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Sensitivity of tekelan leaves (*Chromolaena odorata*) on the growth of *salmonella*

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ABSTRACT

Introduction: Inappropriate use of antibiotics can lead to an increase in side effects and antibiotic toxicity. One of the natural herbal ingredients as an alternative treatment for *Salmonella* infection is the tekelan leaves plant (*Chromolaena odorata*). The purpose of this study was to determine the effect of boiled tekelan leaves (*Chromolaena odorata*) leaves on the growth of *Salmonella*.

Methods: The study was a post-test-only control group design, which examined the effects of tekelan leaf (*Chromolaena odorata*) extract against the growth of *Salmonella* at concentrations of 20%, 40%, 60%, 80%, and 100%. The study's independent variables were the concentrations of ethanol extract from tekelan leaves at 20%, 40%, 60%, 80%, and 100%. The bound variable in this study is the growth of Salmonella typhi based on minimal obstructive power and minimal killing power. Microsoft Excel 2020 was used to examine the data and determine how the effects of each therapy at each concentration differed from one another.

Results: Results of treatment with tekelan leaves decoction showed that concentrations of 20%, 40%, 60%, 80%, and 100% of tekelan leaves decoction (*Chromolaena odorata*) did not form an inhibitory zone, so it was said to be in the resistant category.

Conclusion: The concentration of the boiled leaves of tekelan leaves (*Chromolaena odorata*) showed no effect on the growth of *Salmonella*.

Keywords: *Salmonella*, *tekelan leaves* (*Chromolaena odorata*), herbal medicine, antibiotic.
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INTRODUCTION

Food-borne diseases with complexities are a problem that has the potential to threaten public health worldwide and have a major impact on the economy. One of which is caused by approximately 250 pathogens including bacteria, viruses, and parasitic organisms.[1] Data reported by the Indonesian Food and Medicine Supervisor Agency in 2016 stated that Animals are the group that causes the highest cases of food poisoning with the number of cases reaching 2,426. *Salmonella* species occupy the third rank of poisoning cases, the highest presentation of the main causes of infectious diseases through food in both humans and animals.[2]

Salmonella typhi bacteria are bacteria that can be said to be very easy to live and reproduce due to aerobic and facultative anaerobic growth, at pH 6-8 with a body temperature of around 15-41 degrees Celsius with an optimum growth temperature of 37 degrees Celsius. The growth temperature shows that the growth of *Salmonella typhi* bacteria is very easy considering that the room temperature we know is around 20-25°C, making it easier for bacteria to contaminate food, especially food that is not placed properly.[3]

Antibiotic medications are often used to treat infectious infections. Antibiotic misuse, however, can bring dangers including bacterial resistance. Furthermore, taking antibiotics frequently results in adverse reactions like allergic reactions, toxic reactions, as well as biological and metabolic changes in patients.[4] Therefore, people tend to find and use another kind of treatment, such as plants because they are accessible and have few side effects. People have a tendency to choose medications made from natural ingredients like plants that are commonly used in traditional medicine. One of the plants that can be used as an antibacterial medicine is the leaves of takelan. Takelan leaves (*Chromolaena odorata*) have chemical compounds that potentially have antibacterial properties such as flavonoids, tannins, and saponins.[5] Traditionally, takelan leaves have been used as a medicine in wound healing to treat angina, malaria medication, headache, antidiarrhea, antihypertensive, and anti-inflammatory.[6] This study aimed to determine the effect of boiled tekelan leaves (*Chromolaena odorata*) leaves on the growth of *Salmonella*.

METHODS

Study Design

The study tested the effects of tekelan leaves (*Chromolaena odorata*) extract against the development of Salmonella at doses of 20%, 40%, 60%, 80%, and 100% using a

post-test-only control group design. The concentrations of ethanol extract from tekelan leaves at 20%, 40%, 60%, 80%, and 100% served as the study's independent variables. The growth of *Salmonella* typhi based on minimal obstructive power and minimal killing power is the bound variable in this investigation.

Materials

The materials used are fresh tekelan leaves, light green in color, position 3rd from bud and Media NAS, SSA, MHA (Mueller Hinton agar), Aquadest, Pz, Ciprofloxacin disc paper, and Blank disc paper. The tools used in this study were Petri dishes, scales, pipettes, an autoclave, measuring glass 250, test tubes, tube racks, stirring rods, measuring pipette, erlenmeyer, a round loop, an incubator, a sterile swab, beaker glass 250 ml, 100 ml measuring cup, sterile gauze, balance, thermometer, and micropipette.

Data collection procedures

The procedure for making a decoction of tekelan leaves (*Chromolaena odorata*) carried out in this study was by using 50 grams of tekelan leaves (*Chromolaena odorata*) and 50 ml distilled water and boiled under a temperature of 50°C for 15 minutes. After being boiled, the result of the process was continued under sterilization procedures.

Sample inspection procedures were carried out by preparing Mueller Hinton Agar culture media for *Salmonella* inoculation. Suspension of germs that have been synchronized with the standard Mc. Farland 0.5 was inoculated in MHA (Mueller Hinton Agar) media using the Street plate technique using a sterile swab. For each concentration of tekelan leaf extract, leaf juices were pipetted as much as 50 microliters. Each sample was then incubated for 24 hours at 37°C with the position not reversed. The observation and measuring process of the inhibition zone formed using a millimeter ruler and recording the observed results was carried out after 24 hours.

Data analysis

The data collected is data obtained through direct observation, using a test that measured the zone of clarity which indicated the presence of an inhibitory zone on the MHA (Mueller Hinton Agar) culture media that was given treatment with concentrations of 100%, 80%, 60%, 40%. And 20% of tekelan leaves decoction compared to positive control. The disc paper method is one of the diffusion methods where in this method a blank disc paper is first soaked into a solution of tekelan leaves concentration then put the disc paper on MHA media that has been planted with *Salmonella* MC Farland 0.5 then incubated 1x24 hours 37°C. Next measure the clear zone formed around the well. Data were analyzed using Microsoft Excel 2020 to evaluate the effect differences for each treatment in every concentration.

RESULTS

Data from the results of the study above are data on differences in the inhibition zones of the *Salmonella* based on the concentration of the decoction of tekelan leaves (*Chromolaena odorata*) and positive control, when compared with the standard category of resistance to ciprofloxacin positive control.

Results of treatment with tekelan leaves decoction showed that concentrations of 20%, 40%, 60%, 80%, and 100% of tekelan leaves decoction (*Chromolaena odorata*) did not form an inhibitory zone, so it was said to be in the resistant category.

Based on the results of the study in Table 1, it shows that no inhibition

Table 1. Research data on the inhibition of tekelan leaves leaf juice on *Salmonella* growth

No	Replication	The average results of the inhibition zone of *Salmonella* bacteria based on the concentration of tekelan leaves juice in units (mm)					
		20%	40%	60%	80%	100%	Control (ciprofloxacin)
1.	R1	0	0	0	0	0	30
2.	R2	0	0	0	0	0	30
3.	R3	0	0	0	0	0	30
4.	R4	0	0	0	0	0	30
5.	R5	0	0	0	0	0	30
	Amount	0	0	0	0	0	150
	Average	0	0	0	0	0	30

*The standard categorization of the diameter of the Ciprofloxacin positive control inhibition zone according to CLSI (2011) is as follows: Sensitive (mm): 21 mm; Resistant (mm): 15 mm; Intermediates (mm): 16-20 mm

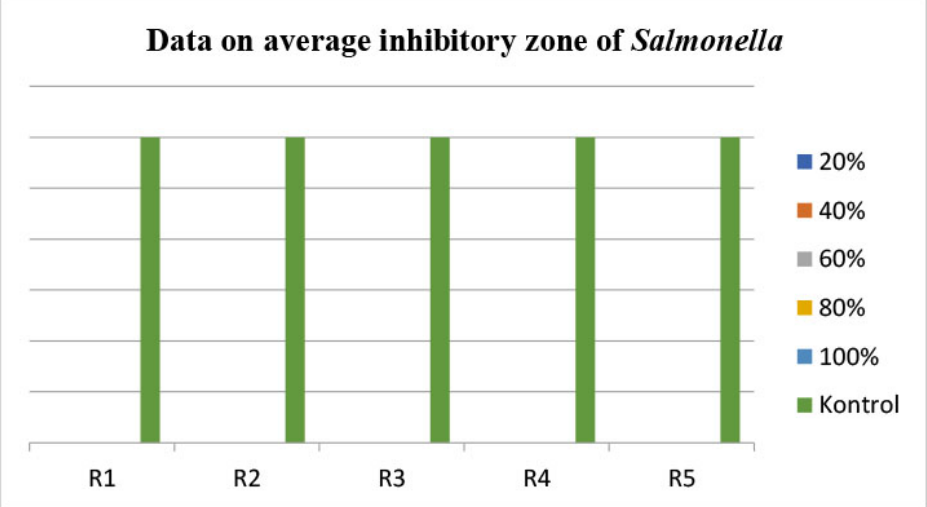

Figure 1. Based on the results of the study, the effect of boiled taken leaves (*Chromolaena odorata*) leaves can be presented in the form of the diagram above.

zone was formed at all concentrations of tekelan leaves (*Chromolaena odorata*) leaf decoction from concentrations of 100%, 80%, 60%, 40%, 20% compared to the control treatment. The result of the inhibition zone of *Salmonella* bacteria at all concentrations was 0 mm, while the positive control was 30 mm. This shows that the whole concentration of tekelan leaves leaf decoction has no effect on *Salmonella* bacteria so its antibacterial activity is said to be resistant.

DISCUSSION

This study did not find any obstacle caused by the administration of tequila leaf extract to *Salmonella* bacteria. This study is not consistent with a study conducted by Fadia et al., 2020 which found maximum eradication of the bacteria. (Fadia 2020).[4] The presence of secondary metabolite chemicals in ethanol leaf extract may contribute to the antibacterial activity of the extract.[7] This is consistent with the findings of phytochemical analyses of tequila leaves, which include several secondary metabolite components, such as alkaloids, flavonoids, saponins, steroids, triterpenoids, and tannins, that can serve as antibacterials. Antibacterial substances can harm in bactericidal (killing bacteria) and bacteriostatic ways.[8]

Salmonella is resistant to tekelan leaves leaf decoction due to many influencing factors, including tekelan leaves leaf decoction using water as a solvent. Water is a polar solvent but the use of water as a solvent has drawbacks compared to ethanol, where the solubility of water is 29.775%, methanol 39.858%, and ethanol 42.375%. The low level of solubility causes the ability of water to remove metabolites to be less, difference in the level of the polarity of the solvent used to make the stew. This difference will affect the characteristics of the active compound as well as antibacterial activity and antioxidant activity.[9]

Polar solvents can extract quaternary alkaloids, phenolic components, carotenoids, tannins, sugars, amino acids, and glycosides. Semi-polar solvents can extract phenolic terpenoid compounds, alkaloids, aglycones, and glycosides. Non-polar solvents can extract chemical compounds such as waxes, lipids, and volatile oils. Water and ethanol have the same polarity, but water has unstable acidic and alkaline properties so that the active compound which acts as an antibacterial cannot inhibit the growth of pathogenic bacteria.[9]

Another factor that causes no inhibition zone to form at all concentrations of 100%, 80%, 60%, 40%, and 20%. In the positive control using the antibiotic Ciprofloxacin, there was an inhibition zone of 30 mm which indicated that the microbe was a sensitive (sensitive) antibiotic. Temperature, incubation time, and lack of homogeneity of the tekelan leaves leaf decoction were all controlled by the researchers. The difference in the size of the inhibition zone was also influenced by the sensitivity level of the test organism, culture medium, incubation time and conditions, the rate of diffusion of antibacterial compounds, and the concentration of antibacterial compounds.[4] This study still consists of several limitations such as in the method and any other compounding variables that might affect the findings.

CONCLUSION

The concentration of the boiled leaves of tekelan leaves (*Chromolaena odorata*) showed no effect on the growth of *Salmonella*. Further studies are needed to re-evaluate these findings.

FUNDING

The authors declare no funding in this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

ETHICAL STATEMENT

This research has been declared to have received an ethical certificate from the Ethical Committee of Faculty of Health, Universitas Nahdlatul Ulama, Indonesia No: 295/EC/KEPK/UNUSA/2021.

AUTHOR CONTRIBUTION

All authors contributed equally to this study.

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