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Antibacterial Test of Teki Grass Extract (*Cyperus Rotundus*) in Inhibiting *Escherichia Coli* and *Salmonella Typhi*

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Abstract: This study aims to determine the antibacterial ability of Cyperus rotundus extract in inhibiting the growth of Escherichia coli and Salmonella typhi by measuring the diameter of the inhibition zone. This research method used a completely randomized design (CRD) and LSD test as a follow-up test with one factor, namely variations in the concentration of C. rotundus extract 20%, 40%, 60%, 80% and 100%, distilled water as a negative control and the antibiotic chloramphenicol as a positive control. The results showed that the extract of C. rotundus could inhibit the growth of Escherichia coli with the largest inhibition zone diameter indicated by a concentration of 100% which was 2.33mm and the smallest inhibition zone diameter was found at a concentration of 20%, namely 1.00 mm. While in Salmonella typhi the largest inhibition zone was shown at a concentration of 100%, namely 3, 03mm and the smallest inhibition zone was indicated by a concentration of 20%, namely 1.07 mm. The results of the ANOVA and LSD tests with 95% confidence test showed that there was a difference in the average diameter of the inhibition zone in each treatment with variations in the concentration of C. rotundus extract with a concentration of 80% as the most effective.

Keywords: ANOVA; Cyperus rotundus; Escherichia coli; LSD; Salmonella typhi

Introduction

Cyperus rotundus (nutgrass) is one of the native Indian plants which is commonly found in India, Asia, Australia, Europe and North America (Akbar, 2020; Sivalan, 2012; Sivalan, 2013), which is used as a traditional medicine used by several countries such as India, China and Japan as a medicine. medication for seizures, stomach disorders and inflammatory stomach diseases, diarrhea, diabetes, inflammation, gastric pain (Kilani-Jaziri et al., 2011; Peerzada et al., 2015; Sarmento et al., 2015).

C. rotundus is known to contain secondary metabolites such as alkaloids, flavonoids, tannins, steroids, sesquiterpenoids, saponins and reducing sugars (Sivalapan, 2013; Gusmailina and Komarayati, 2015; Hamida et al., 2015; Juliantina et al., 2019) which can act as antimalarial substances, abortifacient effects, analgesics, anthelmintics, antialcoholic, anticonvulsant, antihepatotoxic, antihistamine activities, antihypertensive, anti-inflammatory, antioxidant activity, antipyretic, coronary vasodilator activity, hair stimulation effect, lowers uric acid, antifungal and antimicrobial (Jain & Das, 2016; Nurjannah et al., 2018; Sarmento et al., 2015; Sepriana et al., 2017; Setiawan et al., 2014; Rotty et al., 2015). *Salmonella typhi* is a rod, Gram-negative, aerobic and facultative anaerobic bacterium which is the main factor causing Salmonellosis (typhoid fever) with a common symptom, namely gastrointestinal infection. Typhoid fever is still common in developing countries, with around 11-20 million cases of typhoid fever occurring worldwide in 2018 with a mortality rate of 128,000-161,000 people/year (WHO, 2018).

Apart from *Salmonella typhi, Escherichia coli* can also infect the digestive tract of animals and humans. Escherichia coli is a gram-negative, facultative anaerobic bacterium which is a normal flora in the digestive tract

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of animals and humans. But Escherichia coli can also be pathogenic if the amount exceeds normal levels in the intestine, so that it can infect the digestive tract.

Antibiotics are one of the treatments most often given to patients with diarrhea. However, the method of treatment by giving antibiotics that are irrational or with inaccurate prescriptions can cause antibiotic resistance to pathogenic microorganisms (Pusporini, 2019; Brock et al., 2003; Kumar dan Chordia, 2017). Therefore, an alternative way of overcoming this problem is needed by utilizing plants that provide benefits as medicinal plants and contain active antimicrobial ingredients that can slow down or even kill pathogenic microbes. So, based on the description above, the researchers used extract of nutgrass (*Cyperus rotundus*) as an antibacterial in inhibiting the growth of *Escherichia coli* and *Salmonella typhi*.

Method

The material used in this study was sedge grass obtained from Percut Village, Kec. Percut Sei Tuan, the test bacteria were Escherichia coli ATCC 25922 and Salmonella typhi ATCC 19430 obtained from the Pharmacy Laboratory of the University of North Sumatra, 96% ethanol, and Nutreint Agar (NA) media. The tools used in this study were petri dishes, tweezers, calipers/rulers, Rotary Evaporator, loop needles, Autoclave, Beaker Glass, incubator, Erlenmeyer, measuring cups, scales, Bunsen, separating funnel, paper discs and dark bottles.

Production Of Grass Extract (Cyperus Rotundus)

As much as 300 grams of nut grass powder was dissolved and soaked in 96% ethanol solution, covered with aluminum foil and left for 3 days (72 hours) at 25°C-30°C with occasional stirring. Filter the extract soak to produce filtrate and dregs. The dregs were rinsed with 96% ethanol and soaked again for 2 days at room temperature. Then evaporated with a Rotary Evaporator at 50°C for ± 25 minutes, the aim is to obtain a liquid extract of sedge grass. The resulting extract was evaporated over a water bath at 50°C until a thick extract was obtained. Then the extract is put into a closed dark bottle and stored in the refrigerator.

Preparation of Nutrient Agar (NA) Media

A total of 8 grams of NA media was dissolved in 400 ml of distilled water and then heated with occasional stirring until homogeneous. The media was sterilized using an autoclave at 121oC for 15 minutes at a pressure of 1.5 atm, then allowed to stand until the media temperature was warm (40°C-45°C). Then 15 ml of sterile media was put into the petri dish, then the media was allowed to solidify (Ngajow et al., 2013).

Antibacterial Test of Teki Grass Extract (C. rotundus) Against Escherichia coli and Salmonella typhi

The pure culture of the test bacterial culture was inoculated using a loop of wire and put into a test tube containing 10 ml of 0.9% physiological NaCl solution, then homogenized and compared to the turbidity of the bacterial suspension with Mc. Farland with a density of 108 CFU (coloning forming unit). Bacterial suspension was grown into NA media by applying it to the media. Nutgrass extract was dripped with various concentrations of 20%, 40%, 60%, 80% and 100% on sterile disc paper, then inoculated on the surface of the test medium. The test plates were incubated for 24 hours at 37°C. Observations were made by measuring the diameter of the inhibition zone which was signaled by the formation of a clear area around the pathogenic bacterial colonies.

Result and Discussion

Bacteria Test

Antibacterial testing of nut grass extract used *Escherichia coli* and *Salmonella typhi* bacteria as test bacteria. The test bacteria were identified by Gram staining, so that the results were obtained which can be seen in Table 1.

Table 1. Identification results of Escherichia coli and Salmonella typhi bacteria

Bacteria	Shape	Arrangement	Description
Escherichia coli	Basil	Monobasil	Gram (-)
Salmonella typhi	Basil	Monobasil	Gram (-)

Identification was carried out to ascertain whether the test bacteria used were Escherichia coli and Salmonella typhi. The results of the identification showed that both types of test bacteria were in the form of bacilli, red in color with monobacilli cell arrangement.

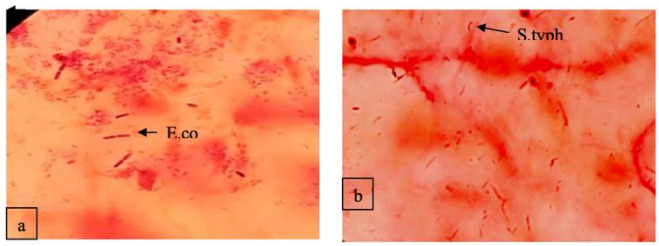

Figure 1. Gram Staining of Bacteria with 100x Magnification. a) Escherichia coli bacteria, b) Salmonella typhi bacteria

The results of Gram staining of Escherichia coli and Salmonella typhi microscopically showed that the two test bacteria were Gram-negative bacilli. Escherichia coli bacteria form short rods or commonly called cocobasil. According to Murwani et al. (2017), Escherichia coli has

a cell length of about 20 µm and a cell width of 0.25-1 µm, a cell volume of 0.6-0.7 µm with a peritrical flagellum and is facultative anaerobic.

While the Salmonella typhi Gram stain test showed that the Salmonella typhi bacteria were in the form of short and thin rods (bacilli), composed singly (monobacilli), Gram negative. Jajere (2019) states that Salmonella typhi has a cell size of around 0.2 -1.5x 2-5 µm with a peritrichous flagellum, which is aerobic and facultative anaerobic.

Antibacterial Test of Teki Grass Extract (Cyperus rotundus) Against Test Bacteria

Antibacterial test results of nutgrass extract (C. rotundus) against Escherichia coli and Salmonella typhi showed that the five variations in extract concentrations namely 20%, 40%, 60%,80% and 100% were able to suppress the growth of the test bacteria with different inhibition zone sizes (Fig. 2).

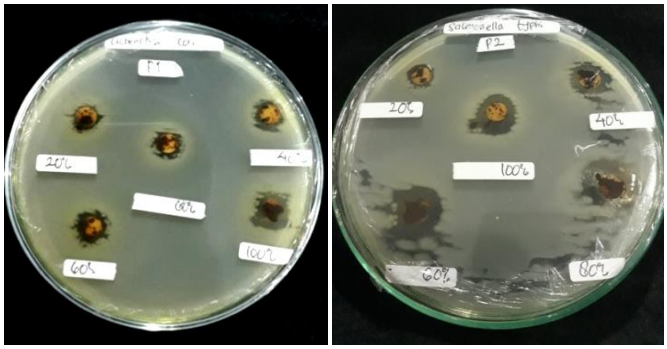

Figure 2. Antibacterial Test Results of Teki Grass Extract with Concentrations of 20%, 40%, 60%, 80% and 100%. a) Escherichia coli, b) Salmonella typhi

The diameter of the Escherichia coli inhibition zone formed due to the application of nutgrass extract (C. rotundus) indicates that the nutgrass extract can inhibit the growth of Escherichia coli bacteria. The average diameter of the Escherichia coli inhibition zone can be seen in Table 2.

The inhibition zone formed by each concentration of nutgrass extract against Escherichia coli showed different results. Based on the data in table 2. it is known that, the greater the concentration of the extract, the stronger the inhibition produced will be. This is in accordance with the statement of Amir et al. (2014) which states that the size of the concentration of antimicrobial substances can affect the activity of antimicrobial compounds, so that the inhibition of antimicrobial substances will be high if the concentration is high. According to Dali et al. (2011) one of the factors that affect the area of the inhibition zone is the amount of active compound composition contained in the extract/solution. Sivapalan & Jeyadevan (2012) and Sivapalan (2013), stated that nutgrass extract contains secondary metabolites in the form of phenolic compounds, flavonoids, glycosides, saponins, alkaloids, tannins, steroids and reducing sugars, some of which have antibacterial effects.

Table 2. Data on the diameter of the inhibition zone (mm) of Escherichia coli on the treatment of sage grass extract (C. rotundus)

Treatment	Repetition			Total	Mean
	1	2	3		
0% (Control -)	0.0	0.0	0.0	0.0	0.00^{d}
20%	1.1	1.0	0.9	3.0	1.00^{c}
40%	1.6	1.4	1.3	4.3	1.43^{c}
60%	1.7	2.4	1.3	5.4	1.80^{bc}
80%	1.8	2.4	2.1	6.3	2.10^{b}
100%	2.5	2.4	2.1	7.0	2.33^{b}
Chloramphenicol (Control +)	10.8	11	11.3	33.1	11.03^{a}

Note: Numbers not marked with different letters show significant differences according to the LSD test results.
Known: LSD 5% (0.486)

The largest inhibition zone is indicated by a concentration of 100% which is 2.33mm, the smallest inhibition zone is found at a concentration of 20% with a diameter of 1.00mm. Meanwhile, the diameter of the inhibition zone produced by the antibiotic Chloramfenicol was 11.03mm. The results of this study are in line with the research of Nurjanah et al. (2018) and Ngajow et al. (2013) which stated that the highest extract concentration in the administration of nut grass extract to *Staphylocccus epidermidis* and *Propionibacterium acnes* was found at a concentration of 100% with a diameter of 19.98 mm in Staphylocccus epidermidis, 30.08 mm in Propionibacterium acnes and the smallest concentration was found at a concentration of 20% with a diameter of 8.03 mm in *Staphylococcus epidermidis*, 11.59 mm in Propionibacterium acnes. The data in table 2 were then analyzed using ANOVA to obtain significant differences with a 95% confidence test for variations in extract concentrations of 20%, 40%, 60%, 80% and 100%.

The results of the ANOVA test of Teki Grass Extract (*Cyperus rotundus*) against *Salmonella typhi* showed that the F count > F table was 0.05 which indicated that there was a significant/significant difference at each concentration of the extract. So it can be proven that the extract of nutgrass (Cyperus rotundus) has antibacterial power or can inhibit the growth of Escherichia coli bacteria. LSD testing was carried out to see which concentration of sedge grass extract was most effective in inhibiting pathogenic bacteria. Based on the LSD test it is known that concentrations of 20%, 40% and 60% are significantly different from concentrations of 80% and 100%. Meanwhile, the concentrations of 80% and 100% were not significantly different, which means that the

two concentrations of nutgrass extract have the same effectiveness in inhibiting *Escherichia coli* bacteria, so the most effective dose is at a concentration of 80%

Table 3. Data on the diameter of the inhibition zone (mm) of *Salmonella typhi* on the treatment of sedge grass extract (*C. rotundus*)

Treatment	Repetition			Total	Mean
	1	2	3		
0% (control -)	0.0	0.0	0.0	0.0	0.00[d]
20%	0.9	1.3	1.0	3.2	1.07[c]
40%	1.7	1.8	1.3	4.8	1.60[c]
60%	2.2	1.9	2.3	6.4	2.13[bc]
80%	2.2	2.3	2.6	7.1	2.37[b]
100%	2.3	2.8	4.0	9.1	3.03[b]
Chloromfenicol (control +)	12.8	12.9	13.3	39.0	13.00[a]

Note: Numbers not marked with different letters indicate a significant difference according to the LSD test results.
Known: LSD 5% (0.486)

The largest inhibition zone is indicated by a concentration of 100% which is 3.03mm, the smallest inhibition zone is found at a concentration of 20% which is 1.07mm. While the diameter of the inhibition zone produced on the antibiotic Chloramfenicol was 13.00mm. The results of this study are in line with the research of Nurjanah et al. (2018) which stated that the highest extract concentration in the administration of C. rotundus extract to Staphylocccus epidermidis and Propionibacterium acnes was found at a concentration of 100% with a diameter of 19.98 mm in Staphylocccus epidermidis, 30.08 mm in Propionibacterium acnes and the smallest concentration was found at a concentration of 20% with a diameter of 8.03 mm in Staphylococcus epidermidis, 11.59 mm in Propionibacterium acnes.

The results of the ANOVA test of the Sesame Grass Extract (Cyperus rotundus) against Salmonella typhi showed that the calculated F value (390.057) > F table was 0.05 (2.848) which indicated that there was a significant difference (real) or had a significant difference at each concentration of the extract. So it can be proven that the sedge grass extract has antibacterial properties that can suppress the growth of Salmonella typhi.

Similar to the antibacterial test of the extract against Escherichia coli bacteria, the LSD test at 0.05 or 5% level showed that there was a significant difference in each treatment of the extract against Salmonella typhi bacteria. Where in this test the results were not significantly different at a concentration of 80% and 100%, which means that both concentrations are equally strong, the extract concentration that is most effective in suppressing the growth of Salmonella typhi bacteria is found at a concentration of 80%, as well as a positive control, namely the antibiotic Chloramphenicol has a greater inhibitory power than the concentration of nut grass extract. (Faisal & Permana et al., 2020; Gansareng et al., 2018; Fredella et al., 2022).

Conclusion

Based on the research that has been done, it can be concluded that sedge grass extract can be used as an antibacterial agent with a concentration of 80%, which is the most effective concentration of sedge grass extract in inhibiting the growth of Escherichia coli and Salmonella typhi.

Author Contributions

Sartini contribution to the concept or design of the article; or the acquisition, analysis, or interpretation of data for the article. Rahmiati contribution to analysis, or interpretation of data for the article. Selvi herliyani and Rianto contribution of drafted the article or revised it critically for important intellectual content. Ellen Panggabean contribution of interpretation of data for the article. Saipul sihotang contribution to analysis and approved the version to be published.

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Conflicts of Interest

The author declares no conflict of interest.

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