeric rhizome extract is obtained.

Making Extract Sample

- 1) 25% Concentration = 25 gr / 100 mL = 0,25 gr / 1 mL
- 2) 50% concentration = 50 gr / 100 mL = 0,5 gr / 1 mL
- 3) 75% Concentration = 75 gr / 100 mL = 0,75 gr / 1 mL
- 4) 100% concentration = 100 gr turmeric extract.

Making Media

1. Agar Nutrient Media (NA) is by pouring 28 gr of Nutrient Agar (NA) and 1L aquadest into Erlenmeyer and then make into a form of solution by heating, then use an autoclave with a temperature of 120°C sterilized for 15 minutes.
2. Media Nutrient Broth (NB) is by inserting 13 grams of Nutrient Broth (NB) and 1L aquadest into Erlenmeyer, then heated until dissolved. Furthermore, further sterilization with an autoclave sterilization is carried out at a temperature of 120°C for 15 minutes. 30-45 degrees until the shape becomes solid at room temperature and stored in the refrigerator at 5°C.

Preparing Inoculum *Bacillus cereus* colony was taken with a sterile OSE from the existing culture stock and then combined to 10 mL NB. Thus, the colony was suspended, and incubated at 35 ± 20C. Spectrophotometer (Depkes RI, 1995).

Antibacterial Effectiveness Test

- 1) Plant in in a sterile room in laminar air flow near Bunsen.
- 2) Take bacterial suspension from NB media on a 0.1 mL micropipet test tube. Treatmet is conducted near Bunsen so that bacteria in the test tube do not come out or vice versa.
- 3) Then transfer the bacterial suspension to a petri dish and fill 15 mL of NA media, then homogenize by forming the number 8.
- 4) Place the disc paper which has been given drops of extract material at different concentrations and a solution of Ciprofloxacin and aquadest on the surface of NA media that has been planted with bacteria. Then Incubation (37°C) for 18-24 hours.
- 5) The clear zone contained is measured as the inhibition zone around the disc paper with calipers.

Data Analysis

Data analysis of antibacterial effectiveness test result statistically analyzed. The software used was SPSS with the One Way Annova test with the Post Hoc Test Tukey.

H0: There is no difference in effect between turmeric rhizome extract and Ciprofloxacin.

Ha: There is a difference in effect between turmeric rhizome extract and Ciprofloxacin.

RESULT AND DISCUSSION

Process of collecting data was conducted in September 2019 at the UNPRI Laboratory by using turmeric rhizome extract samples toward the bacterium *Bacillus cereus* ATCC 14579. Data collected and examined, it can be concluded the results of the study below.

The diameter of the turmeric rhizome inhibitory zone in contact with *Bacillus cereus* ATCC 14579.

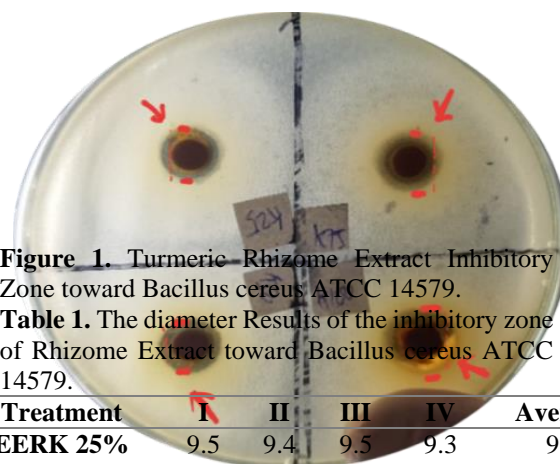


Figure 1. Turmeric Rhizome Extract Inhibitory Zone toward *Bacillus cereus* ATCC 14579.

Table 1. The diameter Results of the inhibitory zone of Rhizome Extract toward *Bacillus cereus* ATCC 14579.

Treatment	I	II	III	IV	Average
EERK 25%	9.5	9.4	9.5	9.3	9.4
EERK 50%	10	9.5	9.8	10.1	9.8
EERK 75%	10.2	10.8	10.5	11	10.6
EERK 100%	11.1	11.3	11.2	11	11.1
KONTROL (+)	31	31	30.9	31.1	31
KONTROL (-)	0	0	0	0	0

Information

EERK: turmeric rhizome ethanol extract

Inhibitory produced by turmeric rhizome extract by using a sensitivity test showed that there was an inhibitory zone in the area of paper discs which had dropped several concentrations. The calipers were used to measure the power of the inhibition zone. To measure the vertical and horizontal parts and then the average is calculated, it was conducted on each treatment and the inhibition zone is

obtained as shown in the figure and table below.

Based on the table and figure above, it shows that the results of research which uses turmeric rhizome extract toward the bacterium *Bacillus cereus* each treatment obtains different results. At a concentration of 25%, it had an average of 9.4 mm, at a concentration of 50% it has an average of 9.8 mm, at a concentration of 75%, it had an average of 10.6 mm, with 100% concentration having an average of 11.1 mm. 25% was the lowest inhibitory zone diameter at treatment, the IV was 9.3 mm and the highest diameter was treated at II with 100% inhibition.

The positive control used was Ciprofloxacin with a inhibition zone of 31.0 mm and the negative control used was Aquadest with a inhibition zone of 0 mm. This shows that the higher the concentration of turmeric rhizome extract, the greater the effectiveness as an antibacterial, as evidenced by the extent of inhibition zones

Ciprofloxacin Inhibition Zone Diameter toward *Bacillus cereus* ATCC 14579 Bacteria.

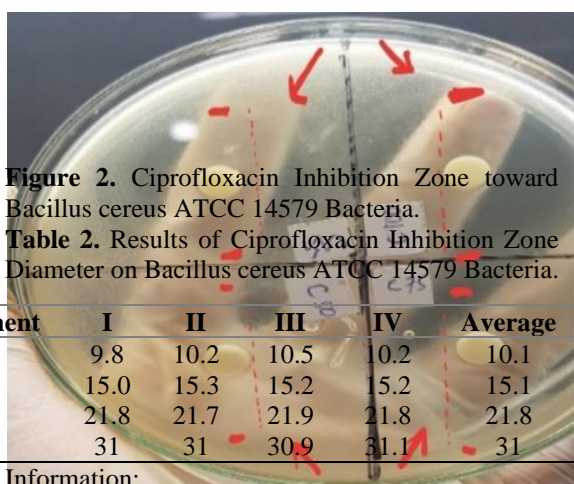


Figure 2. Ciprofloxacin Inhibition Zone toward *Bacillus cereus* ATCC 14579 Bacteria.

Table 2. Results of Ciprofloxacin Inhibition Zone Diameter on *Bacillus cereus* ATCC 14579 Bacteria.

Treatment	I	II	III	IV	Average
C1	9.8	10.2	10.5	10.2	10.1
C2	15.0	15.3	15.2	15.2	15.1
C3	21.8	21.7	21.9	21.8	21.8
C4	31	31	30.9	31.1	31

Information:

C: ciprofloxacin

The inhibitory power of Ciprofloxacin toward *Bacillus cereus* ATCC 14579 could be determined when the inhibitory zone had been formed by looking at it, and measuring it with a calipers by measuring the vertical and horizontal parts and then the average

was calculated. It was conducted at each treatment and the zone was obtained Block like the picture and table below.

Bacillus cereus every treatment given to get different results - different. At a concentration of 25%, it had an average of 10.1 mm, at a concentration of 50% had an average of 15.1 mm, at a concentration of 75% has an average of 21.8 mm, at 100% concentration. There was a diameter of the inhibition zone power of 31.0 mm. at the lowest diameter of the inhibitory zone, it was found a diameter of 10.1 mm conducted at the first treatment with a concentration of 25% and the highest inhibitory zone diameter of 31.1 mm at the IV treatment at a concentration of 100% was 25% on the treatment the. I was 10.1 mm and the highest inhibitory zone diameter is 100%. The negative control used was Aquadest with a inhibition zone of 0 mm.

Comparison of the Inhibition Zone of Turmeric Rhizome Extract with Ciprofloxacin.

From the comparison of turmeric extract zone with ciprofloxacin, it is known that:

H0 : There is no difference in the effectiveness of turmeric rhizome extract with Ciprofloxacin.

Ha : There are differences in the effectiveness of turmeric rhizome extract with Ciprofloxacin.

From the One Way Anova test results, it was obtained a value of $P = 0,000$ which means $p < \alpha$ in which $\alpha < 0.05$ then H_a is accepted and H_0 is rejected. It can be concluded that there are differences in the effectiveness of turmeric extract with ciprofloxacin toward *Bacillus cereus*. Then it was to find out whether there is a difference from each treatment given to bacteria carried out further analysis of Post Hoc Test with Tukey's choice. After conducting the Post Hoc Test, it was found that there were significant differences from each treatment given with a 95% confidence index. In the Post Hoc Tukey test it was also found that the 25%

ciprofloxacin treatment against *Bacillus cereus*, the effectiveness obtained was above 50% turmeric rhizome extract and was below 75% turmeric rhizome extract. As for the concentration of ciprofloxacin 50%, 75%, and 100% have very good effectiveness on the *Bacillus cereus* 95% confidence index.

Discussion

Based on research data result obtained that there is effectiveness of turmeric rhizome extract against *Bacillus cereus* and there was a difference in the effectiveness of turmeric rhizome extract with *Bacillus cereus* ciprofloxacin. The intended effectiveness was the presence of inhibition zones in the surrounding paper discs. With calipers can be measured the inhibitory zone power that has been formed to determine the effectiveness of the treatment. To assess the effectiveness of turmeric extract, greenwood classification is used as shown in the table below.

Table 3. Responses Classification to inhibiting bacterial growth.

Average of inhibition zone diameters	Growth inhibition response
>20 mm	Strong
16-20 mm	Moderate
10-15 mm	Weak
<10 mm	Less effective

Source: (Firdaus, 2014)

The concentration of 100% in turmeric extract was the best concentration which inhibited the growth of *Bacillus cereus* bacteria. This was shown in the second treatment showing a diameter of 11.3 mm which was categorized as a weak inhibitory zone and a concentration of 25% in turmeric extract is the worst concentration inhibiting the growth of *Bacillus cereus* bacteria. This was shown in the fourth treatment showing a diameter of 9.3 mm which was categorized as an ineffective inhibition zone.

In ciprofloxacin, a 100% concentration was the best. This was shown in the fourth treatment showing a diameter of 31.1 mm which was categorized as a strong inhibitory zone and a concentration of 25% was a poor concentration. This was shown

in the first treatment showing a diameter of 10.1 mm categorized as a weak inhibitory zone. The control (-) used was aquadest which did not show an inhibitory zone toward the *Bacillus Cereus* bacteria.

This caused the formation of inhibitory zones because the turmeric rhizome had active compounds that were antimicrobial. Turmeric rhizome had active compounds such as essential oils and yellow dyes (curcuminod). *Curcuma domestica* VALET was a plant that was able to treat infectious diseases.

In this research, it was found that the formation of inhibitory zones in turmeric rhizome extract increased sequentially from the lowest to highest concentrations ranging from 9.4 mm to 11.1 mm. Antibacterial was a beneficial effect found in turmeric rhizome extract to *Bacillus cereus* bacteria and this study was in line with existing research of (Yuliati, 2016), turmeric rhizome extracts studied starting from concentrations of 15%, 30%, 50%, 75% and 100% of the bacteria *Bacillus sp* and *Shigella dysenteriae* both had the best inhibitory zone effect is 14.7 mm which is relatively weak. (Pangemanan et al., 2016), turmeric rhizome extract studied began with concentrations of 5%, 10%, 20%, and 40% of the positive bacteria *Staphylococcus aureus* gram and *Pseudomonas sp* gram negative both of which had inhibitory zone effects which were best 15 mm at a concentration of 40%, which was relatively weak.

Curcumin which has an antimicrobial effect contains polyphenol compounds and it is good for inhibiting thiolase enzymes (sulfhydryl enzymes) as a result of the formation of protein denaturation processes. In addition, polyphenols are compounds which can dissolve in fat. Thus, it can damage the structure of bacterial cell membranes. Essential oils contain terpenoids which work as an antibacterial through the process of destroying the structure of bacterial cell membranes.

According to (Cushine, Cushine, & Lamb, 2014), Alkaloid as an anti-bacterial

works by damaging the constituent constituents of peptidoglycan in bacterial cells by damaging the structure of the outer membrane and cytoplasm, resulting in leakage of cytoplasmic contents⁽¹⁵⁾.

According to (Cushine et al., 2014), flavonoids as antibacterial work processes of flavonoids are divided into 3, as follow:

1. In nucleic acids, there is a buildup of bases to suppress the formation of nucleic acids.
2. The formation of extracellular flavonoid-protein complexes to suppress cell membrane function. This can damage the permeability of cell membranes and suppress the bond between the enzyme ATPase and phospholipase.
3. Besides, it suppress the process of energy metabolism and suppress cytochrome C reductase.

It was different with research by (Muadifah, Eka Putri, & Latifah, 2019), that turmeric rhizome extract as an antibacterial at a concentration of 45%, 55%, 65%, and 75% toward gram-positive *Staphylococcus aureus* bacteria. The best inhibitory zone effect is 11 mm at 45% concentration classified as weak.

CONCLUSION

Turmeric rhizome extract has antibacterial effectiveness toward bacteria *Bacillus cereus* ATCC 14579. Turmeric rhizome extract has a weak effect as inhibitory zone power against *Bacillus cereus* ATCC 14579 bacteria according to inhibition zone classification, the best concentration is 100% average diameter of inhibition zone 11.1 mm. Based on the inhibition zone classification, ciprofloxacin has a strong effect on the bacterium *Bacillus cereus* ATCC 14579 as a growth inhibitor of the bacteria. 100% is the best concentration choice because there is an average inhibition zone diameter of 31.0 mm. The analysis test is carried out with the One Way Anova test and $P = 0,000$ was obtained, which means $p < \alpha$ where $\alpha < 0.05$ then H_a is accepted and H_o is rejected. There are differences in the effectiveness of

turmeric rhizome extract with ciprofloxacin toward *Bacillus cereus* ATCC 14579 bacteria.

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